IRON TOLERANCE AND YIELD OF *Zea mays* (L.) IN A FERRUGINOUS SOIL AFTER EXPOSURE TO BIOSYNTHESIZED COPPER NANOPARTICLES

BY

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SR/2090/RPR/23/26

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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, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

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CERTIFICATION

This is to certify that this work was carried out by Michelle Oluwaseyi FAYEUN in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

Mrs. F. N. Egbenoma (Project Supervisor)	**DATE**
Prof E. D. Vwioko (Head of Department)	**DATE**
External Examiner	**DATE**

DEDICATION

I dedicate this project to God Almighty for his love and to my supportive family for their encouragement.

ACKNOWLEDGEMENTS

I appreciate God Almighty for His guidance, spirit upliftment and strength to complete this research project. I also appreciate my project supervisor, Mrs Egbenoma for her support as well as my Professor, Beckley Ikhajiagbe for his mentorship throughout this study. Thank you for stretching my abilities and for everything you've taught me. I acknowledge the Head of Department, Prof E. D. Vwioko for his leadership and accessibility to the students of the department; and my course advisor, Prof. Eboigbe for his advice and support. Special appreciation to the people who raised me and taught me to always be the best I can be, Mr. and Mrs. Olatunde Fayeun, for their love, protection and emotional and financial support. I love you and thank you for raising me the way that you did, to be exceptional. I appreciate my brothers, Israel and Olumide Fayeun for being my top cheerleaders. To my friends and project colleagues; Samuel Amagbakhen, Adesola Peters, Favour Ugheighele, Jennifer Ediae, Deborah Okereke, Tochukwu Isaiah, John Uvietaire and Osho Ikeghai, thank you for your love, encouragement and every moment spent with you. I feel blessed to have you all as a part of my journey in school. I want to also thank the entire JCIN UNIBEN community for teaching me all I know about leadership and for the opportunities they provided me. I am happy to have found you at the time that I did and I will always remember how you impacted me. In this time and age, I am grateful for life and companionship.

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ABSTRACT

Ferruginous soils pose challenges for plant growth as their high iron content can lead to iron toxicity and reduced crop yields. Copper nanoparticles show promise in alleviating iron toxicity in plants. This project assessed maize plants treated with varying copper nanoparticle concentrations in ferruginous soils with elevated iron. The goal was to evaluate maize tolerance to different iron stress levels and resultant yield. The results revealed enhanced maize growth with copper nanoparticles, especially at 35% and 100% concentrations, in both initial and subsequent growth stages. This indicates a dose-dependent relationship between nanoparticle concentration and maize growth, with higher concentrations conferring increased maize survivability against iron toxicity. Temporal dynamics emerged in the maize response to nanoparticles, underscoring the need to consider exposure duration in applications. During week 2, plants treated with a 35% Cu nanoparticle concentration in soils contaminated at 4 ESV displayed smaller leaf areas than those in 2.5 ESV soils. This suggests higher contamination may hinder the nanoparticles' positive leaf area effects. Overall, the intricate effects of copper nanoparticles on maize morphological characteristics depended on concentration, soil contamination, and specific parameters. Plant height, leaf length/width/area, and sheath length were influenced by treatment, while blocks significantly impacted plant height, leaf length, and sheath length. Copper nanoparticles show the potential to enhance maize survivability in ferruginous soils, offering a promising sustainable agriculture avenue in iron-rich environments.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Yield loss poses a significant obstacle to agricultural productivity, aggravated by the escalating impact of severe environmental stresses stemming from global climate shifts (Ha *et al.,* 2020). These alterations not only impact soil characteristics and processes but also pose a threat to worldwide food security, thereby influencing the global demand for food and fibre resources (Mondal, 2021). The prevalence of ferruginous soil spans approximately 42% of the Earth's surface and this plays a pivotal role in shaping food production, particularly affecting staple crops like Maize. Even in Africa, notably in Nigeria, a leading maize producer with a dense population, regions such as Edo State struggle with substantial ferruginous soil (Doyou *et al.*, 2017). Given these circumstances, there is a pressing need to devise innovative strategies that can sustainably enhance crop survivability in ferruginous soil conditions, especially in nations experiencing rapid population growth. The primary aim of this study is to assess the influence of biosynthesized copper nanoparticles (Cu NPs) on the iron tolerance and survivability of maize grown in ferruginous soil. This study delves into the potential of Cu NPs as a micronutrient to influence maize growth and productivity in iron-rich soils, which pose challenges for crop cultivation due to their high metal content and low fertility. Understanding this holds promise for pioneering innovative agricultural practices that leverage nanotechnology to surmount limitations in ferruginous soils, thereby bolstering food security and advocating sustainable farming practices.

1.2 Research Problem

Maize cultivation spans a wide spectrum of agronomic conditions, encompassing diverse altitudes, latitudes, temperatures and soil types. This versatile crop holds a diverse and pivotal position in the diets of millions across Africa and South Asia while also serving as essential animal feed in East Asia. With its superior yield compared to other cereals, maize stands out as a preferred choice for farmers grappling with land scarcity and burgeoning population densities. Despite its global significance, a substantial portion of maize production, approximately 61% is channelled towards animal consumption, leaving a mere 13% for human consumption (Grote *et al.*, 2021). However, the annual average of 1.127 million tons of maize production worldwide faces significant hurdles in iron-rich soils which impede growth. Iron-rich soils, also known as ferruginous solids, are characterised by high iron content and are prevalent, presenting a formidable challenge for agriculture (Jin *et al.*, 2022).

1.3 Justification of Study

Understanding iron tolerance in maize is important in enhancing its survivability and in developing resilient maize varieties. Research in molecular biology has exposed specific genes responsible for iron uptake and transportation, offering potential for breeding programs to enhance iron tolerance in maize (Shahzad *et al.*, 2021). Given the increasing concerns for climate change, improving iron tolerance of maize in ferruginous soil is a strategy to build agricultural resilience, ensure sustained productivity and address food insecurity. To improve iron tolerance and survivability of maize in ferruginous soil, various methods have been explored by different scientists. Some of these methods include conventional breeding techniques (Lung'aho *et al.*, 2011), germ fraction removal (Keigler *et al.*, 2023), use of rhizobacteria (Amogou *et al.*, 2019), and mycorrhizal fertilization (Koda *et al.*, 2018). Additionally, practices such as combined application of mineral and organic fertilisers, conservation of organic matter and improved soil fertility have been suggested to sustain maize yield (Detchinli and Sogbedji, 2015). While these methods have been explored, nanoparticles have also been shown to enhance plant yield in ferruginous soil conditions that were previously challenging for crop productivity (Ikhajiagbe *et al.,* 2021). Nanoparticles also hold a unique advantage over these methods; have a small size which allows for better penetration into plant tissues, ensuring efficient uptake of nutrients; they can also protect nutrients from leaching or volatilization, increasing their availability to plants over an extended period. Furthermore, they can be tailored to release nutrients in response to specific triggers providing targeted and sustained nutrients to plants (Wang *et al.,* 2019) Nanotechnology is a promising chemical application in agrobiotechnology used to enhance the resilience of crops to non-living environmental stressors and it has become more prominent in the modern world. Nanoparticles (NPs) are natural or manmade materials with at least two dimensions between 1 and 100 nm (Siddiqui *et al.,* 2015). Based on size, physical characteristics and chemical characteristics, it is divided into various categories. The two major categories that represent the carbon-based NPs are Fullerenes and carbon nanotubes (CNTs). Fullerenes comprise nanoparticles made of globular hollow configurations (cage-like) i.e. allotropic carbon forms that are uncharged while carbon nanotubes represent cylindrical forms with high elasticity and thermal conductivity (Nguyen *et al.*, 2021). Nanoparticle biosynthesis, also known as green synthesis involves the use of extracts from plant parts such as fruits, leaves, bark, seeds and roots to synthesise NPs. It also involves the utilisation of biomass waste, viral particles and metabolites from microorganisms such as bacteria, yeast, algae and actinomycetes. This process relies on plant metabolites such as proteins, alkaloids, and terpenoids and their bioactive-reducing abilities. Some secondary metabolites such as poly-hydroxyl groups also synthesise NPs by reducing metal ions (Antonio-Pérez *et al.*, 2023). Nanoscience in agriculture, since it began, has had a significant effect on disease detection, nutrient absorption enhancement and nutrient conduction. It is used to fight plant diseases by delivering pesticides for targeted treatment. Modern research using nanoparticles in crops such as pumpkin, soybean, onion, wheat, lettuce, radish, ryegrass, bitter melon, spinach, alfalfa, cucumber and corn has shown significant improvement in seedling growth, photosynthesis, germination, nitrogen metabolism, protein level and gene expression indicating their potential contributions in crop enhancement. Metals such as Silver, Aluminium, Gold, Iron, Palladium, Zinc, Fullerenes, Carbon and Copper have repeatedly been used in nanoparticle synthesis. Copper-based nanoparticles, in particular, are of interest because of their availability, low cost and other properties (Govindaiah *et al.,* 2014). Copper (Cu) is a vital element that plays a role in plant growth and development, and this includes the development of seeds and the formation of chlorophyll. It is a micronutrient i.e. it is needed in small quantities by plants and has effects on crops (Maize included) when it is deficient. Cu also regulates biochemical reactions in plants and helps in photosynthesis, a process essential for respiration and the metabolism of carbohydrates and proteins (Nguyen *et al.,* 2020). Copper nanoparticles are composed of copper with sizes ranging from 1 to 100 nm and are synthesised chemically or naturally. They have a long history of biological and antibacterial use as a colouring agent, another factor that makes them particularly intriguing (B *et al.*, 2023). Their antimicrobial properties make them useful in plant pathogen control, inhibiting the growth of a variety of plant pathogens such as bacteria, fungi, and viruses. The mechanism used by copper nanoparticles to kill pathogens is believed to be through their ability to generate reactive oxygen species (ROS) which damage their cell wall and membranes. They also inhibit enzymatic properties of pathogens and their use in plant pathogen control has several advantages over traditional chemical pesticides; Copper nanoparticles are less harmful to the environment and thus offer a lower risk in comparison to chemical pesticides. Secondly, due to their targeted action, they are less likely to harm other organisms that are beneficial to plants such as pollinators. Research has also shown the potential of copper nanoparticles' use in pesticide degradation (Kashyap *et al.*, 2023).

1.4 Literature Review

1.4.1 Iron Tolerance in Maize and Its Impact on Growth

Maize, known scientifically as *Zea mays* L. stands as a global agricultural powerhouse, ranking just behind rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) in worldwide cultivation. Revered as the "queen of cereals", maize's exceptional genetic yield sets it apart, being the sole cereal crop adaptable to diverse seasons, applications, and environments. This versatile crop boasts a myriad of varieties, from waxy corn to popcorn, each offering unique characteristics and serving as a vital resource for industrial and economic advancement (ICAR, 2024). Spanning nearly 100 million hectares across 125 developing nations, maize forms the cornerstone of food security in regions like Africa, Latin America and Asia. However, amidst its prominence, maize faces a significant abiotic hurdle in the form of acidic soils, as highlighted by Tandzi *et al.* (2018). Iron (Fe), an indispensable trace element crucial for the growth of all living organisms, including humans and maize, plays a pivotal role in agriculture by enhancing plant vitality and influencing crop productivity and quality (Abbaspour *et al*., 2022). The repercussions of iron deficiency reverberate through maize cultivation, impairing photosynthetic processes, diminishing photosynthate production, and ultimately leading to yield losses. Such deficiencies not only curtail production but also pose a threat to food security, underscoring the critical importance of iron in sustaining maize growth and biochemical adaptations to bolster iron adsorption, ensuring the continuity of its growth and productivity. In cases of iron deficiency in maize, the plant releases mugineic acid, which binds with trivalent iron in the rhizosphere soil and employs a specific transporter to convey the iron from the soil into its root cells. Nevertheless, various maize cultivars exhibit distinct responses to iron stress and mugineic acid secretion, highlighting the significance of breeding iron-efficient maize as a viable approach to mitigate low iron stress and enhance maize survivability in iron-deficient soils (Long *et al.*, 2020). Biologists have researched the impact of iron levels on maize yield under drought stress conditions, revealing that the biological yield of maize increased with higher iron concentrations. This study concluded that iron application is a crucial agricultural technique in semiarid and arid regions to counteract the stress induced by moisture deficiency (Rafsanjani *et al.*, 2019). An investigation demonstrated that, on the fourth day of exposure to partial iron deficiency, maize experienced a 20% reduction in dry biomass, which escalated to 35% under complete iron deficiency, while plant height remained constant (Nenova and Stoyanov, 1993). In contrast to iron-sufficient plants, maize plants lacking in iron exhibited diminished chlorophyll levels in the leaves, reduced catalase activity, and elevated levels of phenolase, aspartate and alanine aminotransferase, and ribonuclease activities. Varying amounts of iron were supplied over 7 days based on the degree of iron deficiency, resulting in a decline in total catalase activity ranging from 20% to 70% (Nenova and Stoyanov, 1995).

1.4.2 Germinability of Maize Seeds and Its Relationship with Soil Properties

The growth and germinability of plants are affected by environmental factors that surround the plant. These environmental factors also affect the maize plant, especially abiotic factors such as rainfall, soil moisture, soil temperature, soil type etc (Nyéki *et al.*, 2022);

1.4.2.1 Soil Texture

Maize seeds grow well in well-drained soils with a good balance of sand, silt, and clay. Sandy soils may limit germination due to insufficient water as they drain quickly. Clayey soils can compact and thus, limit the aeration of seeds. Clayey soils also have the lowest germination rate in maize (Valdés-Rodríguez *et al.*, 2013).

1.4.2.2 Moisture Content

Inadequate water can delay or inhibit the germination of maize. Excessive moisture can inhibit oxygen uptake and waterlog the seeds, leading to seed rot. Soil water potential also controls the rate of germination. At low soil water potential, the rate of germination is delayed and the seeds become infected with pathogens, producing thin and weak plants (Smith-Eskridge, 2014).

1.4.2.3 Temperature

Maize seeds germinate best within a specific temperature range. Germination is delayed by cold soils while excessive high temperatures can dry out the moisture content of the soil and damage the seeds. Soil temperature affects the metabolic processes and enzymatic activity that take place during germination. Studies have shown that the optimal temperature for growing sweet corn is 25 – 28°C (Cherlinka, 2023).

1.4.2.4 Nutrient Availability

Imbalances in levels of nutrients such as nitrogen, phosphorus and potassium influence the metabolic processes and energy required for seed germination and subsequent seedling growth. Organic matter in soil can enhance seed germination as it adds nutrients to the soil (Silva and Uchida, 2000).

1.4.2.5 Soil pH Level

Soil pH levels influence nutrient availability. Maize generally prefers a slightly acidic to neutral soil (pH 6-7) and extreme pH levels can affect nutrient availability, and impact germination and early growth of seedlings (Miller, 2016).

1.4.2.6 Soil Aeration

Aeration and oxygen availability are crucial for germination as they promote root development and overall plant health. Compacted soils or soils with poor aeration limit the intake of oxygen by seed which has damaging consequences and can lead to slow germination or no germination at all (Dasberg and Mendel, 1971).

1.4.3 Role of Copper Nanoparticles in Enhancing Plant Growth and Stress Tolerance

In meeting the growing demand of the rising population for food, agricultural production must also increase steadily in sustainable practices. Global warming and climate change have led to extreme increases in drought and temperature and the use of agrochemicals has become threatening to human health and the environment. As a result, finding ways to grow more high-quality food with limited land and water, without harming the environment, is one of the biggest challenges faced in this century (Hafeez *et. al.,* 2015). In recent times, nanotechnology has been used for plant protection and this has had a great impact on agriculture. At nanoscale, essential components are better conducted in the plants. Studies have shown that soda lime powder with a low melting point containing Cu nanoparticles demonstrates effective antimicrobial activity against fungi and bacteria. This is due to the effects of Ca2+ lixiviated from the glass that inhibit them. A comparative study of the antifungal effects of Cu-based nanoparticles and other commercial agrochemicals on tomato (*Solanum lycopersicum*) infected by *Phytophthora infestans* showed that the synthesised Cu-based nanoparticles were more active in inhibiting the disease than the commercial agrochemicals at low concentrations (Rai *et al.,* 2018). Another research showed that the application of copper nanoparticles to the soil increased the yield and germination performance of wheat by reducing the oxidative stress in wheat (Shi *et al.*, 2016). Cu nanoparticles also increase antioxidant properties, and relative moisture content, and stabilise pigment levels in leaves (El-Saadony *et al.*, 2022).

1.4.4 Previous Studies on the Effects of Nanoparticles On Maize In Ferruginous Soil

I. **Nanoparticle treatment of maize analysed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis (Sillen *et al.,* 2020)**

   This study assessed the use of nanosilver for phytopathogen control with maize and analysed the metatranscriptome of the maize rhizosphere, observing multiple unintended effects of exposure to nanosilver in soil during a specific growth period. It showed that nanosilver could turn out to be negative to crop productivity and ecosystem health in the long term and highlighted the need to include the microbiome when assessing the risk associated with nano-enabled agriculture.

II. **Metal nanoparticles as effective promoters for Maize production (Hoang *et al.*, 2019)**

   This research analysed the use of metal nanoparticles to improve the growth and productivity of corn. The impact was demonstrated throughout the plant development using three metals (Cu, Fe, Co) with Cu having the highest productivity. The increased production was consistent with increased chlorophyll content and drought resistance observed in the early growth stage.

III. **Effect of ZnO Nanoparticles on Growth and Biochemical Responses of Wheat and Maize (Srivastav *et al.*, 2021).**

   This study indicated that ZnO NPs at low doses can act as a seed priming agent, to achieve better germination and seedling growth. It showed that the application of ZnO NPs leads to an increase in amylase activity; however, decreased dehydrogenase activity at higher ZnO NP doses during germination in both maize and wheat. Thus indicating that dose-dependent ZnO NPs can be utilised as a seed priming agent and potential fertiliser for enhancing crop yields in the future (Srivastav *et al.,* 2021).

1.5 Research Aim and Objectives

Aim

To enhance iron survivability of maize in ferruginous soil.

Objectives

The objective of this study is to;

i. Determine the influence of copper nanoparticles on plant height of maize despite exposure to elevated iron in ferruginous soil;
ii. Determine the influence of experimental regimes on folial development;
iii. Examine the changes in folial modal colour as a means of measuring plant response; and
iv. Present visible morphological anomalies due to treatment exposure.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study Area

This study was conducted in front of the Botanical Garden of the Department of Plant Biology and Biotechnology, University of Benn (Ugbowo Campus), Benin City, Edo state.

2.2 Materials

Shovel, wheelbarrow, dried zobo flowers **(***Hibiscus rosa-sinensis* Linn**)**, copper nanoparticles, conical flasks of 1000 ml volume, spray bottles, metre rule, measuring scale, hybrid maize, filter paper, iron sulphate, tarpaulin and black medium-sized polythene bags, measuring cylinder, manual weighing balance, and a pen were among the tools available.

2.3 Experimental Design

The experiment was designed in a randomised full-block model with three well-spaced duplicates.

2.4 Research Methodology

This 3-month research study began at the beginning of the second semester of the school academic year.

2.4.1 Soil Collection

Ferruginous reddish-brown soil was obtained from the vicinity of the Botanical Garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. This soil was obtained using a shovel from three different locations with the coordinates; (6.397264,5.616390); (6.397137,5.616406); and (6.397157,5.616421) to collect the uppermost part of the soil and a wheelbarrow to transport the soil to the site of the experiment where it was homogenised.

2.4.2 Site Preparation

The experimental location was first cleared with a cutlass to remove invasive species. This included cutting off surrounding branches that provided shade on some parts of the site. Then the site was lined with a black tarpaulin. Layers of soil were then poured and spread on the tarpaulin. The purpose of this is for insulation, to help the polythene bag maintain a stable temperature; for anchorage, to help anchor polythene bags in place; for protection, to shield the polythene bag from damage by factors such as sunlight, pests or physical abrasion; and for weed control. 124 perforated polythene bags were then filled with the homogenised soil, labelled appropriately and mounted neatly on the site. The bags were arranged in 4 blocks and the treatments were randomised within these blocks.

2.4.3 Preparation of Iron Sulphate Treatment

The collected soil was put in the planting bags after being weighed at 7 grams of soil per bag. The bagged soils were then treated with iron sulphate to raise the iron content. A weighted sample of iron sulphate crystals at three different concentrations of Ecological screening values; 1, 2.5 and 4 ESV was adopted.

2.4.3.1 Calculations

1 concentration of Ecological screening value of iron (ESV) is equivalent to 200 milligrams of iron in 1 kg of soil. To calculate for 1, 2.5 and 4 ESV for 7g of soil;

(a) For 1 ESV; 200 mg of Fe in 1 kg of soil.

7 kg of soil will need 7 x 200mg

1 ESV = 1400mg of Fe or 1.4g of Fe

(b) For 2.5 ESV; 1ESV x 2.5

= 1.4 g of Fe x 2.5

2.5 ESV = 3.5g of Fe per 7 kg of soil

(c) For 4 ESV; 1 ESV X 4

= 1.4 g of Fe x 4

4 ESV = 5.6g of Fe per 7 kg of soil

For this treatment, the quantity of iron sulphate required to pollute or elevate the ESV for 5 bags of soil was measured together with the manual weighing balance and dissolved in 1 litre (1000 ml) of water. The solution was then divided into 5 parts (1 part for each bag) of the original measurements. 200ml of this solution was measured with a measuring cylinder and poured into a container. Then water was added to make it up to 1000 ml which was poured into the bags.

2.4.4 Preparation of Copper Nanoparticles Treatment

This was done by first pounding 10 grams of dried zobo flowers **(***Hibiscus rosa-sinensis* Linn**)** and rinsing, after which it was put in a conical flask of 100 ml distilled water. Then it was shaken vigorously for 10 minutes to allow the content of the leaves to diffuse into the water, thereby changing its colour to dark red. The extract was filtered using Whatman No. 1 filter paper (Buarki *et al.,* 2022). Separately, 10 mM of copper sulphate was prepared by mixing 1.59609 grams of copper sulphate in 1 litre of distilled water. 10 ml of leaf extract was added to 1 litre of the CuSO4 solution. It was then placed on a magnetic stirrer and allowed to mix until a colour change from dark red to reddish brown was observed (Ramasamy and Selvam, 2015).

2.4.5 Treatment Designation

For the elevation of soil iron content level, 1 ESV, 2.5 ESV, and 4 ESV were adopted. The experiment also constituted the application of Cu NPs in three (3) different concentrations; 35%, 75% and 100%. These were prepared by changing the ratio of the prepared nanoparticle solution to water. The 35% concentration was composed of a 7:13 ratio of the nanoparticle solution to water. The 75% concentration consisted of a 15:5 ratio of the NP solution to water while the 100% concentration consisted of a 1:1 ratio. The nursery bags were labelled according to the treatments they contained i.e. elevated soil iron content and copper nanoparticle treatment. The labels and their representations are as follows;

E_1 - Concentrations of Fe at 1 ESV

E_2 - Concentrations of Fe at 2.5 ESV

E_3 - Concentrations of Fe at 4 ESV

Cu_1 - Copper NP at 35% concentration

Cu_2 - Copper NP at 75% concentration

Cu_3 - Copper NP at 100% concentration

CNTRL - Without either treatment

2.4.6 Planting and Treatment Application

The hybrid maize seeds (OBA SUPER-6) were checked entirely and sorted before being soaked for a few minutes in water. The nursery bags were adequately watered before the maize seeds were placed in them, 5 seeds per bag. Two (2) weeks after planting, the nanoparticle treatment was applied by foliar spray. Readings were taken weekly as the plants were observed and watered regularly for 5 weeks.

2.4.7 Experiment Management

The various practices were carried out at regular intervals;

I. Weeding

This was done by handpicking unwanted surrounding plants every 2 days.

II. Watering

This was done twice a week in between varying periods of rainfall.

2.4.8 Parameter Score

The following parameters were taken from an index plant with a centimetre metre rule at weekly (7 days) intervals during the entire project time; The plant height was measured from the soil surface to the highest point of the arch of the uppermost leaf whose point is tipping down and the leaf length was measured from the node to the tip of the index leaf i.e fifth leaf from the bottom; The leaf width was measured by holding the metre rule across the widest point of the index leaf, from side to side; Leaf area was calculated by multiplying the values of the leaf length and leaf width with a constant of 0.75 i.e Leaf length x Leaf width x 0.75 = Leaf Area; The number of leaves was estimated by starting the count at the base of the plant with the lowermost visible leaf collar (coleoptile) and ending with the uppermost visible leaf and the sheath length was measured from the soil surface to the node of the index leaf. The colour-magnitude of the leaves per week was also observed and compared with a standard scale.

2.5 Data Analysis

The results were provided as the mean of three replicates. For the investigation, a completely randomised experimental design was used, with the assumption that the whole experimental plot was homogeneous when soils were pooled before usage. This data was analysed using a two-way factor analysis of variance (ANOVA). Statistical analysis was carried out using SPSS version 23 and, when applicable, PAST version 2.17c

CHAPTER THREE

RESULTS

The results of the present study are represented in Tables and Figures whereas Plate 3.1 shows the geographical location of the experimental site.

PLATE 3.1: EXPERIMENTAL SITE AT THE INITIAL STAGES OF THE EXPERIMENT

(Mag 0.5x)

Figure 3.1 presents the plant height of maize plants exposed to nanoparticles in ferruginous soil. Cu_1, Cu_2, and Cu_3 represent plants exposed to copper-based nanoparticles at 35%, 75% and 100% respectively and E_1, E_2, and E_3 represent plants at 1, 2.5 and 4 Ecological screening values (ESV) i.e 1.4g, 3.5g and 5.6g respectively. In this experiment, plants that were exposed to 35% (Cu_1) and 100% (Cu_3) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu_1E_1 and Cu_3E_1) had the same height of 5.9 cm during the 1st week and 32cm and 36.5 cm, respectively at week 5 compared to that which was not exposed to nanoparticles treatment but still under similar soil conditions (5.6, and 40.3 cm respectively). At week 2, plants that were exposed to copper-based nanoparticles at 75% (Cu_2) and sown in soils contaminated with iron sulphate at 2.5 ESV (Cu_2E_2) and 4 ESV (Cu_2E_3) had heights of 11.7 cm and 10.5 cm respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (10.9 cm and 9.4 cm respectively); and at week 4, they had heights of 21.2 cm and 21 cm, respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (31.4 cm and 33.7 cm respectively).

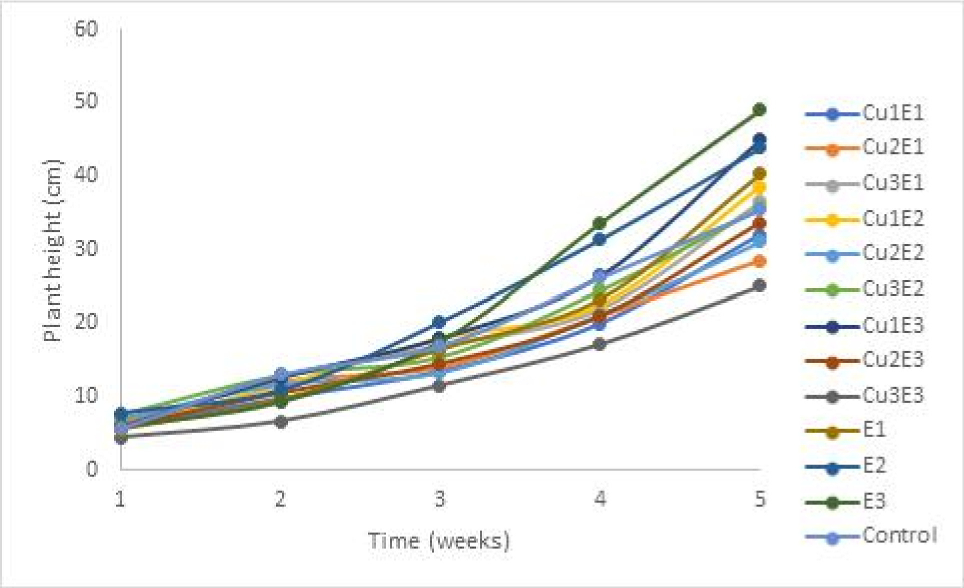


FIGURE 3.1: PLANT HEIGHT OF MAIZE PLANTS

In the experiment, figure 3.2 showed that plants which were exposed to 75% (Cu2) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu2E1) had a leaf length of 12 cm during the 1st week and 74.5 cm at week 5 compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (15 and 63.6 cm respectively). At week 2, plants that were exposed to copper-based nanoparticles at 35% (Cu_1) and sown in soils contaminated with iron sulphate at 2.5 ESV (Cu_1E_2) and 4 ESV (Cu_1E_3) had leaf lengths of 26.4 cm and 10.3 cm respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (32. 9 cm and 36.1 cm respectively); and at week 4, they had leaf lengths of 50.6 cm and 46 cm, respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (56.4 cm and 51.8 cm respectively).

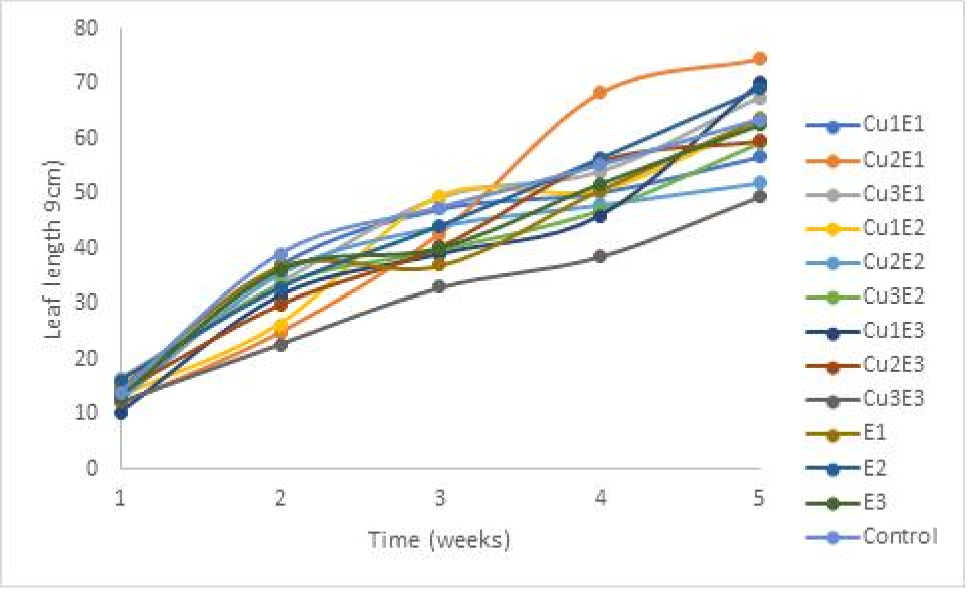


FIGURE 3.2: LEAF LENGTH OF MAIZE PLANTS

Figure 3.3 represents the leaf width of maize plants exposed to nanoparticles in ferruginous soil. The experiment showed that plants that were exposed to 75% (Cu2) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu2E1) had a leaf width of 1.4 cm during the 1st week and 4.4 cm at week 5 compared to those that were not exposed to nanoparticles treatment or iron sulphate contamination i.e control (1.4 and 3.2 cm). At week 2, plants that were exposed to copper-based nanoparticles at 35% (Cu_1) and sown in soils contaminated with iron sulphate at 2.5 ESV (Cu_1E_2) and 4 ESV (Cu_1E_3) had leaf widths of 2.4 cm and 1.8 cm respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (both at 2.2 cm); and at week 4, they had leaf lengths of 3.7 cm and 2.6 cm, respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (both at 2.7 cm).

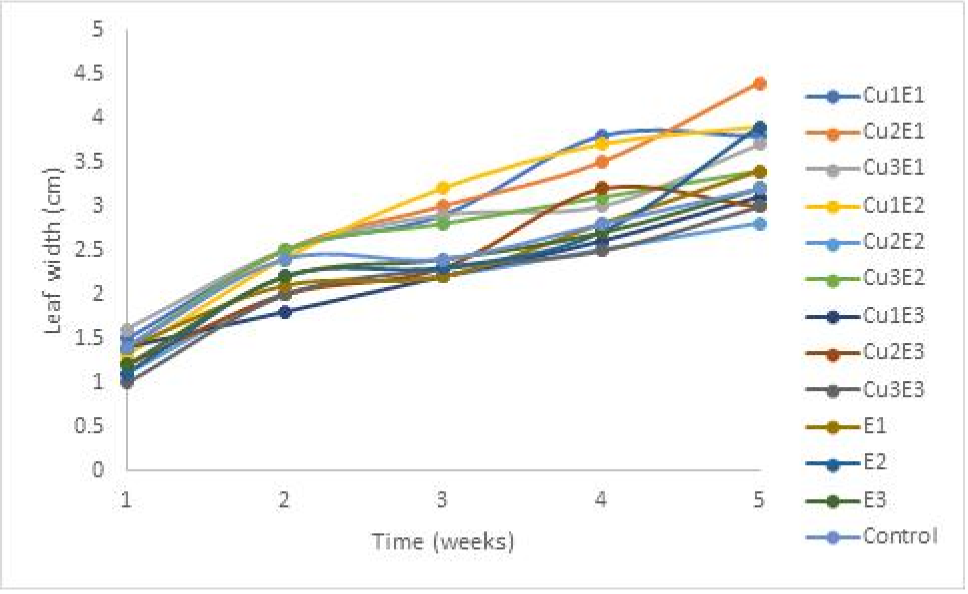


FIGURE 3.3: LEAF WIDTH OF MAIZE PLANTS

Figure 3.4 represents the leaf area of maize plants exposed to nanoparticles in ferruginous soil. The experiment showed that plants that were exposed to 75% (Cu2) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu2E1) had a leaf area of 12.6 cm^2 during the 1st week and 245.85 cm^2 at week 5 compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (15.75 and 162.18 cm^2 respectively). At week 2, plants that were exposed to copper-based nanoparticles at 35% (Cu_1) and sown in soils contaminated with iron sulphate at 2.5 ESV (Cu_1E_2) and 4 ESV (Cu_1E_3) had leaf area of 47.52 cm^2 and 42.5 cm^2 respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (54.29 and 59.57 cm^2 respectively); and at week 4, they had leaf area of 140.41 cm^2 and 89.7 cm^2, respectively compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (114.21 and 104.89 cm^2 respectively).

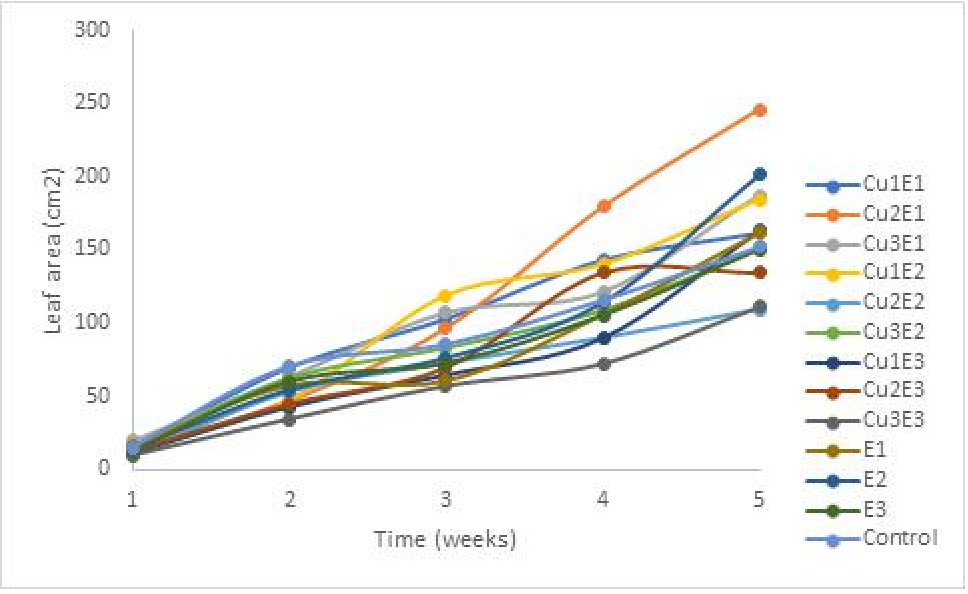


FIGURE 3.4: LEAF AREA OF MAIZE PLANTS

Figure 3.5 represents the number of leaves of maize plants exposed to nanoparticles in ferruginous soil. The experiment showed that plants that were exposed to 75% (Cu2) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu2E1) had 4 leaves during the 1st week and 9 leaves at week 5 compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (5 and 10 leaves). At week 2, plants that were exposed to copper-based nanoparticles at 35% (Cu_1) and sown in soils contaminated with iron sulphate at 2.5 ESV (Cu_1E_2) and 4 ESV (Cu_1E_3) had 6 leaves, the same number of leaves as those that were not exposed to nanoparticles treatment but still under similar soil conditions; and at week 4, they had leaf area of 10 leaves each compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions with 9 leaves each. Plate 3.2 shows the morphological characteristics of maize plants between week 1 to week 4.

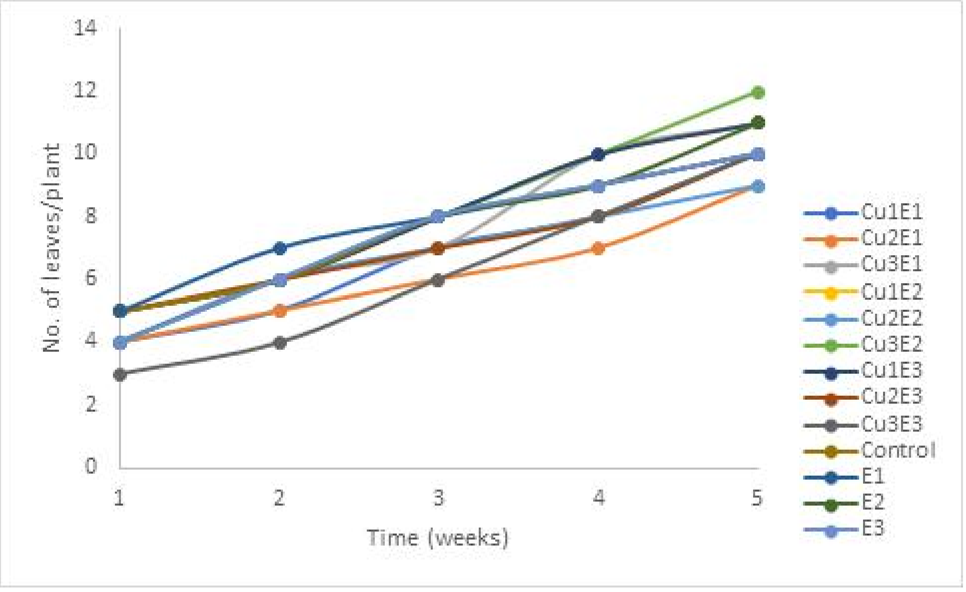
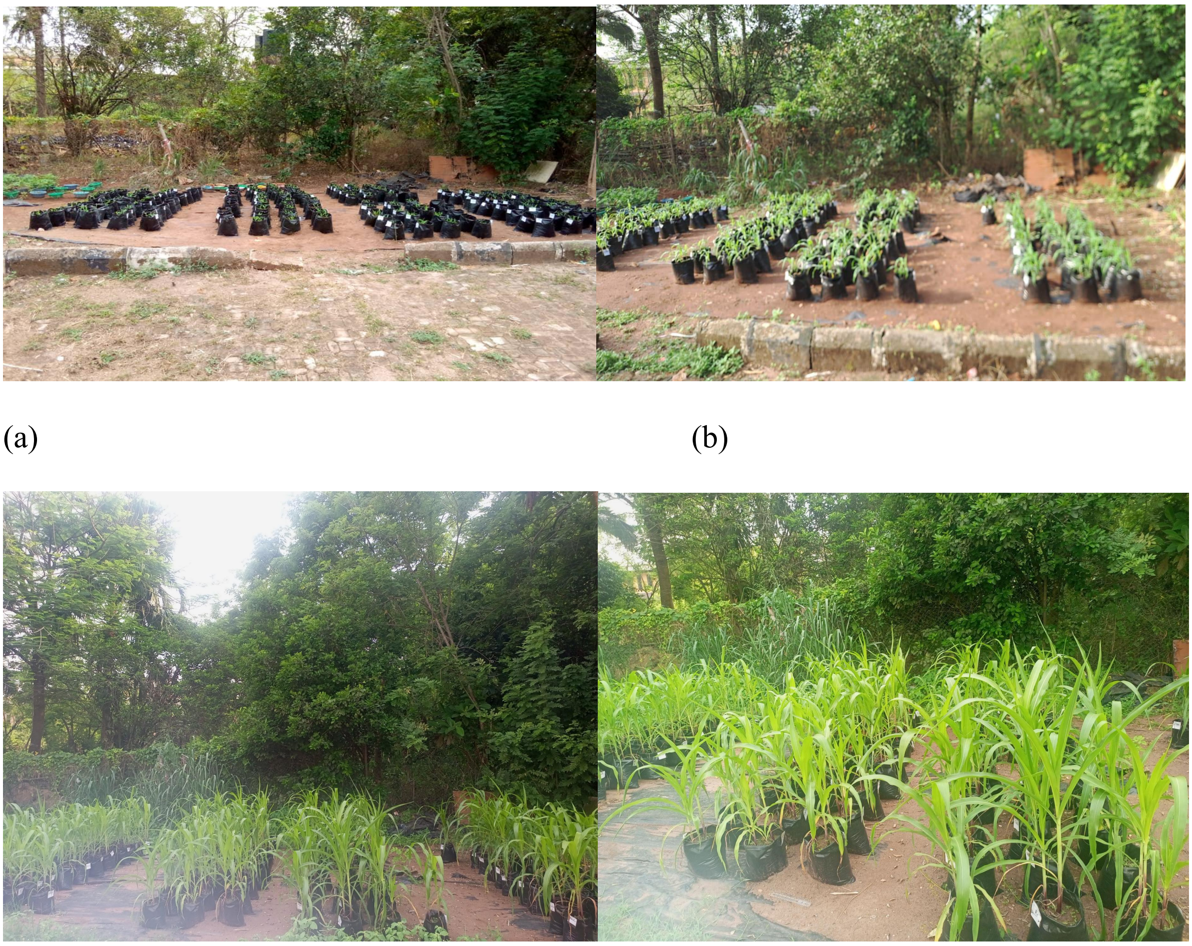


FIGURE 3.5: NUMBER OF LEAVES OF MAIZE PLANTS



(a) (b)

(c) (d)

PLATE 3.2: GROWTH PROGRESS OF MAIZE PLANT FROM WEEK 1 TO 4

(Mag 0.5x)

Table 3.1 shows the sheath length observed for Block 1 on the 4th and 5th week. The experiment showed that plants that were exposed to 75% (Cu2) copper-based nanoparticles and sown in soils contaminated with iron sulphate at 1 ESV (Cu2E1) had a sheath length of 20 cm during the 4th week and 23 cm at week 5 compared to those that were not exposed to nanoparticles treatment but still under similar soil conditions (23.3 and 28.5 cm, respectively). All other treatments showed significant changes in the sheath length as well.

TABLE 3.1: SHEATH LENGTH OF MAIZE PLANTS EXPOSED TO FERRUGINOUS SOIL

Sheath Length	Week 4	Week 5
Cu_1E_1	20	23
Cu_2E_1	21	28.5
Cu_3E_1	18.6	23
Cu_1E_2	19	24
Cu_2E_2	16	24.2
Cu_3E_2	19.5	25.7
Cu_1E_3	19.9	35.5
Cu^2E_3	21	28.2
Cu_3E_3	15	18
Control	18.6	20.3
E_1	23.3	28.5
E_2	29.9	34.7
E_3	30.5	39.4

Figure 3.6 represents the colour-magnitude of leaves of maize plants exposed to nanoparticles in ferruginous soil. The experiment showed fluctuations in the colour-magnitude of leaves of the different treatments. The majority of the plants had colour magnitudes of 1 to 2 at the beginning of the experiment. However, at week 5, they increased to colour magnitudes of 2 to 4. Cu_1E_1 had the deepest colour magnitude of 4 at week 5.

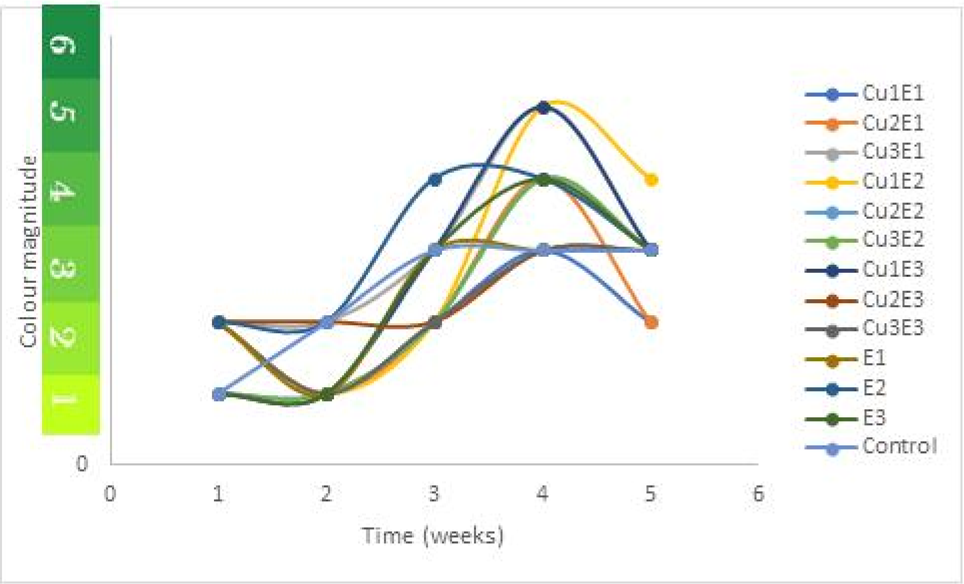


FIGURE 3.6: COLOUR-MAGNITUDE OF LEAVES OF MAIZE PLANTS

TABLE 3.2: MORPHOLOGICAL CHARACTERISTICS OF MAIZE SEEDLINGS AFTER 5 WEEKS

	Plant Height	**Leaf Length**	**Leaf Width**	**Leaf Area**	**Number of Leaves**	**Sheath Length**
Cu_1E_1	32.1a	56.7	3.8	161.595	10	23
Cu_2E_1	28.5a	74.5a	4.4a	245.85a	9	28.5
Cu_3E_1	36.5a	67.5	3.7	187.31a	11	23
Cu_1E_2	38.5a	63.1	3.9	184.56a	11	24
Cu_2E_2	31.8a	52.2a	2.8	109.22	9	24.2
Cu_3E_2	35.7a	59.3	3.4	151.21	12	25.7
Cu_1E_3	45	70.1	3.1	162.98	11	35.5a
Cu_2E_3	33.7a	59.7	3	134.32	10	28.2
Cu_3E_3	25.5a	49.5	3	111.37a	10	18
E_1	35.4a	63.6	3.4	162.18	10	28.5
E_2	40.3	69	3.9	201.82a	11	34.7a
E_3	43.9	62.5	3.2	150.11	10	39.4a
Control	49.1	63.5	3.2	152.43	10	20.3
LSD (0.05)	9.2	11.6	1.2	32.3	3	8.4
p-value	0.094	0.192	0.118	0.047	0.624	<0.001

Means have to be rounded off to the nearest integer

a - Means on same column differ significantly from the control

b - Means on the same column differ significantly from the E_2

Cu_1 - Cu nanoparticle at concentration 1 (35%)

Cu_2 - Cu nanoparticle at concentration 2 (75%)

Cu_3 - Cu nanoparticle at concentration 3 (100%)

E_1 - Concentration at Ecological screening value of Fe (ESV); E_2 - 2.5xESV; E_3 - 4xESV

Table 3.1 analyses the morphological characteristics of maize plants in the 5th week. The plant height of plants with Cu_1E_1 and Cu_1E_2 concentrations was significantly different from the Control, showing a height of 32.1 cm compared to the Control which had 49.1 cm. However, plant height with Cu_1E_3 showed no significant difference from the Control. Plants with Cu_2E_1 and Cu_2E_2 concentrations showed leaf lengths of 75.5 cm and 52.2 cm, respectively which were significantly different from the Control (63.5 cm) but Cu_2E_3 plants showed no significant difference in leaf length. Only Cu_2E_1 showed a significant difference in leaf width from the Control. In the leaf area, plants with Cu_3E_1 and Cu_3E_3 concentrations were 187.3cm^2 and 111. 37 cm^2 respectively, which is significantly different from the Control with 152 cm2. However, Cu_3E_2 plants had no significant difference in leaf area from the Control. Generally, there was no significant difference in the number of leaves of the plants but plants with concentrations at the Ecological screening value of iron at E_2 and E_3 showed significant differences in sheath length (34.7 and 39.4 cm, respectively) from the Control (20.3 cm).

TABLE 3.3: PERCENTAGE CHANGES IN MORPHOLOGICAL CHARACTERISTICS OF MAIZE SEEDLINGS AFTER 5 WEEKS WHEN COMPARED TO THE CONTROL (+ INCREASE, - REDUCTION)

	Plant Height	Leaf Length	Leaf Width	Leaf Area	Number of Leaves	Sheath Length
	Percentage Change (Δ%)					
Cu_1E_1	-34.83	-10.71	18.75	6.03	0	13.3
Cu_2E_1	-41.96	17.32	37.5	61.32	-10	40.39
Cu_3E_1	-25.66	6.3	15.63	22.91	10	13.3
Cu_1E_2	-21.59	-0.63	21.88	21.11	10	18.23
Cu_2E_2	-36.86	-18.11	-12.5	-28.35	-10	19.21
Cu_3E_2	-27.29	-6.61	6.25	-0.78	20	26.6
Cu_1E_3	-8.35	10.39	-3.13	6.94	10	74.88
Cu_2E_3	-31.36	-5.98	-6.25	-11.86	0	38.92
Cu_3E_3	-49.08	-22.05	-6.25	-26.92	0	-11.33

Cu_1 - Cu nanoparticles at concentration 1 (35%)

Cu_2 - Cu nanoparticles at concentration 2 (75%)

Cu_3 - Cu nanoparticles at concentration 3 (100%)

E_1 - Concentration at Ecological screening value of Fe (ESV); E_2 - 2.5xESV; E_3 - 4xESV

Table 3.2 Percentage changes in morphological characteristics of maize seedlings after 5 weeks when compared to the control. In spite of the application of copper nanoparticles in the elevated ferruginous soil, plants with Cu_1E_1 concentration showed a 34% reduction in plant height and 10% in leaf length when compared to the control. However, there was a compensatory growth in leaf width, area and sheath length. Plants with Cu_2E_1 concentration showed a 41% reduction in plant height and 10% in the number of leaves when compared to the control but had a compensatory growth in leaf length, width and area while those with Cu_3E_1 concentration had a 25% reduction in plant height with a compensatory growth of 10% in the number of leaves. Plants with Cu_1E_3 concentration showed an 8% and 3% reduction in plant height and leaf width, respectively with a compensation growth of 74% in sheath length.

TABLE 3.4: SUMMARY 2-WAY ANOVA TABLE FOR SELECTED MORPHOLOGICAL PARAMETERS OF MAIZE SEEDLINGS UNDER CU NANOPARTICLE EXPOSURE (TREATMENT), TESTED AGAINST BLOCKING EFFECTS

Source of Variation	SS	df	MS	F	P-value	F crit
Plant height						
Treatment	404.53	12	33.7	0.745	0.699	2.033
Blocks	542.00	3	180.7	3.995	0.015	2.866
Error	1628.18	36	45.2			
Total	2574.71	51				
Leaf length						
Treatment	1060.50	12	88.4	0.992	0.475	2.033
Blocks	436.23	3	145.4	1.632	0.199	2.866
Error	3207.25	36	89.1			
Total	4703.97	51				
Leaf width						
Treatment	5.92	12	0.5	1.617	0.131	2.033
Blocks	1.51	3	0.5	1.648	0.195	2.866
Error	10.99	36	0.3			
Total	18.43	51				
Leaf area						
Treatment	25792.89	12	2149.4	1.488	0.174	2.033
Blocks	6284.43	3	2094.8	1.450	0.244	2.866
Error	51993.56	36	1444.3			
Total	84070.88	51				
No. of leaves						
Treatment	9.19	12	0.8	0.833	0.617	2.033
Blocks	3.38	3	1.1	1.226	0.314	2.866
Error	33.12	36	0.9			
Total	45.69	51				
Sheath length						
Treatment	697.78	12	58.1	1.780	0.090	2.033
Blocks	91.28	3	30.4	0.931	0.436	2.866
Error	1176.07	36	32.7			
Total	1965.13	51				

Table 3.3 shows a 2-way ANOVA Table for selected morphological parameters of maize seedlings under Cu nanoparticle treatment, tested against blocking. This shows that the blocks significantly affected the plant height i.e. P-value is less than 0.05.

TABLE 3.5: MODAL OBSERVATIONS OF TREATMENTS (+++ INDICATE MAGNITUDE OF REPRESENTATION, - INDICATE MAJOR ABSENCE)

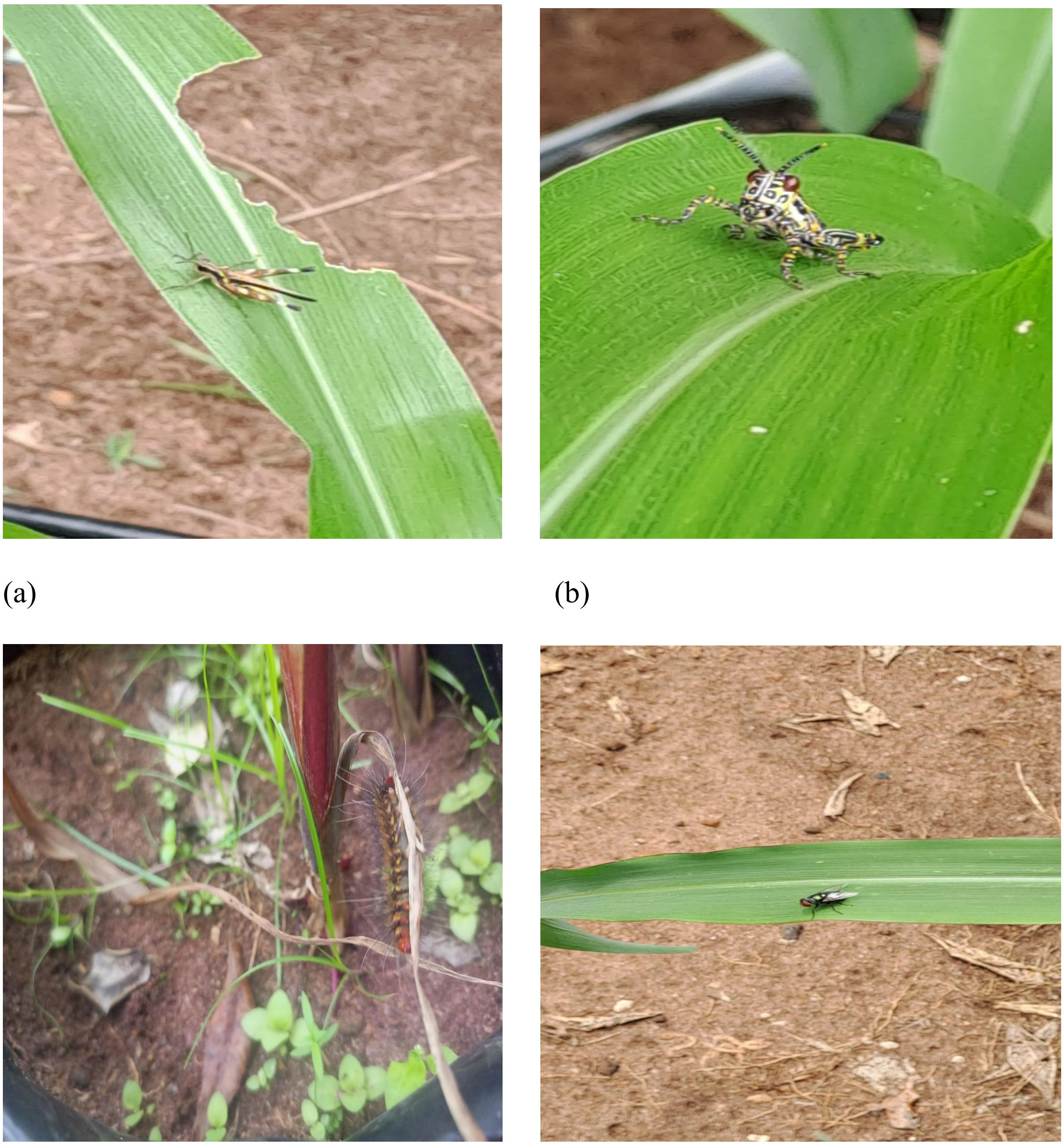
Treatment	Folial Foraging	Presence of insects	Chlorosis	Necrosis
Cu_1E_1	++	++	+	+
Cu_2E_2	++	++	+	++
Cu_3E_3	+++	+++	+	+
Cu_1E_2	+	+	++	++
Cu_2E_2	++	++	++	++
Cu_3E_2	++	++	++	++
Cu_1E_3	+	+	+++	+++
Cu_2E_3	+++	+++	+++	+++
Cu_3E_3	+++	+++	+++	+++
E_1	-	-	++	+
E_2	+	+	++	+
E_3	+	+	+++	+
Control	+	+	-	+

+ indicates minimal magnitude

++ indicate moderate magnitude

+++ indicate high magnitude

- indicate majorly absent



(a) (b)

(c) (d)

PLATE 3.3: FORAGERS FOUND ON MAIZE PLANT (Mag 0.5x)

CHAPTER FOUR

DISCUSSION

Excess iron can severely impact maize plant growth and development. Iron toxicity leads to ultrastructural damage in chloroplasts, reducing key enzymes and slowing carbon flow into sugars (Stocking, 1975). This results in less chlorophyll and delayed growth stages (Rizvi and Khan, 2018). Heavy metal toxicity, including from excess iron, reduces growth and yields while increasing oxidative stress. Collectively, studies show excess iron toxicity can detrimentally impact maize plants, including reducing height, leaf size, leaf area, leaf colour, sheath length, and leaf number (Rizvi and Khan, 2018). This experiment revealed a notable enhancement in maize growth facilitated by the presence of copper nanoparticles, particularly at concentrations of 35% and 100%, spanning both the initial and subsequent growth stages. Evidence from the study showed a clear dose-dependent relationship, wherein varying concentrations of nanoparticles yielded diverse effects on plant height. However, as the study progressed, the plants treated with a 100% concentration (Cu_3) consistently outperformed those treated with a 75% (Cu_2) and 35% concentration (Cu_1). The impact of soil contamination with iron sulphate showcased a nuanced relationship influenced by contamination levels. For instance, by week 2, plants treated with 75% concentration (Cu_2) and sown in soils contaminated at 2.5 ESV (Cu_2E_2) displayed a marginal reduction in height compared to those in soils contaminated at 1 ESV (Cu_1E_1) as earlier concluded by Barbosa (2013) that excessive foliar application of copper can reduce plant height. However, by week 4, the height of plants treated with 75% concentration had caught up, hinting at a potential adaptive response to elevated contamination levels. This was particularly evident in the 100% concentration treatment (Cu_3), implying that while initial growth might be slightly hindered by nanoparticles, the long-term growth trajectory could be more robust, potentially leading to comparable or even superior heights compared to the control group (Ninanma *et al.,* 2023). Similarly, the presence of copper nanoparticles exerted an influence on leaf length in maize plants, albeit with a distinct effect from that on plant height, hinting at divergent responses among growth parameters. The observed dose-dependent trend was consistent here as well, with plants treated with a 75% concentration of Cu_2 displaying longer leaf lengths compared to the control group, both initially and towards the end of the experiment. The impact of soil contamination levels on leaf length responses to copper nanoparticles was also conspicuous. For example, during week 2, plants treated with a 35% concentration (Cu_1) and sown in soils contaminated at 2.5 ESV (Cu_1E_2) exhibited reduced leaf lengths compared to those sown in soils contaminated at 1 ESV (Cu_2E_1). However, by week 4, the leaf lengths of Cu_1E_2 levelled, indicating a potential adaptation to higher contamination levels. Furthermore, the study unveiled a positive effect of copper nanoparticles on leaf area, particularly pronounced by week 5, wherein plants treated with nanoparticles showcased larger leaf areas compared to the control group. Notably, the dose-dependency of the response was evident, with plants treated with higher concentrations generally exhibiting greater leaf areas. During week 2, plants treated with a 35% concentration of Cu_1 and sown in soils contaminated at 4 ESV (Cu_1E_3) displayed smaller leaf areas compared to those sown in soils contaminated at 2.5 ESV (Cu_1E_2), suggesting that either higher contamination levels impeded the positive effects of copper nanoparticles on leaf area (Kasana *et al.*, 2017) or the plant species had a role to play in it (Song *et al.*, 2015). Regarding leaf number, copper nanoparticles exhibited a limited impact, with no substantial deviations observed between treated plants and the control group. The observed temporal variability in the response across different weeks of the experiment suggests nuanced temporal dynamics in the effect of nanoparticles on leaf number. Overall, the effects of copper nanoparticle treatments on various morphological characteristics of maize plants are intricate and influenced by nanoparticle concentration, soil contamination levels, and specific parameters of plant morphology. The morphological parameters of maize seedlings under Cu nanoparticle exposure were significantly affected by both treatment and blocking effects. Specifically, plant height, leaf length, leaf width, leaf area, and sheath length were influenced by treatment, and while blocks had effects on other parameters, it had a significant effect on plant height. Despite contamination, results showed compensatory growth, potentially attributable to activated stress responses. Nanoparticles may increase protective pigments and regulate plant defenses (Nguyen, 2020), triggering physiological responses maintaining growth and function, like enhanced nutrient uptake, photosynthesis, and hormonal signaling (Nair, 2016; Khan, 2021). Inherent plasticity also allows strategic resource allocation, prioritizing certain growth over others. Furthermore, nanoparticle stress may increase growth-promoting compounds, accelerating specific traits (Nguyen *et al.*, 2022). Genetic variation may also contribute differential resilience. Elucidating the specific compensatory mechanisms and implications will provide valuable insights for developing strategies to improve crop resilience and productivity despite environmental stressors.

CONCLUSION

Through a comprehensive investigation spanning various concentrations of copper nanoparticles and soil contamination levels, we observed a clear dose-dependent relationship in the response of maize plants. Higher concentrations of copper nanoparticles generally led to enhanced tolerance to iron toxicity and increased yield, indicating the effectiveness of this nanoparticle-based approach in mitigating the adverse effects of iron-rich soil on crop growth. Furthermore, our findings underscore the importance of considering temporal dynamics in nanoparticle applications, as evidenced by the observed variations in plant response throughout the experiment. This highlights the need for further research to elucidate the mechanisms underlying the long-term effects of copper nanoparticles on plant growth and development. Overall, the results of our study suggest that copper nanoparticles hold promise as a sustainable solution for improving crop productivity in iron-rich soils, offering potential benefits for agricultural practices in regions affected by iron toxicity. However, further investigations are warranted to optimize nanoparticle formulations, application methods, and dosage regimes to maximize their efficacy while minimizing any potential adverse effects on the environment and human health. By continuing to advance our understanding of nanoparticle-mediated plant responses, we can contribute to the development of innovative strategies for enhancing agricultural sustainability and food security in iron-challenged environments.

REFERENCES

Abbaspour, N., Hurrell, R., and Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, **19**(2): 164–174.

Amogou, O., Dagbenonbakin, G., Agbodjato, N. A., Noumavo, P. A., Salako, K., Adoko, M. Y., Kakai, R. G., Adjanohoun, A., and Baba-Moussa, L. (2019). Applying rhizobacteria on maize cultivation in northern Benin: effect on growth and yield. *Agricultural Sciences*, **10**(6): 763-782.

Antonio-Pérez, A., Durán-Armenta, L. F., Pérez-Loredo, M. G. and Torres-Huerta, A. L. (2023). Biosynthesis of copper nanoparticles with medicinal plants extracts: from extraction methods to applications. *Micromachines*, **14**(10): 1882.

Barbosa, R. H., Tabaldi, L. A., Miyazaki, F. R., Pilecco, M., Kassab, S. O., and Bigaton, D. (2013). Foliar copper uptake by maize plants: effects on growth and yield. *Ciência Rural*, **43**(9): 1561–1568.

Buarki, F., AbuHassan, H., AlHannan, F. and Henari, F. Z. (2022). Green synthesis of iron oxide nanoparticles using hibiscus rosa sinensis flowers and their antibacterial activity. *Journal of Nanotechnology*, **2022**: 1–6.

Cherlinka, V. (2023). Soil temperature as a factor of crop development. *EOS Data Analytics*. [Online] [Available at: https://eos.com/blog/soil-temperature/ [Accessed 15/04/24].

Dasberg, S., and Mendel, K. (1971). The effect of soil water and aeration on seed germination. *Journal of Experimental Botany*, **22**(4), 992–998.

Detchinli, K. S., Sogbedji, J. M. (2015). Assessment of the profitability and the effects of three maize-based cropping systems on soil health in western Africa. *American Journal of Agriculture and Forestry*, **2**(6): 321-329

El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Desoky, E. S. M., Babalghith, A. O., El-Tahan, A. M., Ibrahim, O. M., Alia, A. M., El-Mageed, T. A., Elrys, A. S., Elbadawi, A. A., El-Tarabily, K. A., and AbuQamar, S. F. (2022). Role of nanoparticles in enhancing crop tolerance to abiotic stress: A comprehensive review. *Frontiers in Plant Science*, **13**.

Govindaiah, S., Moses, V., Ananda, S. (2014). Biological synthesis of copper nanoparticles and its impact- a review. *International Journal of Pharmaceutical Science Invention* **3**(8): 6–28.

Grote, U., Fasse, A., Nguyen, T. T., and Erenstein, O. (2021). Food security and the dynamics of wheat and maize value chains in Africa and Asia. *Frontiers in Sustainable Food Systems*, **4**.

Ha, C. V., Nguyen, D. V., Huong, N., Le, N. T., Nguyen, H. K., Nguyen. A. T., Dinh, N., and Hoang, S. A. (2020). Copper nanoparticle application enhances plant growth and grain yield in maize under drought-stress conditions. *ResearchGate*, **41**(1): 364-375.

Hafeez, A., Razzaq, A., Mahmood, T., Jhanzab, M. H. (2015) Potential of copper nanoparticles to increase growth and yield of wheat, journal of nanoscience of advanced technology. *ResearchGate* **1**(1): 6-11.

Hoang, S. A., Nguyen, L. Q., Nguyen, N. H., Tran, C. Q., Nguyen, D. V., Le, N. T., Ha, C. V., Vu, Q. N., and Phan, C. M. (2019). Metal nanoparticles as effective promotors for Maize production. *Scientific Reports*, **9**(1).

ICAR- Indian Institute of Maize Research (2024). Maize Biology. [Online] [Available at: https://iimr.icar.gov.in] [Accessed 15/04/24].

Ikhajiagbe, B., Igiebor, F. A., and Ogwu, M. C. (2021). Growth and yield performances of rice (*Oryza sativa* var. America) after exposure to biosynthesized nanoparticles. *Bulletin of the National Research Centre*, **45**(1).

Jin, M., Guan, H., Zhang, W., Tian, D., Wei, J., Kalkhajeh, Y. K., and Gao, H. (2022). High level of iron inhibited maize straw decomposition by suppressing microbial. *Agronomy*, **12**(6): 1286.

Kasana, R. C., Panwar, N. R., Kaul, R. K., and Kumar, P. (2017). Biosynthesis and effects of copper nanoparticles on plants. *Environmental Chemistry Letters,* **15**(2): 233–240.

Kashyap, P., Shirkot, P., Das, R., Pandey, H., and Singh, D. (2023). Biosynthesis and characterization of copper nanoparticles from *Stenotrophomonas maltophilia* and its effect on plant pathogens and pesticide degradation. *Journal of Agriculture and Food Research*, **13**.

Keigler, J. I., Wiesinger, J. A., Flint-Garcia, S. A, and Glahn, R. P. (2023). Iron bioavailability of maize (*Zea mays* l.) after removing the germ fraction. *Frontiers in Plant Science,* **14**.

Khan, A. A. H. (2021). Plant physiological response to engineered nanoparticles. *Plant-Microbes-Engineered Nano-particles (PM-ENPs) Nexus in Agro-Ecosystems.*

Koda, A. D., Dagbenonbakin, G., Assogba, F., Noumavo, P., Agbodjato, N. A., Assogba, S., Aguegue, R. M., Adjanohoun, A., Espinosa, R. R., Novalpons, B. M., and Baba-Moussa, L. (2018). Maize (Zea mays L.) response to mycorrhizal fertilization on ferruginous soil of northern Benin. *Journal of Experimental Biology and Agricultural Sciences,* **6**(6): 919-928.

Long, W., Li, Q., Wan, N., Feng, D., Kong, F., Zhou, Y., and Yuan, J. (2020). Root morphological and physiological characteristics in maize seedlings adapted to low iron stress. *Plos One,* **15(**9), e0239075.

Lung'aho, M. G., Mwaniki, A. M., Szalma, S. J., Hart, J. J., Rutzke, M. A., Kochian, L. V., Glahn, R. P., and Hoekenga, O. A. (2011). *Genetic and physiological analysis of iron biofortification in maize kernels. Plos One*, **6**(6), e20429.

Mller, J. O. (2016) Soil pH affects nutrient availability. *ResearchGate*

Mondal, S. (2021). Impact of climate change on soil fertility. *Climate Change and the Microbiome, Sustenance of the Ecosphere,* (1):551-569.

Nair, R. (2016). Effects of nanoparticles on plant growth and development. *Plant Nanotechnology*.

Nenova, V., and Stoyanov, I. 1993) Physiological and biochemical changes in young maize plants under iron deficiency I. Growth and photosynthesis. *Journal of Plant nutrition*, **16**(5): 835-849.

Nenova, V., and Stoyano, I. (1995) Physiological and biochemical changes in young maize plants under iron deficiency: 2. catalase, peroxidase, and nitrate reductase activities in leaves. *Journal of Plant Nutrition*, **18**(10): 2081-2091.

Nguyen, D. V., Huong, N., Le, N. T., Nguyen, K. H., Nguyen, H. T., Le, H. M., Nguyen, A. T., Dinh, N., Hoang, S. A. and Ha, C. V. (2021). Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. J*ournal of Plant Growth Regulation,* **41**(1): 364–375.

Ninama, J., Rathore, R., Jadhav, V. D., Patil, R. M., Solanke, K. M., Singh, U. P. and Choudhary, D. (2023). Effect of foliar application of nano urea on growth and yield of maize (*Zea mays* L.): *A Review. International Journal of Environment and Climate Change*, **13**(11), 989–992.

Nyéki, A., Daróczy, B., Kerepesi, C., Neményi, M. and Kovács, A. J. (2022). Spatial variability of soil properties and its effect on maize yields within a field—A case study in Hungary. *Agronomy,* **12**(2), 395.

Rafsanjani, K., Madani, A., and Vazin, F. (2019). Effect of iron on yield of corn (*Zea mays* L.) in drought stress. *Cercetari Agronomice in Moldova*, **52**(1): 34–43.

Rai, M., Ingle, A. P., Pandit, R., Paralikar, P., Shende, S., Gupta, I., Biswas, J. K., and DaSilva, S. S. (2018). Copper and copper nanoparticles: role in management of insect pests and pathogenic microbes. *Nanotechnology Reviews*, **7**(4): 303–315.

Ramasamy, S. and Selvam, M. (2015). *Green synthesis of copper nanoparticles from Hibicus rosasinensis and their antimicrobial, antioxidant activities*. *ResearchGate*, **6**(2):1183-1190.

Rizvi, A., and Khan, M. S. (2018). Heavy metal-mediated toxicity to maize: oxidative damage, antioxidant defence response and metal distribution in plant organs. *International Journal of Environmental Science and Technology,* **16**(8): 4873–4886.

S, B. S., Pitchiah, S., Suresh, V., Ramasamy, P. (2023). Biosynthesis of copper nanoparticles from seaweed Ulva lactuca and their in vitro antioxidative potential by container. *Cureus,* **15**(11).

Shahzad, R., Jamil, S., Ahmad, S., Nisar, A., Khan, S., Amina, Z., Kanwal, S., Usmanaslam, H. M, Gill, R. A. and Zhou, W. (2021). Biofortification of cereals and pulses using new breeding techniques: current and future perspectives. *Frontiers in Nutrition*, **8**.

Shi, Y., Zhang, Y., Han, W., Feng, R., Hu, Y., Guo, J., Gong, H. (2016). Silicon enhances water stress tolerance by improving root hydraulic conductance in *Solanum lycopersicum* L. *Front. Plant Sci.* **7**: 196.

Siddiqui, M. H., Al-whaibi, M., Mohammed, F. (2015) Nanotechnology and plant sciences: nanoparticles and their impact on plants. *ResearchGate.*

Sillen, W. M. A., Thijs, S., Abbamondi, G. R., TorreRoche, R., Weyens, N., White, J. C., and Vangronsveld, J. (2020). Nanoparticle treatment of maize analyzed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis. *Microbiome*, **8**(1).

Silva, J., & Uchida, R. (2000). Plant nutrient management in Hawaii's soils: plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture; essential nutrients for plant growth: nutrient functions and deficiency symptoms. *College of Tropical Agriculture and Human Resources, University of Hawaii* pp 31-55

Smith-Eskridge, E. (2014). Assessing the effects of soil moisture on germination assessing the effects of soil moisture on germination of winter wheat of winter wheat. *DigitalCommons@University of Nebraska* pp. 2-22

Song, L., Vijver, M. G., and Peijnenburg W. J. (2015). Comparative toxicity of copper nanoparticles across three Lemnaceae species. *Science of the Total Environment*, pp. 518-519, 217–224.

Srivastav, A., Ganjewala, D., Singhal, R. K., Rajput, V. D., Minkina, T., Voloshina, M., Srivastava, S., and Shrivastava, M. (2021). Effect of ZnO nanoparticles on growth and biochemical responses of wheat and maize. *Plants*, **10**(12): 2556.

Stocking, C. R. (1975). Iron deficiency and the structure and physiology of maize chloroplasts. *Plant Physiology*, **55**(4): 626–631.

Tandzi, L. N., Mutengwa, C. S., Léonard, E., and Gracen, V. (2018). *Breeding Maize for Tolerance to Acidic Soils: A Review. Agronomy*, **8**(6), 84–84.

Valdés-Rodríguez, O. A., Sánchez-Sánchez, O., and Pérez-Vázquez, A. (2013). Effects of soil texture on germination and survival of non-toxic *Jatropha curcas* seeds. *Biomass and Bioenergy,* **48**(1):167–170.

Wang, Y., Cao, A., Jiang, Y., Zhang, H., and Liu, J. (2019). Nanoparticles for plant growth promotion and disease resistance. *Trends in Biotechnology*, **37**(5): 388-394.