QUANTITTATIVE GROWTH ASSESSMENT OF MAIZE [ZEA MAYS] AFTER COPPER NANOPARTICLE INTERVENTION

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BENIN CITY.

FEBRUARY, 2025.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc.) IN PLANT BIOLOGY AND BIOTECHNOLOGY

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this work was done by **Favour OMOLABI** in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

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DATE

Prof E. D. Vwioko
(Head of Department)

DATE

DEDICATION

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

ACKNOWLEDGEMENTS

I appreciate God Almighty for His guidance and strength to complete this research project. I also appreciate my project supervisor, Mrs Egbenoma for her support and understanding all through this study. I acknowledge the Head of Department, Prof E. D. Vwioko for his leadership to the students of the department. Special appreciation to the person who raised me and taught me to always be the best I can be, Mrs. Omolabi Kate, for her love, guidance, emotional and financial support. I love you and appreciate you for raising me the way you did. I appreciate my brother, Engineer Paul Airaoje for making my education his topmost priority, thanks for seeing me through school. To my friends ; Eseosa Oronsaye, Favour Obi, Treasure Edigue, Innocent Blessing thank you for your love, encouragement, support and every moment spent with you. I feel blessed to have you all as a part of my journey in school.

TABLE OF CONTENTS

TITLE PAGE ii
CERTIFICATION iii
DEDICATION iv
ACKNOWLEDGEMENTS v
TABLE OF CONTENTS vi
LIST OF TABLES ix
LIST OF FIGURES x
ABSTRACT xi
CHAPTER ONE 1
1.0 INTRODUCTION 1
1.1 Background of Study 1
1.2 Statement of Problem 2
1.3 Justification of the Study 3
1.4 Literature Review 3
1.4.1 Maize (Corn) 3
1.4.2 Taxonomy of maize 4
1.4.3 Maize Morphology 5
1.4.3-1 Maize Plant 5
1.4.3.2 Root System 6
1.4.3.3 Stem 7
1.4.3.4 Leaf 7
1.4.3.5 Flower 7
1.4.3.6 Fruit and Seed 9
1.4.3.7 Fruit wall: 9
1.4.3.8 Endosperm: 9

1.4.3.9 Embryo: 10

1.4.4 Reproduction System in Maize 10

1.4.5 Gamete Formation 11

1.4.6 Pollen Shed and Dispersal 11

1.4.7 Pollination and Fertilization 12

1.4.8 Embryogenesis 12

1.4.9 Seed Dispersal 13

1.4.10 Seed Dormancy 13

1.4.11 Germination 14

1.4.12 Adaptation of Maize and Yield Potential 14

1.4.13 Nutritional value of maize 16

1.4.14 Phytochemical content of maize 16

1.4.14.2 Phenolic compounds: 17

1.4.14.3 Phytosterols: 17

1.4.15 Health benefits of maize 18

1.4.16 Overview of Nanotechnology 20

1.4.17 Need for Nanotechnology in Plant Science and Agriculture 21

1.4.18 Current Applications of Nanotechnology in Plant Science 23

1.4.18.1 Biosensors 23

1.4.18.2 Controlled Release of Agrochemicals and Nutrients 24

1.4.18.3 Nanomaterials for Plant Genetic Engineering 26

1.4.19 Importance of Nanotechnology in Plant Science 27

1.5 Research Aim and Objectives 27

1.5.1 Research Aim 27

1.5.2 Research Objectives 28

1.6 Research Questions 28

CHAPTER TWO **29**

MATERIALS AND METHOD **29**

2.1 Study Area 29

2.2 Materials 29

2.3 Experimental Design 29

2.4 Research Methodology 29

2.4.1 Soil Collection 30

2.4.2 Site Preparation 30

2.4.3 Preparation of Iron Sulphate Treatment 30

2.4.3.1 Calculations 31

2.4.4 Preparation of Copper Nanoparticles Treatment 31

2.4.5 Treatment Designation 33

2.4.6 Planting and Treatment Application 34

2.4.7 Experiment Management 34

2.4.8 Parameter Score 34

CHAPTER THREE **36**

RESULTS **36**

CHAPTER 4 **45**

DISCUSSION **45**

CONCLUSION **48**

REFERENCES **49**

LIST OF TABLES

Table Page

3.1 Sheath length of maize plants exposed to ferruginous soil 41

3.2 Morphological characteristics of maize seedlings after 5 weeks 42

LIST OF FIGURES

Figures Pages

1: Annotated image of a mature maize plant showing the various parts 6

3.1: Stem Height of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate 35

3.2: Leaf length of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate 36

3.3: Leaf width of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate 37

3.4: Leaf area of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate 38

3.5: Leaf number of *Zea mays* plant introduced to the various concentration of Cu-based nanoparticle and Iron sulphate 39

3.6: Copper uptake of *Zea mays* in different treatments 40

ABSTRACT

Ferruginous soils present challenges for plant growth due to their elevated iron levels, which can cause iron toxicity and negatively impact crop productivity. Copper nanoparticles demonstrate potential in mitigating iron toxicity in plants. This research studied maize plants polluted with different copper nanoparticle concentrations in ferruginous soils with elevated iron. The aim was to ascertain maize tolerance to various iron stress levels and its resultant yield. The results indicated improved maize growth with copper nanoparticles, significantly at 35% and 100% concentration in the early and late development phases. This infers a dose-dependent relationship between nanoparticle concentration and maize growth, with higher concentrations conveying increased maize sensitivity to excessive iron levels. The dynamic response of maize to nanoparticles over time emphasizes the need for exposure duration. In week 2, plants polluted with a 35% Cu nanoparticle concentration in soils alleviated at 4 ESV portrayed smaller leaf areas in contrast to those in 2.5 ESV soils. This infers that higher contamination may hamper the nanoparticles' positive leaf area effects. The complex impact of copper nanoparticles on maize morphology was influenced by a combination of factors including concentration, soil contamination, and specific parameters. Plant height, leaf length, leaf width, leaf area, and sheath length were modified by treatment, while blocks substantially affected plant height, leaf length, and sheath length. Copper nanoparticles demonstrate the potential to improve maize resilience in ferruginous soils, presenting a viable sustainable agriculture solutions in iron-rich environments.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Nanotechnology is an emerging technology based on producing and utilizing structures of materials of dimensions less than 100 nanometres (Baig *et al*., 2021). As an interdisciplinary field, it has rapidly expanding applications in various sciences and industries. In the field of soil science, it has found applications for effective plant nutrition management through the use of nanofertilizers, controlling soil-borne diseases with nanopesticides, remediation of salinity and removal of pollutants from soil using nanoparticles and porous nanosorbents, enhancing soil moisture-retaining capacity through superabsorbent nanomaterials, stabilizing erodible soils using nanosilicates and nanopolymers, and providing various chemical and biosensors for precise soil measurement (Liu and Lal, 2015; Chaudhry *et al*., 2018). Thus, nanotechnology offers new solutions for improving food security and is capable of revolutionizing diverse aspects of farming (Tarafdar and Raliya, 2011)

Currently, there is a mismatch between population growth and food requirements globally. This challenge necessitates the use of modern and technologically advanced technologies in agriculture and food production. The importance of nanotechnology as an interdisciplinary and pioneering science becomes evident. Possessing the capability to boost the performance of farming products throughout, cultivation, harvesting, and storage processes, as well as optimize production conditions and food preservation, nanotechnology has the potential to revolutionise the agricultural food production. Presently, approximately 70% of the top 10 priorities for nanotechnology in the world are directly or indirectly related to agricultural sciences (Mukhopadhyay, 2014).

Maize (*Zea mays*) is a staple food that is consumed in many parts of the world. It is the third leading crop of the world after rice and wheat (Rouf Shah *et al.,* 2016). Maize has several economic and health benefits (Rouf Shah *et al.,* 2016). Therefore, understanding the effect of nanoparticle intervention on maize production may ensure its proper application in order to boost food production and overcome the challenges associated with conventional agricultural practices in maize farming.

1.2 Statement of Problem

Nanotechnology, as a source of new industrial materials, has been widely used in recent years and has appeared in almost every field. Currently, nanoparticles have been applied in many areas such as textiles, cosmetics, pharmaceuticals, and automotive industries. Utilisation of nanoparticles has increased in recent years, but their effects on plant production and the ecosystem at large are only beginning to be known.

Daghan (2018) investigated the effect of application of increasing concentrations of TiO_2 nanoparticle to maize plants on biomass production, nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu), and chlorophyll content significantly reduced with increasing TiO_2 nanoparticle applications. The results showed that plants' shoot and root biomass and leaf chlorophyll content were significantly reduced with increasing TiO_2 nanoparticle application, while N, P, and K macronutrients as well as Zn, Mn, and Cu micronutrients were positively affected, demonstrating that TiO_2 nanoparticle treatment increased nutrient uptake of maize and decreased biomass at high doses.

Another study has demonstrated evidence of the potential of TiO_2 nanoparticle application as an efficient and sustainable alternative for enhancing crop tolerance in nickel-contaminated areas as it reduces ultrastructural damage caused by Ni stress in maize (Rehman *et al.,* 2024).

Being an emerging technology, limited studies have been carried out on the effect of nanoparticle application to maize production. Therefore, more studies are recommended to conclusively elucidate the effect on nanoparticle intervention on maize production, particularly as it relates to optimum concentration for better outcomes.

1.3 Justification of the Study

Nanotechnology offers new solutions for improving food security and is capable of revolutionizing diverse aspects of farming (Tarafdar and Raliya, 2011). As an interdisciplinary field, it has a broad spectrum of uses in the farming industry, leading to significant outcomes such as increased harvest yield, reduced utilization of plant nutrients and insecticides, an extended shelf-life of agricultural products, and perhaps a transformative impact on all stages, inputs, and agricultural tools, aiming for improvement (Pandey, 2020).

Soil is considered a primary resource for agricultural production, and therefore, preserving its health and fertility is of utmost importance for sustainable food production. It is crucial to maintain the optimal levels of nutrients and moisture in the soil while minimizing the effects of pollutants like heavy metals and toxins. Nanotechnology can significantly contribute to improving this process.

1.4 Literature Review

1.4.1 Maize (Corn)

Maize or corn (*Zea mays* L.) is a useful annual cereal crop of the world belonging to family Poaceae. The term *Zea* is an ancient Greek word, which means “sustaining life”, and *mays* is a word from Taino language meaning “life giver.” The word “maize” is from the Spanish “maiz” (Kumar and Jhariya, 2013). It is considered as a staple food in many parts of the world. It is a third leading crop of the world after rice and wheat (Rouf Shah *et al.,* 2016). It is globally regarded as “queen of cereals” due to its highest yield potential among the cereals. Maize is commonly used for animal feed. It is generally processed into various products, including cornmeal, grits, starch, flour, tortillas, snacks, and breakfast cereals. Maize flour is used to make flat breads that are eaten particularly in India (Rouf Shah *et al.,* 2016).

1.4.2 Taxonomy of maize

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae

Subfamily: Panicoideae

Tribe: Andropogoneae

Genus: *Zea*

Species: *Zea mays*

The genus *Zea* consists of four species of which *Zea mays* L. is economically important. The other *Zea* species, referred to as teosintes, are largely wild grasses native to Mexico and Central America. The number of chromosomes in *Zea mays* is $2n = 20$.

1.4.3 Maize Morphology

1.4.3-1 Maize Plant

Maize is a tall, determinate annual plant belonging to monocotyledon class and is monoecious with separate male and female flowering organs but on the same plant. Shanks develop in the leaf axis and will mature into female inflorescence (an ear). Depending on the variety, more than one shanks may develop on one maize plant but usually only 1-2 may develop into economic ears (cobs). Ears are covered by a number of leaves (husks) and each cob has even number of rows (8-30) of kernels (Awata, *et al.,* 2019). Each ovary contains one ovule which will mature into a kernel. One ear of maize contains between 300-1000 kernels (AGOGTR, 2008). The apical meristem of maize stalk develops into a tassel which consists of central spike and up to 40 lateral branches carrying male flowers. The tassel structure is erected on top of the plant by a strong peduncle. Maize stem has protective epidermis that covers layers of sclerenchyma tissues resulting into strong stalk. Generally, tropical/sub-tropical varieties are taller unlike their temperate counterparts (AGOGTR, 2008).

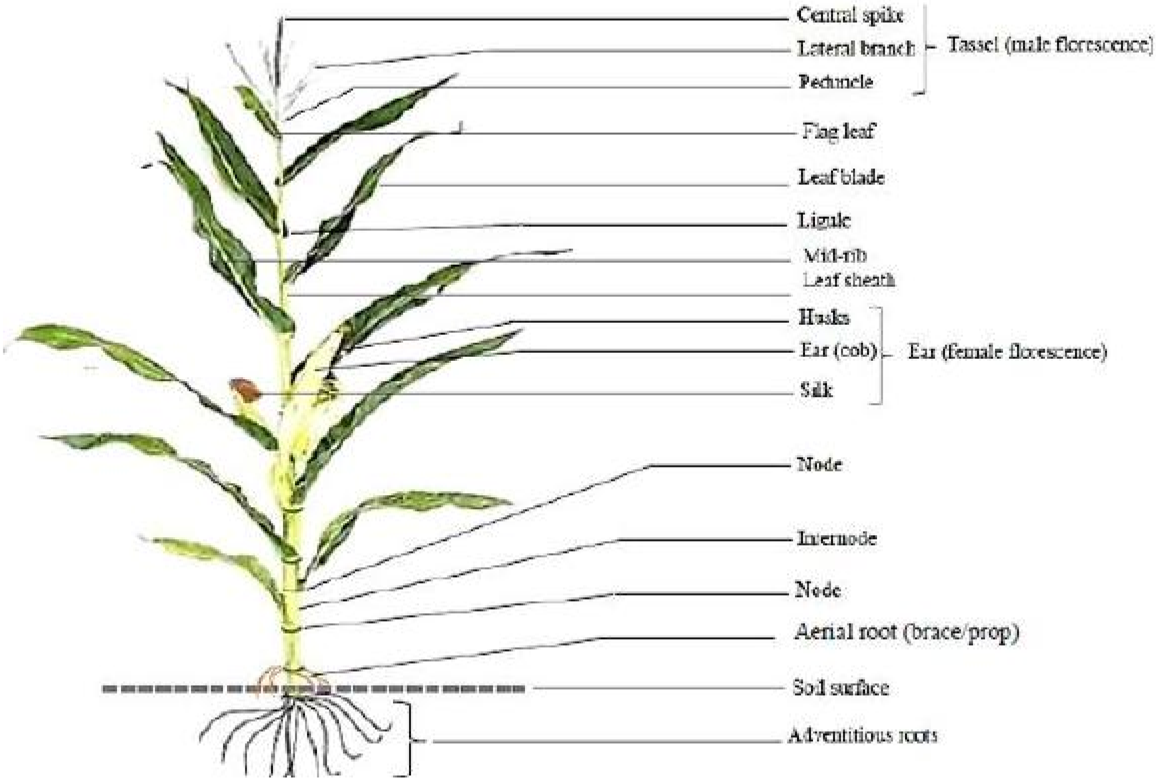

Figure 1: Annotated image of a mature maize plant showing the various parts

Source: (Awata, *et al.,* 2019).

1.4.3.2 Root System

Maize roots are very shallow due to their adventitious nature where no tap root is observed. Normal maize plant can develop 4-6 adventitious roots of almost equal sizes. The adventitious roots develop from nodes below the soil surface. Root length can reach 1.5 m laterally and about 2.0 m deep (Espinoza and Ross, 2010). The roots are instrumental for absorption of water and nutrients from the soil.

1.4.3.3 Stem

Depending on the variety and environmental conditions under which the plant is grown, normal maize plant has a single stem of about 0.5-5 m tall (measured from the soil surface to the point where flag leaf is attached to the peduncle). The stem is cylindrical, solid and divided into nodes separated by internodes. Internodes are cylindrical in the upper part, and alternately grooved on the lower part of the stem with a bud in each groove, with one or occasionally two lateral branches in the leaf axils in the upper part of the plant. Grooves are required for proper positioning of the ears (Awata, *et al.,* 2019).

1.4.3.4 Leaf

Leaves are the photosynthetic organs responsible for food production. The upper leaves are more responsible for light interception and are major contributors of photosynthate for grain filling. About 8-30 leaves may form on one plant and are arranged spirally on the stem. Stomata occur in rows along the entire leaf surface and more are found on the underside of the leaf than on the upper surface (Zarinkamar, 2006). During moist conditions, cells rapidly absorb water, become turgid and unfold the leaf. However, under warm, dry weather conditions, the cells quickly lose their turgor and as a result, the leaves curl inwards hence water loss due to evaporation is minimized since smaller leaf surface is exposed to the air (Awata, *et al.,* 2019).

1.4.3.5 Flower

Maize is a typical monoecious plant, that is, it produces two morphologically incomplete (male and female) flowers. The male flower only contains stamen while the female flower has pistil. Though the two reproductive organs are on the same plant, they are situated on different parts of the plant.

Male flower (tassel): Male flower is called tassel and is borne on the top of a maize plant, supported by a peduncle. Tassel is developed from the apical meristem of maize stalk (AGOGTR, 2008). Normally, one maize plant carries only one tassel made up of central spike (tassel rachis) and about 20-50 tassel arms (branches). Each arm carries several male stamens and each male stamen contains 3 anthers dangling out on slender filament. Anthers are the male organs responsible for production and dispersal of millions of pollen into the air (Hofmann *et al.,* 2016).

Female flower (ear): Ear is the female reproductive part of maize and it grows from the shanks (stalk-like structures) that are developed from axillary bud towards the middle of the stem length. Lateral shoot carrying the main ear emerges from the groove in the 8th node above the soil surface. One or two nodes below the 8th bud may produce rudimentary lateral shoots where one or two of them may develop into mature ears (AGOGTR, 2008). Sometimes, maize plant may initiate many ears up to 12th or 14th node but normally the upper most will grow to a full ear. Ear contains cob (rachis) with rows of sessile bearing spikelets that eventually grow into kernels and silks (Iltis, 2000). First, silks at the base of the cob emerge followed by those towards the tip. Silks can remain viable for up to 10 to 14 days so as to allow ample time for pollination. Excessive heat, moisture and senescence can affect silking. Silks are attached to ovaries arranged in rows (8-30) found on a cob, and covered in leaves (husks). Each ovary contains one ovule which matures into a kernel. Typically, an ear contains up to 1000 ovules and all silks must be pollinated so that the 1000 ovules mature into kernels. However, due to missing pollination, number of mature kernels per cob may be low (AGOGTR, 2008).

1.4.3.6 Fruit and Seed

After the elapse of fertilization, maize seed, which is a combination of both fruit and seed (also called kernel or grain), is formed. Seed contains approximately 72% starch, 10% protein, 4% fat and 1.4% ash, supplying an energy density of 365 Kcal/100 g (Ranum *et al*., 2014). In addition, maize seed contains vitamins A and E, as well as riboflavin and nicotinic acid. It is a dry indehiscent single-seeded fruit (caryopsis) containing three main compartments: fruit wall (brand), endosperm and embryo.

1.4.3.7 Fruit wall:

Is a structure tightly adhered to the fruit, is formed out of pericarp (ovary wall) and testa (seed coat) which provides protection to the seed. Seed coat (testa) is the outer layer of the seed consisting of membranous structures that are fused with fruit wall (hull) to envelope the embryo and endosperm. Seed coat is responsible for internal protection against biotic stresses, mechanical injury and desiccation. It is also important for gas exchange, water uptake and control of nutrients for embryo and endosperm. The coat plays key roles in seed viability, longevity, dormancy and germinability (Sliwinska and Bewley, 2014). Pericarp is a protective cover develops from the ovary wall.

1.4.3.8 Endosperm:

Is a thick component (80-85%) of grain, consisting of stored food reserves that is used by the seedling before the plant establishes its own photosynthetic structures (Ognakossan *et al*., 2018). Maize endosperm is separated from the outer aleurone and sub-aleurone layers by a radial symmetry which is composed of three sections: (i) embryo-surrounding region; (ii) the central (largest portion of the endosperm); and (iii) the basal endosperm transfer layer (Sliwinska and Bewley, 2014). Endosperm (tip) cap acts as a closer for radicle tip and prevents radicle emergence, and enhances seed dormancy. Size of endosperm varies among maize varieties depending on how much reserve food has been transferred to the endosperm during developmental stages.

1.4.3.9 Embryo:

Embryo (germ) constitutes about 9-10% of seed volume and contains most of the nutrients in grain (33% fat, 19% proteins, minerals and vitamins B complex and E), and is rich in unsaturated fatty acids (oleic and linoleic acids) (Ognakossan *et al*., 2018). This is the germ of maize consisting of one embryonic axis (complex) with only one cotyledon (monocotyledon), situated in a groove at one end of the endosperm. Embryo is vital for seed germination. The embryonic axis composed of four parts: (1) the radicle; (2) hypocotyl; (3) epicotyl; and (4) plumule (shoot apex), with a transitional zone between the radicle and hypocotyl. The radicle is located close to micropyle and it contains root meristem which develops into embryonic root when germination is complete. Hypocotyl is a stem-like region of the embryonic axis terminated by radicle at the basal end and by cotyledon at the proximal end. Epicotyl is the first shoot segment above the cotyledons. In maize, cotyledons may be well developed and serve as storage organs for reserves, or remain thin and flattened (endospermic seeds). Scutellum is a large shield-shaped body formed as a result of shrinkage in cotyledon structure followed by elongation of the basal sheath of the cotyledon to form a coleoptile that covers the first leaves. Coleorhiza is a protective sheath enclosing embryonic root structure and it provides protection against damages.

1.4.4 Reproduction System in Maize

Maize plant is a sexually reproducing organism with well-developed male and female reproductive systems. No report of asexual reproduction has been found in maize however, under advanced laboratory conditions, vegetative parts of maize such as embryos can be manipulated using tissue culture techniques to grow into a maize plant with complete morphology (Jones, 2009; Wang *et al.*, 2012).

1.4.5 Gamete Formation

Both male and female reproductive organs contain mature sporophytes. During gamete development, male sporophyte (2n) undergoes meiosis cell division to produce microspore (n). The microspore further divides mitotically (male gametogenesis) to produce microgametophyte which contains 3 gametic cells (2 sperm cells and 1 vegetative nucleus) (Dumas and Mogensen, 1993). Similarly, female sporophyte (2n) divides through meiosis to generate megaspore (n) which then undergo three successive non-nuclear mitosis divisions (female gametogenesis) to produce megagametophyte with two cells: central cell containing 2 nuclei, and egg cell with 1 nucleus (Sliwinska and Bewley, 2014; AGOGTR, 2008).

1.4.6 Pollen Shed and Dispersal

As soon as the tassel is fully emerged, pollen shed (anthesis) begins and may continue up to 2 weeks. Normally, anthesis lasts for 5 to 8 days with peak of pollen shed on the third day. Ideal period for pollen shedding is morning hours because hot/dry weather and excessive humidity delay flowering and affect pollen viability (AGOGTR, 2008). Under normal cultural practices and favourable environmental conditions, huge amount of pollen (1010 to 1013 pollen grains/plant) can be produced. Total amount of pollen disposed in the same field is about 23.3 million pollen grains/m^2 (Hofmann *et al.*, 2014). Pollen grain is dispersed by wind or animals (especially flower-sacking insects) and can be transported up to a distance of 300 meters from the point of disposition (AGOGTR, 2008). When deposition occurs at higher altitudes, pollen grains can travel as far as 3.3 to 4.45 km. Factors such as wind direction, field size, plant density, maize variety, growing conditions, agricultural management and weather conditions affect pollen disposition and dispersal (Hofmann *et al.*, 2014). After disposition, pollen grain remains viable in the field for 1-4 h depending on the weather conditions. However, pollen can be stored for longer period under cold conditions such as in laboratory (Bannert, 2006).

1.4.7 Pollination and Fertilization

Pollination refers to the transfer of pollen grain from the anther to the silk. Fertilization is the process by which male gamete from the pollen unites with female gamete from the ovule to form a zygote. Fertilization can occur only when cell division is complete. First, pollen grain is carried onto the female reproductive organ (silk) by wind and animals (especially insects) or by direct physical contacts between the plants. Unlike other organisms, maize exhibits double-fertilization: embryo fertilization and endosperm fertilization (Faure *et al.,* 2003). One of the 2 sperm cells fertilizes egg cell to form an embryo with 2n (embryo fertilization) while the remaining sperm cell fuses with central cell nuclei to form an endosperm with 3n (endosperm fertilization). Fertilization occurs 12-28 h after pollination. During pollination and fertilization period, enough water and nutrients are required and therefore, supplementary use of irrigation and application of fertilizers are needed. Maize is an out-crossing crop thus, only 5% of kernels may be fertilized with pollen from the same plant. The silk can remain receptive up to 10 days after emergence however, silk begins to die off 7-8 days after emergence depending on the environmental conditions (Bannert, 2006).

1.4.8 Embryogenesis

Embryogenesis is the process in which a fertilized egg cell (zygote) develops into a mature embryo. First phase of embryogenesis occurs within 100 h after fertilization in which a proembryo structure with 12-24 cells is produced. The basal cell divides into large vacuolated cells while the apical cell divides to generate 9-18 smaller cells (AGOGTR, 2008). Second phase of embryogenesis begins 8-9 days after fertilization, followed by formation of meristem and embryogenic axis at 13 days from the day of fertilization (Fong *et al.,* 1983). This stage is followed by formation of a coleoptile-like structure within 14-15 days after fertilization, which then differentiates to form scutellum, coleoptile, coleorhiza as well as root and shoot apical meristems. First leaf primordium emerges 16 days after fertilization resulting to stage 1 embryo of about 1 mm long. This is followed by more leaf primordia and primary and secondary root primordia development, leading to complete embryo formation covered by scutellum about 30-40 days after first leaf primordium (AGOGTR, 2008).

1.4.9 Seed Dispersal

Maize structure does not allow seed to naturally disperse. Mature maize grain dries on the cob, making it difficult to move away. Dry maize plant usually falls down together with the cob containing the seeds (especially when harvest is over delayed). As a result, seed usually germinates at the same spot provided favourable conditions prevail (AGOGTR, 2008). However, forced seed dispersal can occur in maize through the aid of animals and man, whereby maize grain can be transported over long distances or even across continents.

1.4.10 Seed Dormancy

Seed dormancy refers to genetic characteristics of plant that allow manipulation of environmental conditions so as to prevent seed germination within a given period. Seed dormancy normally occurs in maize grain due to accumulation of carotenoid and abscisic acid (ABA) which are important compounds for prevention of preharvest sprouting (vivipary growth) and germination. At dormancy, maize seed is alive but is in a quiescence state in which metabolic respiration rate, seed water content and synthetic activities (for example, RNA synthesis) are very low (Walle and Bernier, 1976). Seed respiration can be minimized by keeping the seed under cool and drier conditions.

1.4.11 Germination

Germination is the physiological process during seed transition from a dormant state to a vital active state. Seed germination requires favourable environmental conditions to allow changes of chemical and biological factors within the seed prior to initiation of germination (Rajjou *et al.,* 2012). Germination occurs when the substrate moisture is 30% or higher, where the seed first imbibes water depending on seed-substrate contact during planting. Maize seed imbibes 1.5 to 2 times its dry weigh for germination to take place (Belfield and Brown, 2008). Minimum and maximum temperatures for maize seed germination are 10 and 30°C respectively. Variation in soil temperature affects days to germination. For example, at 10°C the seed takes 25 days to germinate; at 13-16°C the seed takes 10-14 days to germinate; and at 18-21°C the seed needs only 5-8 days to germinate. Oxygen supply is very important during germination because the seed requires enough oxygen for respiration. Water-logged soil causes seed suffocation and seed death since oxygen is blocked. Maize seed germination is hypogeal (the energy storage part of the seed remains below the ground) where the plumule, covered in a protective coleoptile, is pushed through the soil to the surface (Espinoza and Ross, 2010). Sowing depth of more than 8 cm may delay seed germination. If seed is planted in drier soil with higher temperature, the seed may die if no moisture is availed (Belfield and Brown, 2008).

1.4.12 Adaptation of Maize and Yield Potential

Adaptation in maize is referred to as good performance in terms of yield and other agronomic characteristics under given environmental conditions (Brown *et al.,* 1985). Maize is a universal cereal adapted to diverse agroecologies of both temperate and tropical regions of the world from as far as 58° North up to 30° South, and can grow even at higher altitude of up to 4000 masl. It It is a short-day plant with photoperiod of 12.5 h and relative humidity of

85 to 100%. Maize poorly performs under saline soils especially during flowering (Belfield and Brown, 2008). Optimum soil pH for maize growth is 5.5 to 7 (AGOGTR, 2008). Suitable temperature for optimum maize production ranges between 17 to 33°C and a minimum soil temperature of 12°C for germination. Maize is a widely adapted crop with high yield potentials (Ortega *et al.,* 1980). Global average yield is reported at 4 t/ha (VIB, 2017). High productivity of maize is partly attributed to it being a C4 plant; thus, it has modified anatomical and biochemical mechanisms that allow efficient use of carbon dioxide for photosynthesis (Furbank, 2011). Also, the bundle sheath cells have larger and richer chloroplasts which are useful for photosynthesis (AGOGTR, 2008). Maize plant has high water use efficiency of about 450 to 700 mm of water per season, which is mainly absorbed from the soil moisture content (Hamad *et al*., 2011; Nafziger, 2008). Single plant can consume up to 250 L of water during a life span with about 15.0 kg of grain produced per each millimetre of water consumed, provided normal agronomic practices are observed (Awata, *et al.,* 2019). Total leaf area at maturity may exceed one square metre per plant. Nutrient uptake by maize is highest at flowering stage such that at maturity the plant might have assimilated about 8.7 g of nitrogen, 5.1 g of phosphorus and 4.0 g of potassium respectively (Awata, *et al.,* 2019). In addition to efficient use of water, maize uses sunlight more efficiently than any other crop, resulting into highest yield (kg/ha). Number of kernel rows per cob varies with a maximum of about 40 rows based on the maize varieties, and a total of 1000 kernels can be produced by a single maize cob. Number of cobs per plant normally ranges from 1 to 4 though other maize cultivars my bear up to 5 cobs per plant (Hoofpen and Maiga, 2012). United States of America, China, Brazil and Mexico are the leading producing countries in the world with an average of more than 4 t/ha, contributing to about 563 million tonnes of the global total production of 717 million tonnes/year (*Ranum et al.,* 2014). In SSA, average maize yield is lagging at about 2 t/ha, resulting into over 20% of

the annual requirement being met through imports (VIB, 2017). Leading maize producers in Africa include South Africa, Nigeria, Ethiopia, Tanzania and Egypt (VIB, 2017).

1.4.13 Nutritional value of maize

Maize kernel is an edible and nutritive part of the plant. It contains carbohydrates, protein, fat, fibre, ash, moisture, phosphorus, sodium, sulphur, amino acids, minerals, calcium, iron, potassium, thiamine, vitamin C, magnesium, and copper (Shah *et al.*, 2016). It also contains vitamin C, vitamin E, vitamin K, vitamin B1 (thiamine), vitamin B2 (niacin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folic acid, selenium, N-p-coumaryl tryptamine, and N-ferrulyl tryptamine (Shah *et al.*, 2016). Potassium is a major nutrient present in significant amounts as an average human diet is potassium-deficient (Kumar and Jhariya, 2013).

Maize germ contains about 45–50% of oil that is used in cooking salads and is obtained by wet milling (Orthoefer *et al.,* 2003). The oil contains 14% saturated fatty acids, 30% monounsaturated fatty acids, and 56% polyunsaturated fatty acids. The refined maize oil contains linoleic acid 54–60%, oleic acid 25–31%, palmitic acid 11–13%, stearic acid 2–3% and linolenic acid 1% (Corn Refiners Association, 2006). The two main forms of vitamin E present in our diet are alpha (α) and gamma (γ) tocopherols. Maize oil is amongst the rich sources of these tocopherols, especially γ-tocopherol and their reported concentration was 21.3 and 94.1 mg/100 g, respectively (Sen *et al.,* 2006). Maize silk contains various constituents essential for our diet such as maizenic acid, fixed oils, resin, sugar, mucilage, salt, and fibres (Kumar and Jhariya, 2013).

1.4.14 Phytochemical content of maize

Phytochemicals are bioactive chemical compounds naturally present in plants that provide human health benefits and have the potential for reducing the risk of major chronic diseases

(Liu, 2004). Maize is an essential source of various major phytochemicals such as carotenoids, phenolic compounds, and phytosterols (Lopez-Martinez *et al.,* 2009).

1.4.14.1 Carotenoids: Carotenoids belong to a family of red, orange, and yellow pigments. There is a large quantity of carotenoid pigments present in yellow maize grains, especially in the horny and floury endosperm (Liu, 2007). These pigments are divided into two classes: carotenes, which are purely hydrocarbons containing no oxygen, and xanthophylls (lutein and zeaxanthin) which are hydrocarbons containing oxygen.

1.4.14.2 Phenolic compounds:

Phenolic compounds are a widely distributed category of phytochemicals in the plant kingdom (Saxena *et al.,* 2013). They are specified as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Liu, 2004). These compounds are abundantly present in maize, especially in bran (Zhao *et al.,* 2005). The major phenolic compounds from maize are ferulic acid (FA) or 4-hydroxy-3-methoxycinnamic acid and anthocyanins. Anthocyanins are common class of phenolic compounds collectively known as flavonoids. They are the largest group of water-soluble plant pigments which are reddish to purple in color. Maize has the second highest concentration of anthocyanins (Abdel-Aal *et al.,* 2006).

1.4.14.3 Phytosterols:

Phytosterols are an essential component of plant cell walls and membranes (Piironen *et al.*, 2000). Maize oil is very rich in phytosterols (Verleyen *et al.,* 2002). The most commonly consumed phytosterols from maize oil are sitosterol, stigmasterol, and campesterol. Their distribution varies in different fractions of maize kernel such as endosperm, pericarp, and germ (Harrabi *et al.,* 2008).

1.4.15 Health benefits of maize

Resistant starch (RS) from maize has various health beneficial effects. Maize endosperm contains 39.4 mg/100 g RS (Jiang, 2010). It escapes digestion and its consumption helps in altering microbial populations, lowering cholesterol and enhancing its faecal excretion, increasing the fermentation and short-chain fatty acid production in large intestine, reducing symptoms of diarrhoea, which altogether reduce the risk of caecal cancer, atherosclerosis, and obesity-related complications (Murphy *et al.*, 2008). Its consumption influences cholesterol metabolism, lowers body fat storage therefore reduces the risk of atherosclerosis, hyperlipidaemia, diabetes, and obesity (Higgins, 2004). It can significantly shorten the intestinal transit time that leads to elimination of waste material through faeces in a quicker time (Kim *et al.,*2003).

RS as dietary fibre helps in weight control as it reduces food intake by diluting energy density of the diet as well as by modulating certain gene expressions. A study was carried on rats which explained that the inclusion of RS from maize in their diet can affect the energy balance through its effect as a fibre, a stimulator of gut peptide tyrosine-tyrosine (peptide YY), an expressor of glucagon-like peptide-1, as well as other genes in hypothalamic area of brain which are the key factors for maintaining energy homeostasis and reducing the food intake by increasing satiety (Keenan *et al*., 2006; Shen *et al.,* 2009). Another investigation was carried out to examine the effects of different high-fibre foods on the satiety of healthy human subjects. The results showed that eating muffins containing RS and maize bran had a major impact on satiety compared with foods containing other fibres (Willis *et al.*, 2009). RS has also been suggested to be potentially beneficial for improving insulin sensitivity in both animal and human subjects (Deng *et al.*, 2010; Johnston *et al.*, 2010).

Maize is an essential source of various phytochemicals that play an important role in our health (Kopsell *et al.,* 2009). Research has suggested that phytochemicals in grains due to their potent antioxidant activities demonstrate significant beneficial contribution in reducing the risk of many diseases (Shahidi, 2009). Maize grains, especially yellow variety contains large quantities of the carotenoid pigments and has a vital significance in the diet as human beings are not able to biosynthesize carotenoids. Carotene has many health benefits associated with it. Alpha (α) and beta (β) carotene possess provitamin A activity. High concentration of β-carotene has been observed to act as a pro-antioxidant and induces apoptosis of colon cancer cells, leukemia cells, melanoma cancer cells, and gastric cancer cells, thus rendering potent chemopreventive effect (Jang *et al.*, 2009).

Xanthophylls (lutein and zeaxanthin) in maize have some pivotal and specific biological functions. Lutein supplementation in food at dose-dependent manner increases tumour latency, inhibits mammary tumour growth, enhances lymphocyte proliferation, lowers the incidence of palpable tumour, and significantly protects cells against oxidant-induced damages (Shah *et al.,* 2016). Lutein and zeaxanthin are found to be the only carotenoids in the macula of the retina that are responsible for sharp and detailed vision. They also appear to protect humans against phototoxic damage; also play a role in protection against age-related macular degeneration and age-related cataract formation. Supplementing lutein to the subjects' diets for a period showed a significant enhancement in macular pigment optical density and notable protection of the macula from light damage (Shah *et al.,* 2016).

Anthocyanins have been well known for their health-promoting benefits such as anti-carcinogenic, anti-atherogenic, lipid lowering, anti-diabetic, antimicrobial, and anti-inflammatory properties (Shah *et al*, 2016). Due to the potent antioxidant properties, they are able to decrease capillary permeability and fragility, immune system stimulation, and inhibit platelet aggregation (Ghosh and Konishi, 2007). The pigments from black glutinous maize cob have shown to possess potent anti-hyperlipidaemic effects in high-fat-fed mice by improving the serum lipids profile and reducing the atherogenic index (Zhang *et al.*, 2010).

Phytosterols have many health benefits. Dietary consumption of phytosterol is negatively related to cholesterol absorption, serum total, and LDL cholesterol (Jiang and Wang, 2005). The major mechanism involved in the health benefits of dietary phytosterols is the inhibition of cholesterol absorption through intestine and stimulation of cholesterol synthesis resulting in the enhanced elimination of cholesterol in stools. The consumption of corn oil in a long-term period can reduce cholesterol concentrations and prevent atherosclerotic disease (Shah *et al.,* 2016).

Maize acts as a nanoscale biomaterial that has unique solubility and film-forming properties. It has novel applications in pharmaceutical and nutraceutical areas to coat nanoparticles, develop promising nanocomposite antimicrobial agents, produce novel food packaging, encapsulate nutrients, and provide target delivery with controlled release (Luo *et al.,* 2011; Rouf Shah *et al.,* 2016).

1.4.16 Overview of Nanotechnology

Nanotechnology emerged as one of the most rapidly advancing science of twenty first century. It is the science of manipulating materials at the nanoscale (Baig *et al*., 2021). As a field, nanotechnology explores working with particles at the smallest possible size and promises to increase productivity, cut production costs, and create novel materials with special qualities. Diversified application of nanotechnology in various fields have been found to uplift the entire scenario of industry and agricultural sector including information technology, medicine, disease detection and diagnosis, food safety and security, pest and disease management, environmental science and many more. It possesses marvellous application as antimicrobial and therapeutic compounds, targeted drug delivery, high sensitivity disease detection and diagnosis and thus likely to enhance agricultural productivity due to decline in cost associated with agricultural production practices (Dutta *et al.,* 2021;

Dutta *et al.,* 2022). The small size of nanoparticles (<100 nm), greater surface area to volume ratio and high reactivity favours its wide-scale application in the field of human and plant pathology (Jeevanandam *et al*., 2018).

1.4.17 Need for Nanotechnology in Plant Science and Agriculture

Currently, there is a global concern about food security due to the exponential growth of human population together with the ongoing climate crisis. The traditional approach for improvement of crop productivity to sustain the growing population is by applying bulk chemical fertilizers (sometimes referred to as synthetic, inorganic, and mineral fertilizers) and pesticides. Nevertheless, evidence shows that only a fraction of the chemical fertilizers and pesticides applied contribute to aiding crop production (Raliya *et al.,* 2018; Tudi *et al.,* 2021). Even worse, the residues of these chemical fertilisers and pesticides, to a significant extent, pollute the environment and groundwater via leaching. These pollutants cause soil degradation in form of acidification and eutrophication, which are usually hazardous to aquatic and agro-ecosystems (Tudi *et al.,* 2021).

In spite of the potential yield benefits associated with bulk chemical fertilization, it is capable of changing the chemical properties of the soil and does not improve the richness, diversity, or abundance of soil microbial communities, which are generally indicators of soil fertility (Dinca *et al.,* 2022). Excessive concentrations of chemical fertilisers were reported to impact the viability and metabolic activity of bacterial and fungal species in the soil (Dinca *et al.*, 2022). On the other hand, pesticides may hinder vital cellular processes of non-target microorganisms and other soil biota which inadvertently results in reduced chemical and biological soil fertility (Vischetti *et al.,* 2020; Tudi *et al.,* 2021).

From agriculture point of view, the major concern related to soil and environmental health includes: increased pesticide residue in soil and water bodies, decline in soil beneficial organisms, alteration of soil physical and chemical properties, pesticide resistance in pathogens and many more (Sharma *et al.,* 2019).

Therefore, the use of nanotechnology in agriculture has been recently explored as an alternative to the conventional use of bulk chemical fertilizers and pesticides (Kalwani *et al.,* 2022). Nanoparticles can potentially provide various benefits over conventional agricultural practices such as large surface area to volume ratios, mass transfer abilities as well as slow, controlled and targeted delivery of lower nutrient or pesticide concentrations to enhance crop productivity, if used appropriately (Hussain *et al.,* 2023). Although the application of nanoparticles has been revolutionary for crop productivity, the response of plant-associated microorganisms to nano-based amendments remains unclear. Similarly to bulk chemical fertilizers or pesticides, nano-based agricultural amendments may have an impact on plant-associated microorganisms. Exposure of plant-associated microorganisms to nanoparticles can either be beneficial or harmful depending on various factors. Hence, further investigation on the interaction and response of plant-associated microorganisms to nanoparticles is warranted to ensure sustainable precision agricultural practices.

With nanotechnology, attempts are being made to enhance the efficacy of applied fertilizers by using nanoparticles. Efforts are focused on restoring soil fertility by facilitating the controlled release of fixed nutrients (Sun *et al.,* 2021). The farming community frequently concentrates on reducing the cost of agricultural inputs and increase earnings. To accomplish this goal, farmers use fertilizers, herbicides, and fungicides to maximize crop productivity. Nano-structured formulations, with their ability to employ mechanisms such as targeted delivery or slow/controlled release, offer the potential for the precise release of active ingredients in response to environmental stimuli and biological demands. Empirical studies show evidence that using nano fertilizers leads to enhanced nutrient utilization efficiency, diminished soil toxicity, mitigated risks associated with excessive dosages, and reduced application frequency (Muller *et al*., 2017). Hence, nanotechnology has a high potential for achieving sustainable agriculture, especially in developing countries.

Furthermore, the challenges posed by pests, and diseases in the agricultural production persist, necessitating the continual exploration of alternative remedies. Although modern agricultural practices propose various solutions, the outcomes are only sometimes replicated across farms due to the individual characteristics of each farm, including its distinct topography, soil composition, available technological resources, and anticipated crop yields. (Majumdar *et al*., 2021). In order to increase crop productivity nanoscale materials and devices, such as nanoscale transporters, nano sensors, nano fertilizers (NFs), nano herbicides, and nano pesticides, can help in crucial fields like fertilizer delivery, pest management, monitoring the environment, variable rate technology, automated machinery, and data analytics by combining current technologies and data-driven decision-making tools with them. Improved nutrient implementation, efficient and targeted application of pesticides, continual evaluation of soil and plant parameters, and precise input delivery are all possible outcomes of integration. (Yadav *et al.,* 2023). The farming community may eliminate agrochemicals while maintaining good crop output, safeguarding the health of the soil and water, and making a positive contribution to a cleaner environment by implementing precision agriculture practices based on nanotechnology.

1.4.18 Current Applications of Nanotechnology in Plant Science

1.4.18.1 Biosensors

Nanomaterials (NMs) have been applied to develop biosensors or “sensing materials” in the fields of crop biotechnology, agriculture, and food industry (Duhan *et al*., 2017; Chaudhry *et al*., 2018). Different categories of nanosensor types have been tested in plants, including plasmonic nanosensors, fluorescence resonance energy transfer (FRET)-based nanosensors, carbon-based electrochemical nanosensors, nanowire nanosensors and antibody nanosensors. Although the use of nanosensors in plants is at an initial stage (Rai *et al.*, 2012), interesting reports have proposed the use of NMs as tools for detection and quantification of plant metabolic flux, residual of pesticides in food and bacteria, viral and fungal pathogens.

NMs-based biosensors are very promising as they allow rapid detection and precise quantification of fungi, bacteria and viruses in plants (Duhan *et al.*, 2017). For example, fluorescent silica NPs combined with antibody was designed for diagnosing Xanthomonas axonopodis pv. vesicatoria, which causes bacterial spot disease in Solanaceae plants (Yao *et al.*, 2009). Recently, Au NPs have been proposed from Lau *et al.* as DNA biochemical labels to detect Pseudomonas syringae in A. thaliana by differential pulse voltammetry (DPV) on disposable screen-printed carbon electrodes (Lau *et al.,* 2017). Similarly, fluorescently labeled-DNA oligonucleotide conjugated to Au NPs were employed in the diagnosis of the phytoplasma associated with the flavescence dorée disease of grapevine (Firrao *et al.*, 2005).

1.4.18.2 Controlled Release of Agrochemicals and Nutrients

NMs can be applied to the soil as nanostructured fertilizers (nanofertilizers, as for Fe, Mn, Zn, Cu, Mo NPs) or can be used as enhanced delivery systems to improve the uptake and the performance of conventional fertilizers (nutrients and phosphates) (Liu and Lal, 2015). Even though nanofertilizers and NM-enhanced fertilizers are very promising for agriculture, the use of nanotechnology in fertilizer supply is very scanty (DeRosa *et al.*, 2010).

Hydroxyapatite nanoparticles, used as phosphorous nanofertilizers, enhance the soybean growth rate and seed yield by 33 and 20%, compared to a regular P fertilizer (Liu and Lal, 2015). In addition, nanofertilizers can be released at slower rates which may contribute to maintain the soil fertility by reducing the transport of these nutrients into a runoff or ground water and decreasing the risks of environmental pollution and toxic effects due to their over-application (Liu and Lal, 2015). Metallic nanoparticles based on Iron oxide, ZnO, TiO2, and copper have been directly applied as nanofertilizers in soil by irrigation or via foliar applications in different plants, such as mung bean plant, cucumber and rape (Gao *et al.*, 2006; Tarafdar *et al.*, 2014; Saharan *et al.*, 2016; Verma *et al.*, 2018).

Similarly, MWNTs used as soil supplements increased twice the number of flowers and fruits in tomato plants likely through the activation of genes/proteins essential for plant growth and development (Khodakovskaya *et al.*, 2013). Despite these intriguing evidences, the use of nanofertilizers is still debatable. Accumulation in treated soils may pose a threat to soil microbial communities such as small invertebrates, bacteria and fungi (Shen *et al.*, 2015; Simonin *et al.*, 2016; Goncalves *et al.*, 2017). This impact on the agro ecosystem reasonably discourages the use of metallic nanoparticles in agriculture.

Only recently, a natural polymer, such as chitosan NPs, have been used for controlled release of nitrogen, phosphorus and potassium in wheat by foliar uptake (Abdel-Aziz *et al.*, 2016). The use of organic NPs is more acceptable in terms of environmental pollution. However, their effective advantages for nutrient supply over traditional fertilization methods need more robust evidence (Liu and Lal, 2015). On the other hand, pesticides delivered by nanomaterials generally have increased stability and solubility and enable slow release and effective targeted delivery in pest management (Duhan *et al.*, 2017). Organic and polymeric NPs in the form of nanospheres or nano capsules have been used as nanocarriers for herbicide distribution (Tanaka *et al.*, 2012). In particular, polymeric NPs, such as Poly(epsilon-caprolactone), present good properties of biocompatibility and have been repeatedly used for the encapsulation of atrazine herbicide (Tanaka *et al.*, 2012). In another study, chitosan nanoparticles loaded with three triazine herbicides have shown reduced environmental impact and low genotoxic effects in Allium cepa (Grillo *et al.*, 2015).

1.4.18.3 Nanomaterials for Plant Genetic Engineering

Cell wall constitutes a barrier to the delivery of exogenous biomolecules into plant cells. To circumvent this barrier and achieve plant genetic transformation, different strategies based on Agrobacterium transformation or biolistic methods are worldwide used for DNA delivery in plant cells. Limitations to these approaches rely on narrow host range and plant extensive damages, which often inhibit plant development.

Results reported recently in plants are proving that NMs may overcome the barrier of the cell wall in adult plants and reduce the drawbacks associated with current transgene delivery systems. One seminal study proved that dsRNA of different plant viruses can be loaded on non-toxic, degradable, layered double hydroxide (LDH) clay nanosheets or BioClay. The dsRNAs and/or their RNA breakdown products provide protection against the Cauliflower Mosaic Virus (CMV) in sprayed tobacco leaves, but they also confer systemic protection to newly emerged, unsprayed leaves on viral challenge 20 days after a single spray treatment in tobacco (Mitter *et al*., 2017). This is a proof of concept for species-independent and passive delivery of genetic material, without transgene integration, into plant cells for different biotechnology applications in plants.

A successful stable genetic transformation has been achieved in cotton plants via magnetic nanoparticles (MNPs). b-glucuronidase (GUS) reporter gene-MNP complex were infiltrated into cotton pollen grains by magnetic force, without compromising pollen viability. Through pollination with magnetofected pollen, cotton transgenic plants were successfully generated and exogenous DNA was successfully integrated into the genome, effectively expressed, and stably inherited in the offspring obtained by selfing (Zhao *et al*., 2017).

In another recent paper, carbon nanotubes scaffolds applied to external plant tissue by infusion were used to deliver linear and plasmid DNA, as well as siRNA, in *Nicotiana benthamiana*, *Eruca sativa, Triticum aestivum*, and *Gossypium hirsutum* leaves and in *E.*

sativa protoplasts, resulting in a strong transient Green Fluorescent Protein (GFP) expression. Moreover, the same authors reported that small interfering RNA (siRNA) was delivered to *N. benthamiana* plants constitutively expressing GFP, causing a 95% silencing of this gene (Demirer *et al.*, 2018). The first and promising approach of genome editing mediated by mesoporous silica nanoparticles (MSNs) has been recently proposed. MSNs have used as carriers to deliver Cre recombinase in *Zea mays* immature embryos, carrying *loxP* sites integrated into chromosomal DNA. After the biolistic introduction of engineered MSNs in plant tissues, the *loxP* was correctly recombined establishing a successful genome editing (Valenstein *et al*., 2013).

1.4.19 Importance of Nanotechnology in Plant Science

The use of nano-encapsulated fertilizers and pesticides can reduce the amounts of chemical fumigants and pesticides reaching the soil surface as compared to conventional formulations and also prolongs protection to plants against various phytopathogens. Nano-based materials can also act as cargo molecule and can release the active ingredients owing to its greater surface area to volume ratio. The effect on non-target organisms can also be reduced as they are highly target specific (Din *et al.,* 2017). The disease tolerance ability of plants can also be enhanced thereby improving plant health. Thus, it may be predicted that integrity between timely and accurate disease diagnosis and management can be established in near future by exploiting the science of nanotechnology (Mahmood *et al.,* 2017).

1.5 Research Aim and Objectives

1.5.1 Research Aim

The aim of this study was to quantitatively assess the growth of maize plants following application of nanoparticle intervention.

1.5.2 Research Objectives

The specific objectives of this study include to:

I. Assess growth parameters of maize plants following application of nanoparticle intervention

II. Determine whether maize plants grow better or worse following application of nanoparticle intervention compared to maize grown without nanoparticles.

III. Determine the advantages, if any, of application of nanoparticle intervention in maize production.

1.6 Research Questions

1. What are the effects of application of nanoparticle intervention on quantitative growth of maize?
2. Do maize produced following the application of nanoparticle intervention exhibit better quantitative growth parameters?

CHAPTER TWO

MATERIALS AND METHOD

2.1 Study Area

This study was carried out 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, oven 0-200c,50ml pyrex beaker, glass stirring rod, filter paper; Whatman No 1, 50ml volumetric flask, cotton wool, knife, milling machine, polythene bag, envelope; khaki, 10ml pipettes, 25ml volumetric flask, wheelbarrow, zobo flowers **(***Hibiscus rosa-sinensis* Linn**)**, copper nanoparticles, conical flasks of 1000 ml voldume, spray bottles, metre rule, measuring scale, hybrid maize (OBA SUPER 6), 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 in a three properly-spaced duplicates.

2.4 Research Methodology

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

2.4.1 Soil Collection

Ferruginous reddish-brown soil was procured from the premises of the Botanical Garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. This soil was collected using a shovel from three different locations to collect the topmost layer of the soil and a wheelbarrow to transport the soil to the experimental site where it was thoroughly mixed.

2.4.2 Site Preparation

The experimental location was initially cleared thoroughly of invasive vegetation using a cutlass which includes cutting off surrounding branches and trees serving as shade on some part of the site. The experimental location was lined with a black tarpaulin, soil layers were subsequently poured onto the tarpaulin and evenly distributed. The aim of doing this is for insulation, to enhance a stable temperature on the polythene bag; for firm support, to help keep polythene bags in place; for protection, to shield the polythene bag from being destroyed by sunlight, pests or physical abrasions.

124 perforated polythene bags were then filled with the homogenised soil, labelled accurately and placed 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 procured soil was poured in the planting bags after being weighed accurately at 7 grams of soil per bag. These bagged soils were then treated with iron sulphate to increase the iron content. A weighed sample of iron sulphate crystals at three various concentrations of Ecological screening values; 1, 2.5 and 4 ESV.

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 amount of iron sulphate needed to elevate the ESV for 5 bags of soil was measured simultaneously with the manual weighing balance and dissolved in 1 litre (1000 ml) of water. The solution was further divided into 5 parts i.e 1 part for each bag of the initial measurements. 200ml of this solution was measured using a measuring cylinder and poured into a container. Then water was used in diluting to make it up to 1000 ml which was poured into the bags.

2.4.4 Preparation of Copper Nanoparticles Treatment

This was carried out by first pounding 10 grams of zobo flowers **(***Hibiscus rosa-sinensis* Linn**)** and rinsing, after which it was poured in a conical flask of 100 ml distilled water. The mixture was shaken vigorously for 10 minutes to allow the content of the leaves to diffuse into the water, therefore changing its colour to dark red. Separately, 10 mM of copper sulphate was prepared by diluting 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 further placed on a magnetic stirrer and allowed to mix properly until a colour change from dark red to reddish brown was observed.

Procedure

For Dried Plant Material

The plant material was clean thoroughly with cotton wool that has been dipped in water. The material was chopped into small pieces with a knife. Some pieces were put into an envelope (khaki) and dried in an oven at 85°C to constant weight (this takes 2-3 days). Was then removed from the oven and grind in a Milling Machine to powder form and preserved in a polythene bag.

Procedure

1g of the Plant Sample (grinded) was placed in a beaker 10ml Aqua Regia: (Measure 75ml of Conc. HCl and 25ml conc. HNO_3 into 100ml Volumetric Flask, 3:1) was added. Then, it was stirred to dissolve completely using a glass stirring rod. The solution was then cooled and filtered into a 100ml flask and further diluted to mark with distilled water.

ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

The atomic absorption spectrometry was used for the determination of Heavy Metals. The source of radiation is a Hallow lamp, which contains a Cathode constructed of same metals as that being analysed. This emits the wavelength characteristic of the metal; and a different lamp was used for each metal. The light from the lamp was directed through a flame and onto a monochromator, which selects the preferred analytical wavelength. The light monochromator was detected by a photomultiplier tube and converted to an electrical signal. The sample was aspirated in the flame where the solution was evaporated and the metal –

containing compounds volatilized and dissociated into ground state atoms. The ground state atoms absorbed the radiation from the hallow lamp and excited to higher energy levels. Some atoms were also thermally excited but their fraction was so small that it caused no error in the analysis. An acetylene-air flame was used.

2.4.5 Treatment Designation

For the increase of soil iron content level, 1 ESV, 2.5 ESV, and 4 ESV were adopted. The experiment also involved the application of Cu NPs in three (3) different concentrations; 35%, 75% and 100%. These concentration were made by changing the ratio of the prepared nanoparticle solution to water. The 35% concentration was made of a 7:13 ratio of the nanoparticle solution to water. The 75% concentration was made 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 appropriately labelled according to the treatments applied to it i.e. elevated soil iron content and copper nanoparticle treatment. The labels and their representations are as follows;

E1 - Concentrations of Fe at 1 ESV

E2 - Concentrations of Fe at 2 ESV

E3 - Concentrations of Fe at 3 ESV

Cu1 - Copper NP at 35% concentration

Cu2 - Copper NP at 75% concentration

Cu3 - 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 properly sorted before being soaked for a few minutes in water. The planting bags were adequately watered before the maize seeds were placed in them, 5 seeds per bag. Nanoparticle treatment was applied using foliar spray two (2) weeks after planting. Readings were taken weekly as the plants were monitored and watered regularly for 5 weeks.

2.4.7 Experiment Management

The various practices were carried out at regular intervals;

I. Weeding

This was carried out by handpicking invasive species 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 using a centimetre metre rule at 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 topmost 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 measurement of the leaf width was taken 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 lead 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.

CHAPTER THREE

RESULTS

Figure 3.1 presents the plant height of maize plants introduced to nanoparticles in ferruginous soil. Cu1, Cu2, and Cu3 signifies plants introduced to copper-based nanoparticles at 35%, 75% and 100% concentrations respectively and E1, E2, and E3 indicates plants at 1, 2.5 and 4 Ecological screening values (ESV) of iron i.e 1.4g, 3.5g and 5.6 respectively. In this study, maize that were introduced to 35% (Cu1) and 1.4g iron sulphate (E1) had stem height of 32.1cm, while the maize that was sown in 100% (Cu3) copper based nanoparticles and polluted with iron sulphate at 1 ESV had an increase in stem length of 38.5cm, among the various treatment apart from the control which showed the highest stem height (49.1cm) followed by Cu1E3 which depicted that 45cm. The lowest stem length was noticed in plant introduced to 100% copper-based nanoparticle (Cu3) and 5.6 iron sulphate (E3). Notable difference was observed in the stem of length of the treatments.

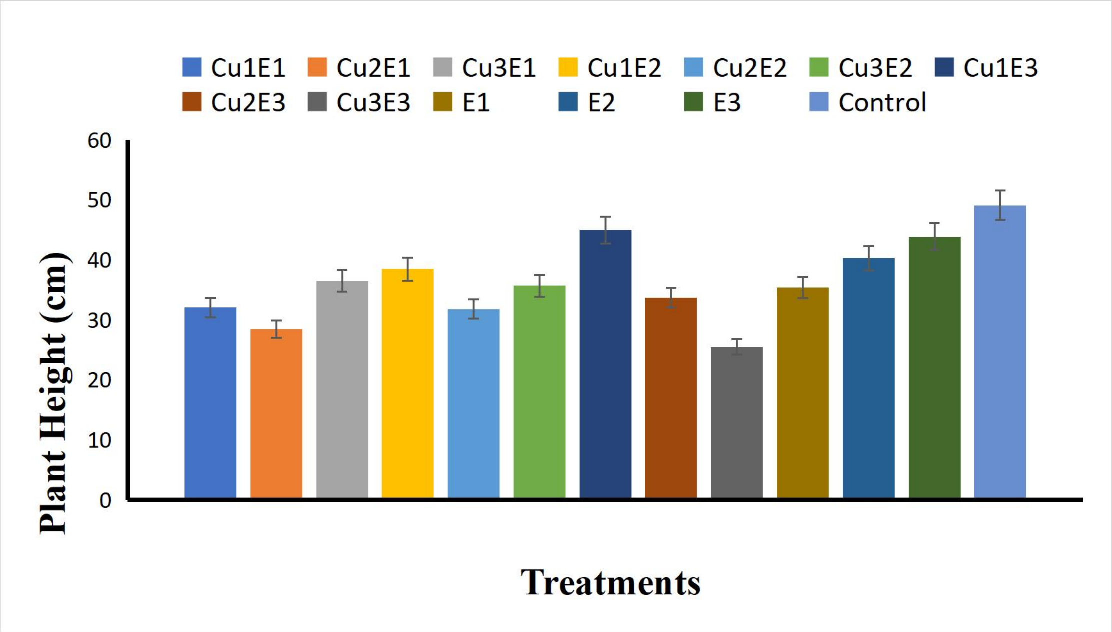

Figure 3.1: Stem Height of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate

Figure 3.2 shows the leaf length of plants introduced to the various concentration of Cu-based nanoparticles (Cu1, Cu2, Cu3) and different treatments of iron sulphate (ESV 1, ESV 2, ESV 3) at 1.4g, 3.5g, 5.6g respectively. The results infers that maize which were introduced to 75% (Cu2) copper-based nanoparticles and planted in soils polluted with iron sulphate at 1 ESV (Cu2E1) had a leaf length of 74.5cm, followed by plants exposed to 35% Cu-based nanoparticles (Cu1) and 3ESV of iron sulphate elevation which had leaf length of 70.1cm. The lowest leaf length (49.5cm) were noticed in plants that were introduced to 100% Cu-based nanoparticles and 3 ESV of iron sulphate in contrast to those that were not introduced to nanoparticles treatment but still under same soil conditions (E3) which measured leaf length of 62.5cm.

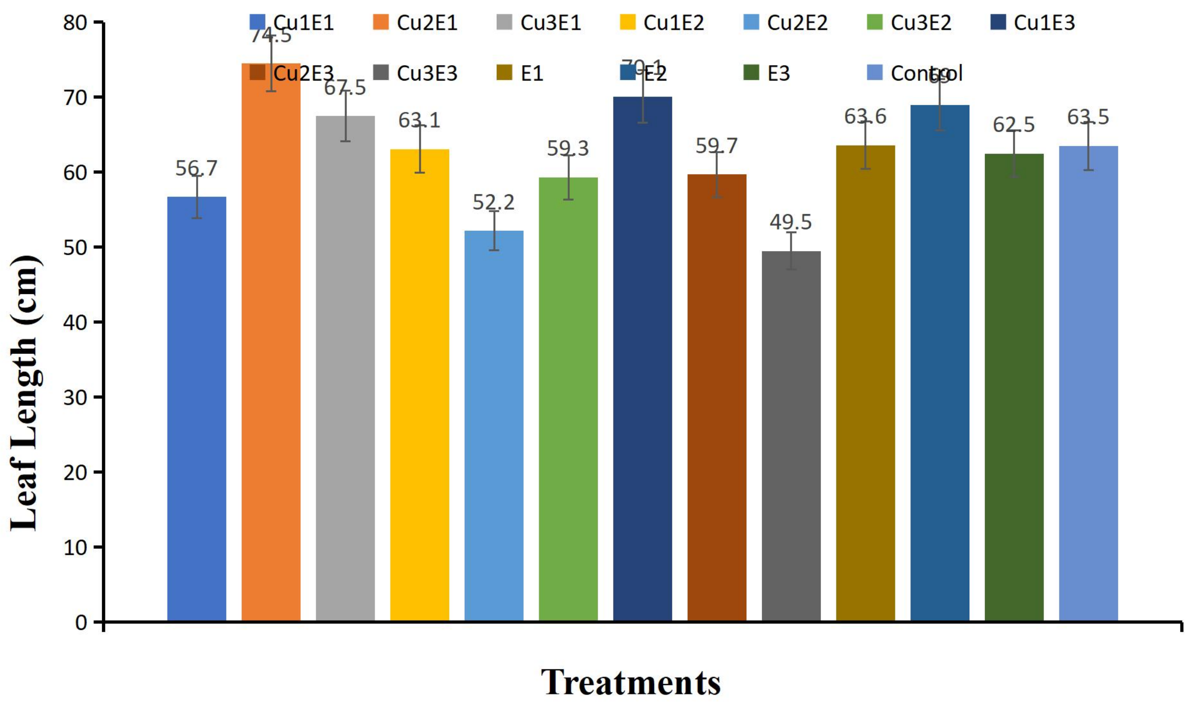

Figure 3.2: Leaf length of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate

Figure 3.3 presents the leaf width of *Zea mays* plant introduced to the various concentration of Cu-based nanoparticle and Iron sulphate. The findings infers that plant introduced to 75% Cu-based nanoparticles and 1 ESV of iron sulphate measured the highest leaf width, followed by Cu1E2 and E2 which had leaf width of 3.9cm respectively. Control treatment measured leaf length of 3.2cm which was not apparently different from the other treatments. However, the lowest leaf width was noticed in plants introduced to 75% Cu-based nanoparticles and 2ESV of iron sulphate elevation.

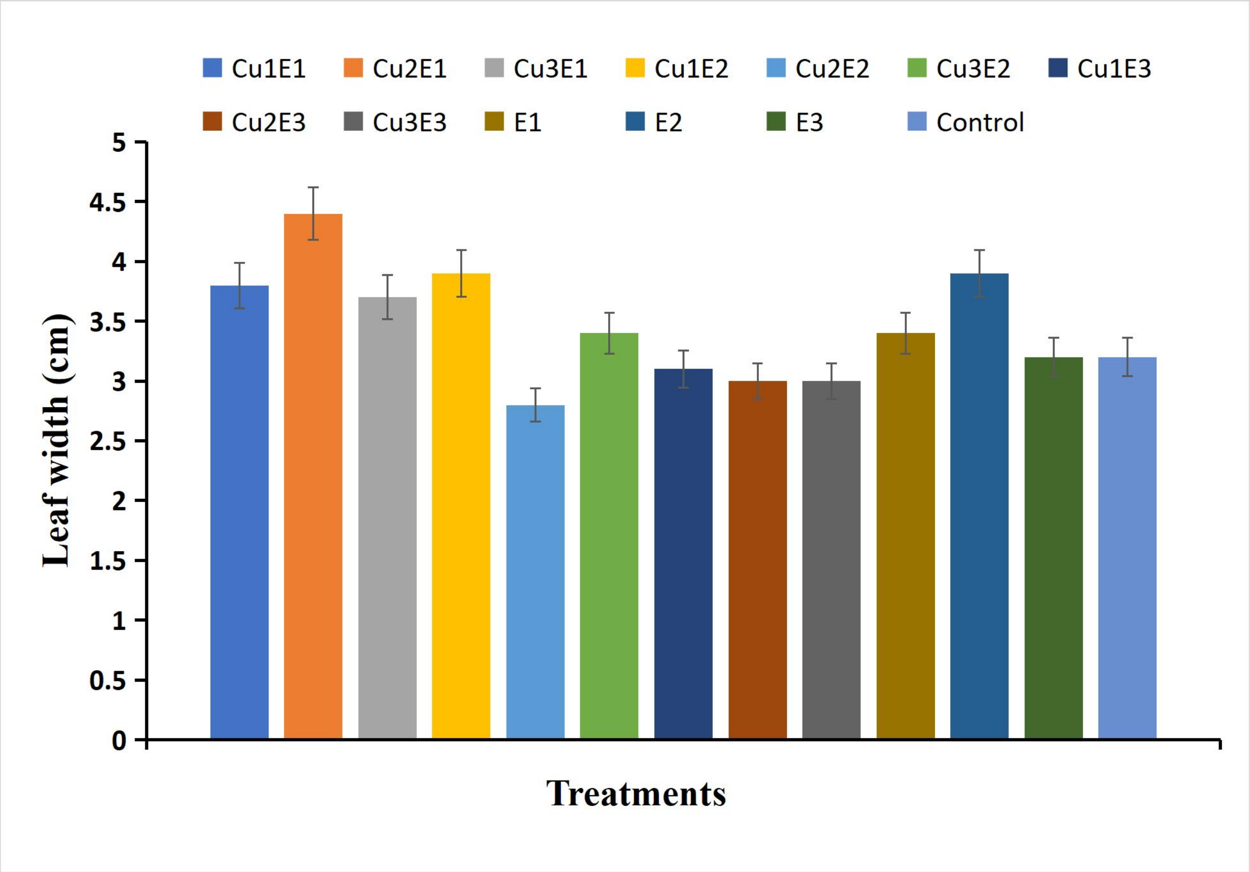

Figure 3.3: Leaf width of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate

Figure 3.4 represents the leaf area of maize plants introduced to nanoparticles in ferruginous soil. The study revealed that plants that were introduced to 75% (Cu2) copper-based nanoparticles and grown in soils polluted with iron sulphate at 1 ESV (Cu2E1) had a leaf area of 245.85 cm² during the 5th week in contrast to the control treatment which had leaf area of 152.43cm². Plants that were not introduced to nanoparticles treatment but was grown in iron sulphate elevated soil E1, E2 and E3 still under same soil conditions had leaf area of (162.18, 201.82 and 150.11cm² respectively). The lowest leaf area were noticed in plants introduced to CU2E2 which had leaf area of 109.22 cm².

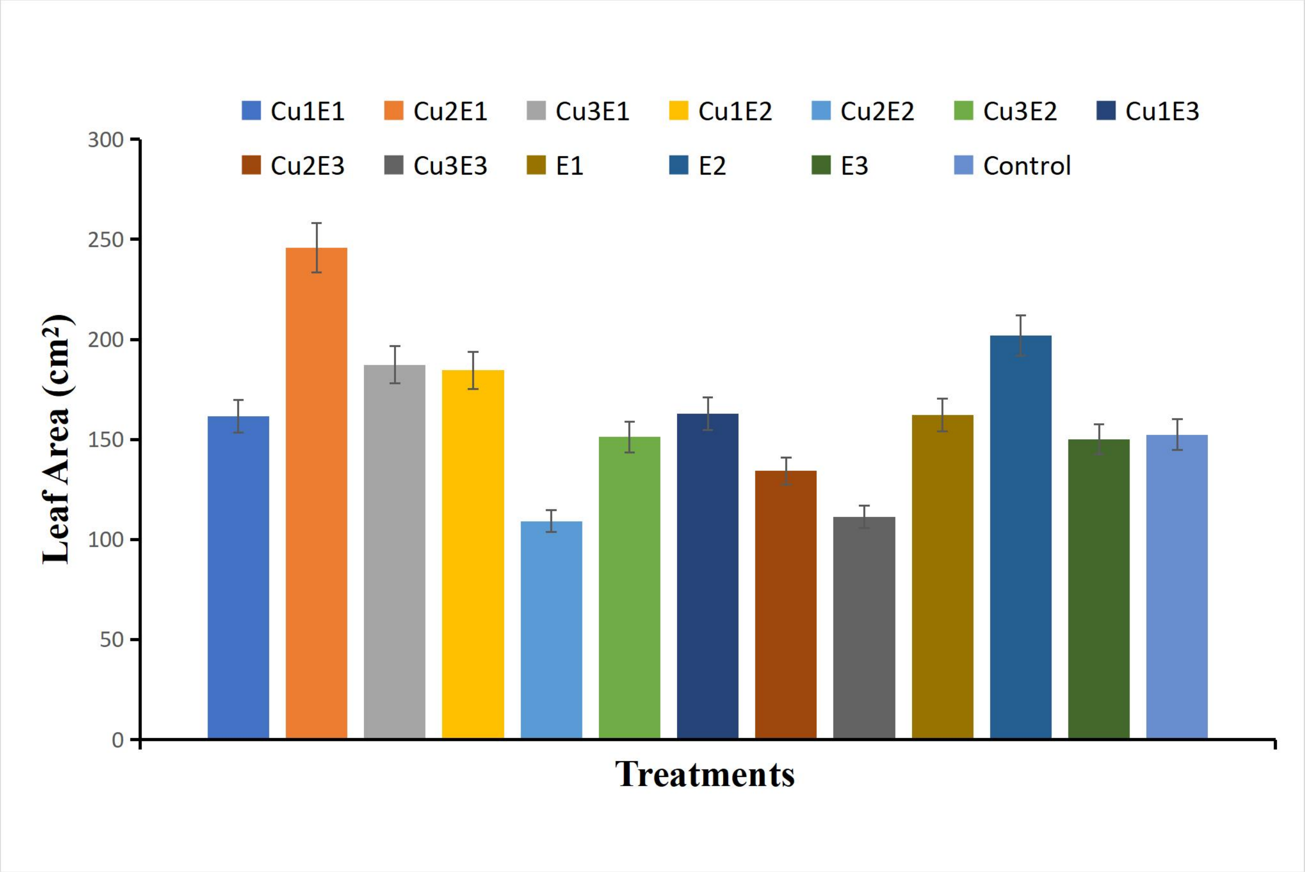

Figure 3.4: Leaf area of *Zea mays* plant exposed to different concentration of Cu-based nanoparticle and Iron sulphate

Figure 3.5 represents the Leaf number of *Zea mays* plant introduced to the various concentration of Cu-based nanoparticle and Iron sulphate alleviation. The findings revealed that plants with the highest number of leaves were plants that were introduced to 100% (Cu3) copper-based nanoparticles and grown in soils polluted with iron sulphate at 2 ESV (Cu3E2), followed by Cu3E1, Cu1E2, Cu1E3 and E2 which had leaf number of 11 in contrast to Cu2E1 and Cu2E2 which had 9 leaves during the 5th (fifth) week of the experiment.

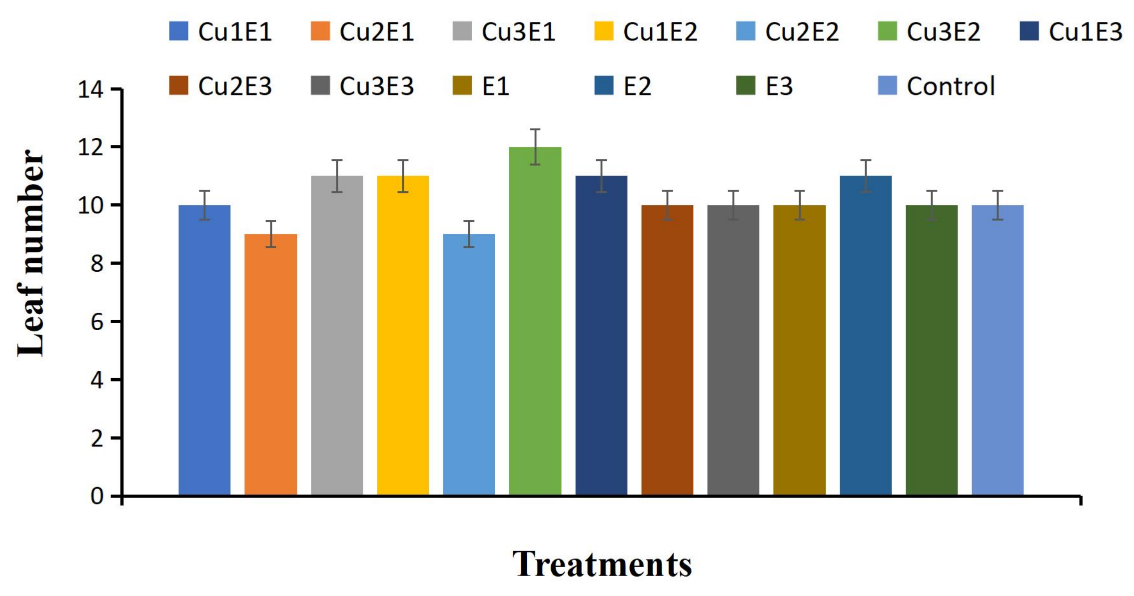

Figure 3.5: Leaf number of *Zea mays* plant introduced to the various concentration of Cu-based nanoparticle and Iron sulphate

Figure 3.6 shows the results of the Copper uptake of *Zea mays* in different treatments. The experiment shows that plants that were exposed to 35% (Cu) Copper-based nanoparticles and sown in soils contaminated at 1 ESV of iron sulphate showed 21.1mg/kg Copper content in the stem, 33.1mg/kg Copper contents in the leaves, and 10.2mg/kg Copper in the root of the plant. While plants that were exposed to 75% (Cu) Copper-based nanoparticles and sown in soils contaminated at 2 ESV of iron sulphate (Cu2E2) had an average Copper content of 15.8mg/kg in the stem, 33.45mg/kg Copper in the leaves and 10.3mg/kg of Copper in the root of *the* plant. Furthermore, plants exposed to 100% (Cu) Copper-based nanoparticles and sown in soil contaminated with 3 ESV of iron sulphate, showed an average Copper content of 13.4mg/kg in the stem, 10.3mg/kg roots and 31.7mg/kg in the leaves. The highest Copper contents were observed in the leaves of (Cu2E2) plants.

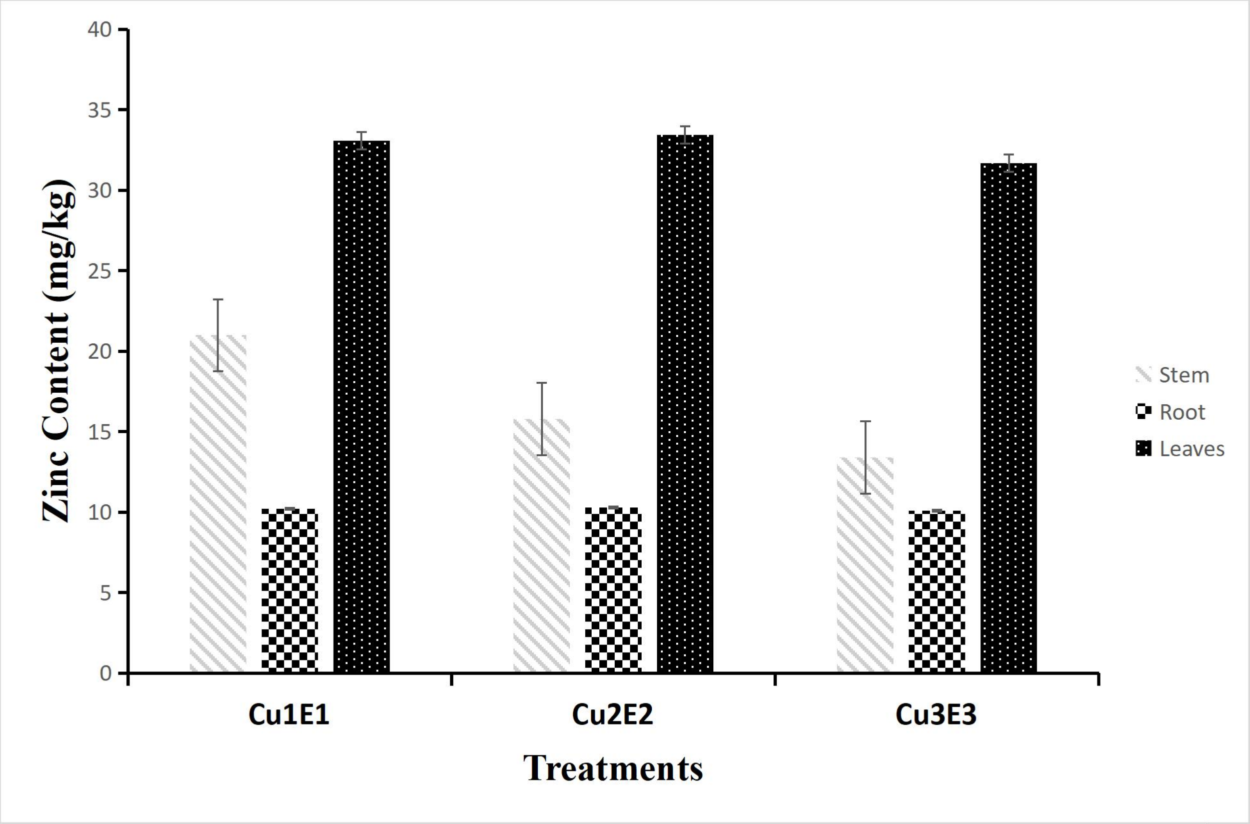

Figure 3.6: Copper uptake of *Zea mays* in different treatments.

Table 3.1: Sheath length of maize plants exposed to ferruginous soil

Sheath Length	Week 4	Week 5
Cu1E1	20	23
Cu2E1	21	28.5
Cu3E1	18.6	23
Cu1E2	19	24
Cu2E2	16	24.2
Cu3E2	19.5	25.7
Cu1E3	19.9	35.5
Cu2E3	21	28.2
Cu3E3	15	18
Control	18.6	20.3
E1	23.3	28.5
E2	29.9	34.7
E3	30.5	39.4

	Plant Height	Leaf Length	Leaf Width	Leaf Area	Number of Leaves	Sheath Length
Cu1E1	32.1a	56.7	3.8	161.595	10	23
Cu2E1	28.5a	74.5a	4.4a	245.85a	9	28.5
Cu3E1	36.5a	67.5	3.7	187.31a	11	23
Cu1E2	38.5a	63.1	3.9	184.56a	11	24
Cu2E2	31.8a	52.2a	2.8	109.22	9	24.2
Cu3E2	35.7a	59.3	3.4	151.21	12	25.7
Cu1E3	45	70.1	3.1	162.98	11	35.5a
Cu2E3	33.7a	59.7	3	134.32	10	28.2
Cu3E3	25.5a	49.5	3	111.37a	10	18
E1	35.4a	63.6	3.4	162.18	10	28.5
E2	40.3	69	3.9	201.82a	11	34.7a
E3	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

Table 3.2: Morphological characteristics of maize seedlings after 5 weeks

CHAPTER 4

DISCUSSION

Excessive iron can significantly hinder maize plant growth and development. Iron toxicity causes ultrastructural damage to chloroplasts, leading to a decline in essential enzymes and slowing the conversion of carbon into sugars (Stocking, 1975). As a result, chlorophyll production decreases, and growth stages are delayed (Rizvi and Khan, 2018). The presence of heavy metal toxicity, including excess iron, reduces plant growth and yield while increasing oxidative stress. Research indicates that excessive iron toxicity negatively affects maize by reducing height, leaf size, leaf area, leaf color, sheath length, and leaf count (Rizvi and Khan, 2018).

This experiment demonstrated a significant improvement in maize growth due to copper nanoparticles, particularly at concentrations of 35% and 100%, across both early and later growth stages. The study provided evidence of a dose-dependent effect, where different nanoparticle concentrations influenced plant height. However, as the experiment progressed, plants treated with a 100% concentration (Cu3) consistently exhibited better growth than those treated with a 35% concentration (Cu1). The impact of soil contamination with iron sulfate was complex and dependent on contamination levels. By the second week, plants treated with a 75% concentration (Cu2) and grown in soil contaminated at 2.5 ESV (Cu2E2) showed slightly lower heights compared to those in soil contaminated at 1 ESV (Cu1E1), aligning with Barbosa's (2013) conclusion that excessive copper foliar application can reduce plant height. However, by the fourth week, plants in the 75% treatment group had caught up in height, suggesting a possible adaptive response to increased contamination levels. This trend was especially evident in the 100% concentration group (Cu3), implying that while nanoparticles may initially hinder growth, their long-term impact could be beneficial, potentially leading to growth comparable to or even exceeding that of the control group (Ninanma et al., 2023).

Similarly, copper nanoparticles influenced leaf length in maize, though the effect differed from that on plant height, indicating distinct responses across growth parameters. A dose-dependent pattern was also observed, as plants treated with a 75% concentration (Cu2) exhibited longer leaves than the control group, both initially and towards the experiment's end. The level of soil contamination also played a role in leaf length responses. For instance, in the second week, plants treated with a 35% concentration (Cu1) and grown in soil contaminated at 2.5 ESV (Cu1E2) had shorter leaves than those in soil contaminated at 1 ESV (Cu2E1). However, by the fourth week, leaf length had evened out, indicating potential adaptation to higher contamination levels.

The study further revealed that copper nanoparticles positively influenced leaf area, particularly noticeable by the fifth week, as treated plants had larger leaf areas than those in the control group. A clear dose-dependent trend was present, with higher nanoparticle concentrations generally resulting in greater leaf areas. In the second week, plants treated with a 35% concentration (Cu1) and grown in soil contaminated at 4 ESV (Cu1E3) exhibited smaller leaf areas compared to those in soil contaminated at 2.5 ESV (Cu1E2). This suggests that higher contamination levels might have inhibited the beneficial effects of copper nanoparticles on leaf area (Kasana et al., 2017) or that plant species played a role in this outcome (Song et al., 2015).

Regarding leaf count, copper nanoparticles had a minimal effect, with no significant differences between treated and control plants. The observed variations across different weeks indicate a complex interaction between nanoparticles and leaf number. Overall, the impact of copper nanoparticles on maize morphology was intricate, influenced by nanoparticle concentration, soil contamination levels, and specific plant traits.

The morphological characteristics of maize seedlings exposed to copper nanoparticles were significantly affected by both treatment and blocking effects. Factors such as plant height, leaf length, leaf width, leaf area, and sheath length were influenced by treatment, while blocking effects notably impacted plant height, leaf length, and sheath length. Despite contamination, plants exhibited compensatory growth, likely due to activated stress responses. Nanoparticles may enhance protective pigment production and regulate plant defense mechanisms (Nguyen, 2020), triggering physiological responses that sustain growth and function, including improved nutrient uptake, photosynthesis, and hormonal signaling (Nair, 2016; Khan, 2021). The inherent plasticity of maize enables strategic resource allocation, prioritizing certain growth parameters over others. Additionally, nanoparticle-induced stress might stimulate the production of growth-promoting compounds, accelerating specific traits (Nguyen et al., 2022). Genetic variability may also contribute to different levels of resilience. Understanding the compensatory mechanisms and their implications will offer valuable insights into improving crop resilience and productivity under environmental stress.

CONCLUSION

Through a comprehensive study investigating various concentrations of copper nanoparticles and soil contamination levels, we established a clear dose-dependent response in maize plants. Higher concentrations of copper nanoparticles generally enhanced tolerance to iron toxicity and boosted crop yield, highlighting the potential of this approach in mitigating the harmful effects of iron-rich soils on maize growth. Additionally, our findings emphasize the importance of considering temporal changes in plant responses to nanoparticles, as indicated by the observed variations throughout the experiment. This underscores the necessity for further research to explore the long-term effects of copper nanoparticles on plant growth and development.

Overall, our study suggests that copper nanoparticles could serve as a sustainable solution to enhance crop productivity in iron-rich soils, offering promising benefits for agricultural practices in regions affected by iron toxicity. However, further studies are needed to refine nanoparticle formulations, application methods, and dosage strategies to maximize effectiveness while minimizing potential environmental and health risks. By deepening our understanding of nanoparticle-mediated plant responses, we can contribute to the development of innovative strategies for improving agricultural sustainability and food security in iron-stressed environments.

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