

IMPACT OF NANOPARTICLES UPTAKE AND ITS ACCUMULATION IN *Vigna mungo*

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Nisha K (39230029) who carried out the project "**IMPACT OF NANOPARTICLES UPTAKE AND ITS ACCUMULATION IN *Vigna mungo***" under my supervision from September 2022 to April 2023.

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I, Nisha K (39230029) hereby declare that the Project report entitled "**IMPACT OF NANOPARTICLES UPTAKE AND ITS ACCUMULATION IN *Vigna mungo***" done by me under the guidance of Dr. V Ramesh Kumar and Dr. U Sudha at the International Research Centre, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of a Bachelor of Technology degree in biotechnology.

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ABSTRACT

The increasing population of the world requires novel techniques to feed everyone, which can replace or work along with traditional methods to increase production of agricultural crops. Plant cells are frequently challenged with a wide range of adverse environmental conditions that restrict plant growth and limit the productivity of agricultural crops. In recent times, nanotechnology is considered as a promising and emerging approach to be incorporated in agriculture to improve productivity of different crops by the administration of nanoparticles through seed treatment, foliar spray on plants, nano-fertilizers for balanced crop nutrition, nano-herbicides for effective weed control, Nano insecticides for plant protection, early detection of plant diseases and nutrient deficiencies using diagnostics kits, and nano-pheromones for effective monitoring of pests. ZnO, Fe₃O₄ and CuO nanoparticles for accessing its impact in improving germination and seedling vigor in black gram (*Vigna mungo*) is reported herein. Invigoration studies have been conducted under in vitro conditions where seeds treated with different concentrations (0,1,3, 5, 7, 15 and 20 mg/L) of ZnO, Fe₃O₄ and CuO nanoparticles prepared in distilled water and sonicator for 15 minutes are used for the treatment in *Vigna mungo* seeds to study the effect on bioavailability of seed germination and observed early seedling growth and growth characteristics of *Vigna mungo*. The experiment was carried out under greenhouse conditions. ZnO nanoparticles at 5mg/l, 7mg/l and CuO nanoparticles at 5mg/l and Fe₃O₄ nanoparticles at 5mg/l, 7mg/l has shown significant improve in seed quality of black gram. ZnO, Fe₃O₄ and CuO NPs were synthesized by wet chemical method and has been characterized by XRD, SEM, FTIR and UV spectroscopy. The shoot and root length were low when the concentration of the nanoparticles increased.

Keywords: seed germination, nanoparticles concentration, toxicity, growth characteristics

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LIST OF ABBREVIATIONS:

FTIR	Fourier Transmission Infrared Spectroscopy
QDs	Quantum Dots
SEM	Scanning Electron Microscopy
XRD	X- ray Diffraction
ZnO	Zinc oxide
NPs	Nanoparticles
PVP	Polyvinylpyrrolidone
RSG	Relative Seed germination rate
RRG	Relative Root Growth
GI	Germination Index
GR	Germination Rate
SPIONs	Superparamagnetic Iron Oxide Nanoparticles
GST	Glutathione S- transferases
SOD	Superoxide dismutases
POD	Peroxidase
CAT	Chloramphenicol acetyltransferase

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1. INTRODUCTION

Global population rate and food demand are increasing proportionally at a rapid pace. It is estimated that population may reach 9.7 billion by the year 2050, thereby increasing food demand by 70% than that of consumed today (Rakshit *et al.* 2020; Cole *et al.* 2018). Agriculture land have been losing its nutrient content due to repeated cultivation, thereby affecting a countries' economy. Plant cells are frequently challenged with a wide range of adverse environmental conditions that restrict plant growth and limit the productivity of agricultural crops (Hossain *et al.*, 2020). Agriculture is the largest interface between humans and the environment, and is a major cause of soil and ecosystem modification. Despite the extensive use of chemicals, direct yield losses caused by pathogens, animals, and weeds, are altogether responsible for losses ranging between 20 and 40% or even over 50% of global agricultural productivity (Pestovsky *et al.* 2017). The natural surroundings of plants are composed of a complex set of environmental stresses such as drought, flood, disease, abiotic stress, salinity, temperature stress, excessive water, heavy metal stress, UV stress and pesticide resistant insects have affected growth of crops, that may exhibit significant impact on crop production resulting in disturbance in food chain. Several techniques were applied such as organic fertilizers, genetically modified crops, pesticides, to tackle such problems. But the side effects of such techniques are also considered crucial. It is estimated that there can be further decline in crop production in near future due to continued reduction in agricultural land, reduction of water resources, global warming and climate changes (Arora 2019; Usman *et al.* 2020; Singh *et al.* 2021). In India, agricultural growth is decreased to 2% in 1995–2005 from 3.6% in 1985–1995 and annual food grain production per capita is also decreased to 179 kg in 2014–2017 from 207 kg in 1991–1995 (Ministry of Agriculture, Government of India, 2017–2018). (N. Singh, Bhuker, and Jeevanadam *et al.* 2021). Thus, newer developments in the agricultural sector are mandatory such as applying new technologies and continuous innovation in agriculture is highly needed.

Nanotechnology is one of the most promising and innovative tools in the current scenario and has been acknowledged as “Key Enabling Technology” by the European Commission (Parisi, Vigani *et al.* 2015). They also have higher solubility and surface reactivity than large size nanoparticles due to their large surface area. Nanotechnology

has the ability to transform the agricultural and food industry with the help of various innovative tools for the molecular management of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients (Farooqui et al. 2016). For further advancements in the era of climate change, polluted environment and global food insecurity, investigations in the area of nanotechnology and its application in the field of agronomy can serve as a key alternative to achieve the goal of sustainable agriculture. . In Nanotechnology, nanoparticles (NPs) are used, which are substances of 1– 100 nm dimension; bioactive in nature and exhibit hybrid quantum effects (Khan, Saeed, and Khan *et al* 2019; Ma *et al.* 2010). Several physiochemical properties of nanoparticles (NPs) that make them versatile such as high reactivity, high surface area, particle shape, crystallinity and variable pore size, magnetic property, antimicrobial activity, and optical, electronic, as well as high enzymatic activity, and aggregating capabilities, enable them to penetrate cellular barriers (Guo *et al.* 2014; Kaphle *et al.* 2018; Martínez-Ballesta *et al.* 2018; Prasad *et al.* 2017). Various NPs are applied in a different industry, including agriculture, coatings, consumer goods, cosmetics, chemicals, electronics, and optics, as well as for environmental remediation, food processing, fuel additives, textile manufacturing, medical applications, paints, plastics, and wastewater treatment (Salata et al. 2004; Weir et al. 2012; Keller and Lazareva et al. 2013). Recent research has shown the promising potential of nanotechnology to improve the agriculture sector by increasing the efficiency of agricultural inputs and offering solutions to agricultural and environment problems for improving food productivity and security (Usman *et al.* 2020; Fincheira *et al.* 2020). Zinc is one of the essential micronutrients for living organisms; so, the right performance of several enzymes depends on this element. This micronutrient is a regulator of phytohormones and chlorophyll synthesis, and also, it is an essential element for the carbohydrates' metabolisms in plants. Considering the relatively high solubility of ZnO-Nanoparticles and also the ability of plants to uptake and accumulate these nanoparticles in their biomass, ZnO-Nanoparticles can be used as an effective nanofertilizer for plants' growth. Zinc oxide, copper oxide, iron oxide nanoparticles synthesized using chemical method and the effect of these nanoparticles on seeds' germination, seedlings' rootlet, seedlings' plumule, and seedling's vigour index in *Vigna mungo* were investigated. For the detailed characterization of the nanoparticles, the

techniques of UV-visible spectroscopy, SEM, FTIR, XRD analysis had been made use of. Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties.

The mechanism of NPs uptake by plant roots is not clearly understood. Studies have shown that depending on the size, NPs may enter in plant cell through carrier proteins, ion channels, via fluid phase endocytosis, plasmodesmata transport, or entry may be facilitated through natural organic matter or root exudates and formation of new pores (Singh et al. 2021). The uptake and transport of NPs are size dependent, and they move actively as well as passively throughout the plant. Furthermore, it was discovered that once NPs enter different tissues, they are effective at enhancing specific morpho-physiological parameters like height and flower count, fruit yield fresh and dry weights, overall growth and biomass, early flowering, total crop yield, quantity and quality in soybean and mung bean, seed germination, and uptake of macro and micronutrients (Pnas et al. 2011; Kole et al. 2013; Ali et al. 2019; Alsaeedi et al. 2019; Sahandi et al. 2011; Sheykhbaglou et al. 2010; Dhoke et al. 2013; Feizi et al. 2012). ZnO NPs increase biomass and yield in tomato, onion, and many legumes (Jayarambabu et al. 2015), Cu oxide NPs and CeO₂ NPs improve yield and nutritional parameters in cereals and legumes (MAHMOOD et al. 2015; Rico et al. 2014) and Iron oxide NPs increase crop production, quantity, and quality of soybean (Sheykhbaglou et al. 2010). In a different investigation, Fe, Co, and Cu nanocrystalline powders increased the germination rates of soybean seeds (Noori et al. 2020). ZnO, CuO and Fe₃O₄ NPs has shown improved growth in Black gramme (*Vigna mungo* (Linn.) Hepper), mung bean (*Vigna radiata* L.) increasing shoot and root biomass, germination rate (Raja et al. 2019; Rajiv et al. 2017). The formation, size, shape, and function of the NPs are highly depends on physical and chemical methods of their synthesis. Reaction temperature, stirring rate, the ratio of gold to reducing agent are some of those constraints. For improved production and to increase macronutrient uptake in crop plants, a variety of metal oxide nanoparticles, including zinc oxide, iron oxide, and copper oxide nanoparticles, were induced in plants. NPs have different effects on different kinds and types of plants.

1.1 NUTRIENT DEFICIENCY

Widespread nutrient shortage in soils has a negative impact on soil health, lowers the nutritional value of farm products, incurring economic losses for the farmer. Large amounts of fertilizer are used to increase crop output, but because most macronutrients are not easily absorbed by plants, crops typically only utilize around half of the fertilizer applied. Additionally, because most macronutrients are not soluble in soil, plants cannot use them. The unused portions of the residual fertilizer that was applied are either leached or run off, which pollutes the soil, air, and water. Hence, applying chemical fertilizers to enhance productivity may result in short-term economic gains but has a long-term harmful effect on the agroecosystem (Chugh et al. 2021). The excessive use of chemical fertilizers damages soil health and microflora and disturbs belowground food webs, leading to genetic mutations and changes in ecosystem ecology (Rai et al. 2015). Numerous factors such as nutrient immobilization, leaching, surface run-off, the excessive use of pesticides and herbicides, soil erosion, poor soil fertility, temperature, and moisture influence the general nutritional status of the plant (Mohanty et al. 2019). The addition of macronutrients such as N, P, and K and various micronutrients is gradually hindering plant growth and development in agricultural fields worldwide (Jha et al. 2011). Hence, sustainable alternatives to chemical fertilizers need to be developed that scaffold fundamental science and apply innovation to improve the functional value of crops and their related biota for better and more efficient nutrient use, value-adding, and environmental remediation. Nanotechnology can systematically overcome the obstacle of different agricultural domains, including food security and productivity (Pradhan et al. 2017). Micronutrients and macronutrients assume critical roles in the protection of plants against disease. Using NPs, the nutritional status of plants can be enhanced to improve yields, tolerate stress, and withstand attack from pathogens (Zhao et al. 2020). This raises a need for nanotechnological interventions that are highly targeted, site-specific, and release the carrier material (nutrient) in a controlled manner to effectively address nutrient deficiency in agricultural soils.

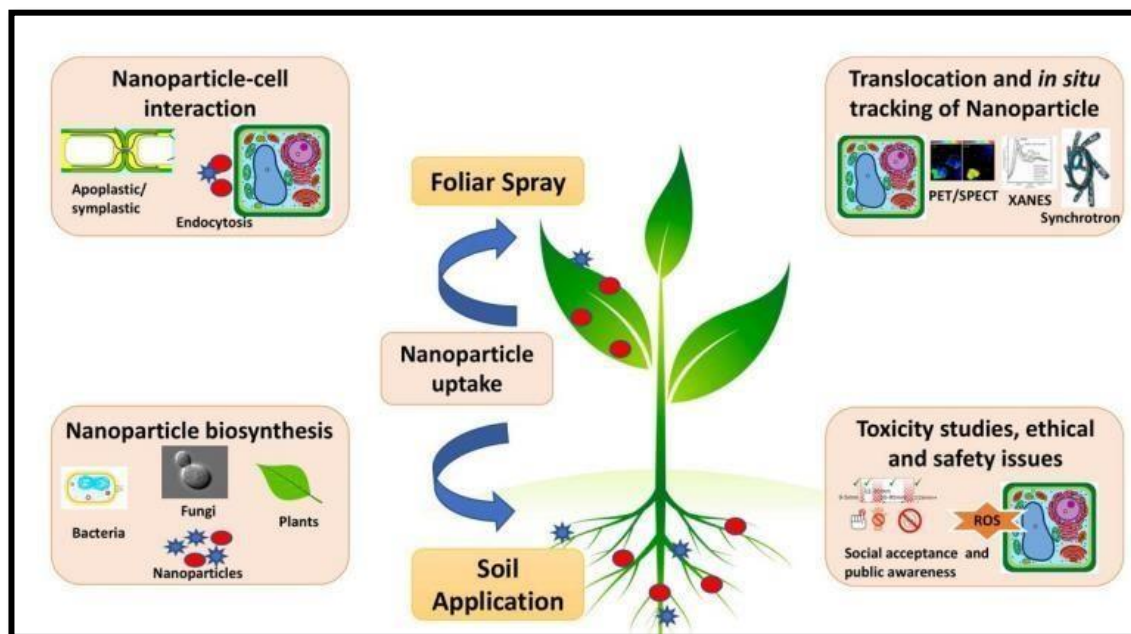


Fig 1.1: Application of Nanobiotechnology in agriculture for combating nutrient deficiencies with nanotoxicity challenges.

1.2 NANO TOXICOLOGY STUDY

Thorough and systematic physicochemical characterization of nanomaterials is essential prior to studying their toxic effects in vivo. One of the main reasons is to ensure the accuracy and reproducibility of the observed effects of toxicity with the studied nanoparticles. Toxicity of nanoparticles is significantly influenced by their physicochemical properties such as size, shape, surface charge, charge density, composition, density of structure, presence of pores, and surface activating sites. Hence documenting the characteristics of the nanoparticle under evaluation for in vivo toxicity becomes crucial in order to correlate the observed biological effects in vivo. Knowledge of nanoparticle properties is indispensable for understanding their biological behavior in a complex in vivo environment. Importantly characterization data is important to facilitate comparison of toxicity results of a given nanoparticle with other nanoconstructs. Instrumentation techniques have developed considerably in the past decade to sufficiently characterize the physicochemical properties of nanoparticles including dynamic light scattering techniques for size determination, zeta potential measurements for relative particle charge, electron (scanning and transmission) microscopy to

qualitatively analyze size and shape, nuclear magnetic resonance to accurately determine chemical structures, X-ray photoelectron spectroscopy to measure chemical composition, chemical and electronic states, mass spectrometry for mass, and elemental analysis and spectroscopy techniques that measure absorption, emission, or scattering of either wavelength or frequency. An important note of caution while characterizing nanomaterials is to evaluate these properties under physiologically relevant conditions. (Oberdörster et al. 2005; Greish et al. 2012).

1.3 NANOPARTICLE UPTAKE MECHANISM

Vigna mungo seeds were washed using 2.5% of sodium hypochloride solution for 15 min. After washing the seeds, they were rinsed in distilled water three times and were soaked in various concentrations (3, 5, 7, 15 and 20 ppm) of aqueous iron oxide, zinc oxide and copper oxide nanoparticles for soaking period of 24 hrs at laboratory condition ($30 \pm 1^\circ \text{C}$, 63% RH) in an incubator (dark condition). A thin layer of cotton was placed on each petri dish (90 mm X 15 mm), 3 ml of each nanoparticles suspensions or distilled water were added. 20 seeds were transferred to each petri dish and then it was covered with parafilm and kept for incubation at room temperature. After 3 days of incubation, the seed germination was recorded by counting the germinated seeds and the root and shoot length by measuring. Each treatment has three replicates and the mean values were recorded. Nanoparticles bind to the carrier protein via ion channels, aquaporin and endocytosis, form new pores and enter the plant cell. After nanoparticles enter the plant cell, they can be transported from one cell to another via plasmodesmata via apoplast or symplast. Uptake of the nanoparticles by the cellular systems occurs with a process known as endocytosis and is influenced by the physicochemical characteristics of nanoparticles such as size, shape and surface chemistry as well as the employed experimental conditions.

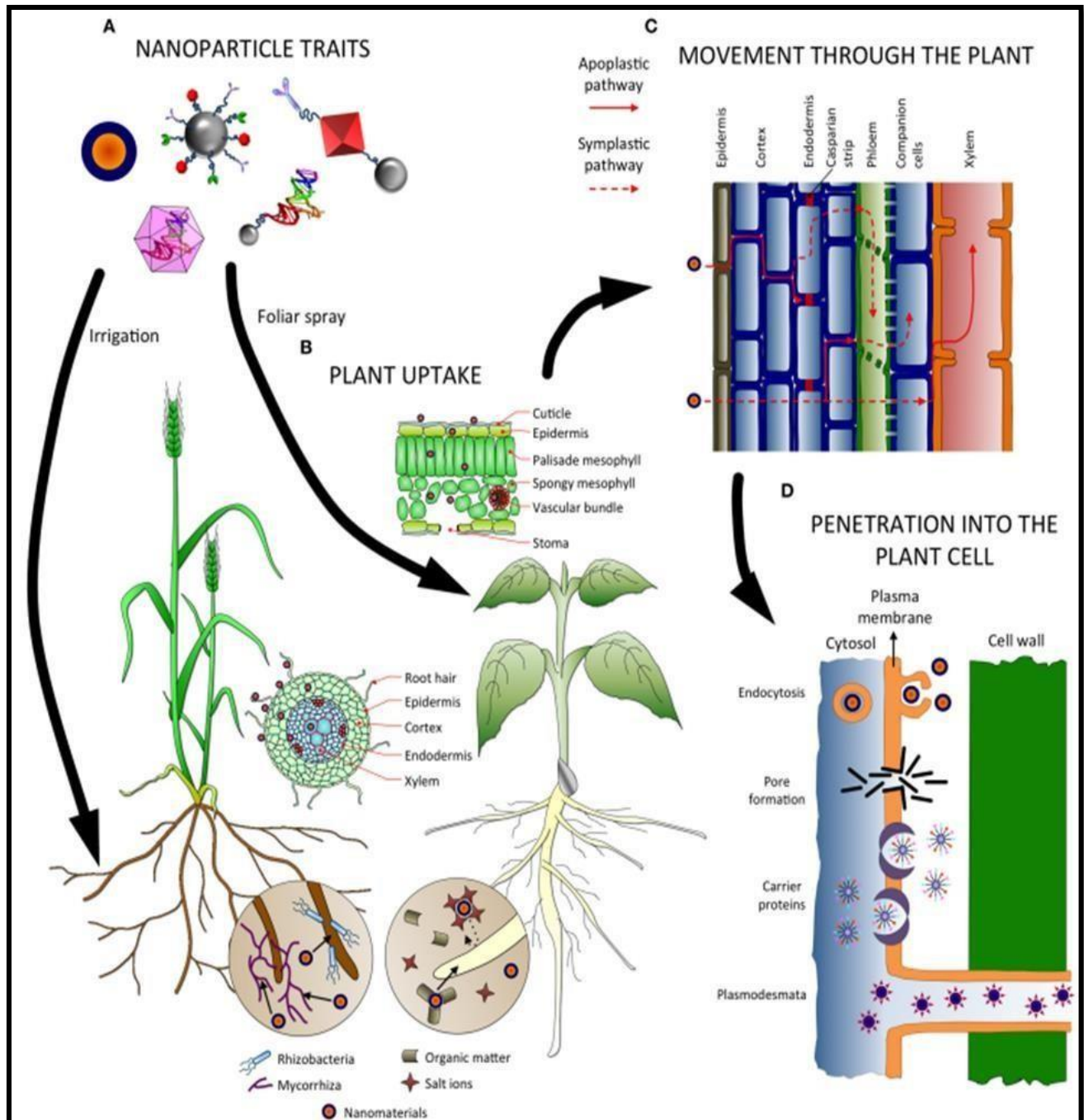


Fig1.2: Factors influencing absorption, uptake, transport and penetration of nanoparticles in plants.

(A) Nanoparticle traits affect how they are uptaken and translocated in the plant, as well as the application method. (B) In the soil, nanoparticles can interact with microorganisms and compounds, which might facilitate or hamper their absorption. Several tissues

(epidermis, endodermis...) and barriers (Casparian strip, cuticle...) must be crossed before reaching the vascular tissues, depending on the entry point (roots or leaves). (C) Nanomaterials can follow the apoplastic and/or the symplastic pathways for moving up and down the plant, and radial movement for changing from one pathway to the other. (D) Several mechanisms have been proposed for the internalization of nanoparticles inside the cells, such as endocytosis, pore formation, mediated by carrier proteins, and through plasmodesmata

1.4 INTERACTION OF METAL OXIDE NANOPARTICLES WITH DIFFERENT CROPS

Copper oxide nanoparticles (CuO NPs), one of the many forms of metallic NPs, have mostly been employed as starting materials because of their natural abundance, ease of manufacture and favorable electrical and optical properties. The maintenance of leaf hydration status, chlorophyll content, and carotenoid content by copper nanoparticles in *Zea mays* (Maize) promotes plant development and biomass (Van Nguyen *et al.* 2022). The effect of nanoparticles on seeds varies depending on their concentration. The impact of 3 and 25 mg/L concentrations of positive (PC) and negative (NC) charged iron oxide (Fe_2O_3) NPs (IONPs) on the physiology of *Arabidopsis thaliana* Plants treated with 3 mg/L did not exhibit any observable impacts on seeding or root length. However, the 25 mg/L treatment decreased the length of the roots and seedlings (positive 48% and negative 0%, respectively) (Farooqui *et al.* 2016). Without exhibiting any toxicity, superparamagnetic iron oxide nanoparticles (SPIONs) increased soybean chlorophyll levels (Mohammad H. *et al.* 2013). Studies have shown that Fe_3O_4 nanoparticles increase the activities of antioxidant enzymes (GST, SOD, POD, and CAT) in *Triticum aestivum* L. (Wheat), and *Dracocephalum moldavica* L. (Ajowan) as well as improve relative water content (RWC), leaf pigmentation, and root and shoot growth. One of the crucial elements needed for plant growth and development is zinc (Zn). Better drought-tolerant cultivar in *Solanum melongena* (Semida *et al.*, 2021) improved grain yield (22-183%), increased drought stress tolerance in *Sorghum bicolor* (Dimkpa *et al.*, 2019) and improved drought stress tolerance in *Oryza sativa* L. enhanced photosynthesis and biomass; decreased Cd accumulation (Ali *et al.*, 2019). According to studies, ZnO nanoparticles have a larger capacity for growing roots and shoots biomass yield.

2. LITERATURE SURVEY

Alabdallah et al. 2021 Green synthesized metal oxide nanoparticles mediate growth regulation and physiology of crop plants under drought stress

Metal oxide nanoparticles (MONPs) such as iron oxide, copper oxide, zinc oxide boost plant development in drought-stressed conditions, as well as their potential application in agricultural production. A significant step in the application of nanotechnology in sustainable farming will be a move from testing/using MONPs in plants to creating MONPs based on agricultural demands. Nevertheless, MONPs mobility and environmental impact should be extensively studied to ensure their safe usage in agricultural production. Size and concentration of these nanoparticles and the cultivation technique will comprehend how the MONPs increases plant productivity and drought stress tolerance.

Sathya Moorthy et al. 2021 Engineered zinc oxide quantum dots for enhancing seed germination and seedling vigour in blackgram (*Vigna mungo*).

Apart from nanoparticles, ZnO QDs are also capable of entering the seeds through pores present on the seed coat during water imbibition, induce water uptake ability of seeds, enhance enzymatic activity and free radical scavenging system, lower the oxidative damage and eventually improve seed germination, seedling length, seedling dry weight and seedling vigour.

Dimkpa et al. 2019 Zinc oxide nanoparticles alleviate drought-induced alterations in sorghum performance, nutrient acquisition, and grain fortification

ZnO nanoparticles as a soil fertilizer amendment at judicious doses can increase the resilience of cropping systems to climate change events, increase the use efficiency of N, while promoting both the yield and Zn nutrition of crops under otherwise adverse production conditions. It is clear that the management of N and other nutrients must become much more efficient; efforts such as those described in this paper will be critical to agricultural sustainability and food and nutrition security.

Ealias et al. 2017 A review on the classification, characterisation, synthesis of nanoparticles and their application

Nanotechnology is improving our everyday lives by enhancing the performance and efficiency of everyday objects. It provides a clean environment by providing safer air and water, and clean renewable energy for a sustainable future. Nanotechnology has gained a wide attention where more investment is made for the research and development by top institutions, industries and organisations. Nanotechnology has established to be an advanced field of science where extensive research is carried out to implement the technology. It is being tested for various new applications to increase the efficiency and performance of the object or process and subsequently reduce the cost so that it is accessible for everyone.

Elizabeth et al. 2017 Effect of different concentrations of iron oxide and zinc oxide nanoparticles on growth and yield of carrot (*Daucus carota* L.)

Zinc oxide and iron oxide nanoparticles has shown better results enhancing the growth of crop and improving its ability to uptake nutrients from soil. Similar studies can also be observed in *Vigna mungo*.

Faizan et al. 2021 Zinc Oxide Nanoparticles Help to Enhance Plant Growth and Alleviate Abiotic Stress: A Review

The application of ZnO-NPs is one of the most effectual options for considerable enhancement of agricultural yield globally under stressful conditions. ZnO-NPs can transform the agricultural and food industry with the help of several innovative tools in reversing oxidative stress symptoms induced by abiotic stresses. In addition, the effect of ZnO-NPs on physiological, biochemical, and antioxidative activities in various plants has also been examined properly. This review summarizes the current understanding and the future possibilities of plant-ZnO-NPs research.

Farooqui et al. 2016 Role of nanoparticles in growth and development of plants: a review.

The function of nanoparticles in agriculture aims to reduce applications of plant protection products, minimize nutrient losses and increase yields. Zinc oxide nanoparticles serve as one of the most flexible materials, due to their miscellaneous properties, functionalities, and applications. ZnO NPs have tremendous physical, optical and antimicrobial properties. Lower concentration of ZnO NPs has higher seed germination. The effect of NPs on germination depends on concentrations of NPs.

Ghorbani et al. 2015 Synthesis of ZnO nanoparticles by precipitation method.

ZnO nanoparticles were synthesized by direct precipitation method using zinc nitrate as zinc source and NaOH as precipitating agent in aqueous solution. The size range of the generated ZnO powder was estimated by SEM, TEM analysis.

Hossain et al. 2020 Nanoparticles: synthesis, morphophysiological effects, and proteomic responses of crop plants.

Nanoparticles can have both positive and negative effects on agricultural land. Morphophysiological as well as proteomic studies on NPs-induced phytotoxicity reveal that particle size, concentration, and chemistry of NPs, as well as the type of plant species used, are the key factors determining the type and magnitude of the cellular responses.

P et al. 2022 Bioefficacy of Fe₂O₃ quantum dots on enhancing seed germination and seedling vigour in black gram (*Vigna mungo*)

QDs are capable of entering the seeds through pores present on the seed coat during water imbibition, induce water uptake ability of seeds, and enhance enzymatic activity. The present study demonstrates that application of Fe₂O₃ Quantum dots to improve Black gram seed quality. At the concentration of 500 ppm, Fe₂O₃ Quantum dots improve the seed germination, seedling length, seedling dry weight and seedling vigour. When the concentration of QD increases beyond 500 ppm, it shows antagonistic effect on the seed parameters.

Jayarambabu et al. 2015 Beneficial role of zinc oxide nanoparticles on green crop production.

Zinc oxide nanoparticles penetrate easily inside the plant cell wall and causes effects on biomass and growth parameters of mung bean plant. Deposition of small size nanoparticles inside the cell wall and vacuoles causes disturbance in metabolic activity of plant. The effect can be minimizing by limiting the concentration of biological synthesized zinc oxide nanoparticles solution used in different activity. Mung bean were economically important crop plant, nanoparticles can easily find their way in human body through food chain.

Sabir et al. 2014 Biogenesis of ZnO nanoparticles for revolutionizing agriculture: A step towards anti -infection and growth promotion in plants

ZnO nanoparticles produced through biogenesis mode have been confirmed to be elevated with various physico-chemical parameters. The synthesized ZnO nanoparticles had an average crystallite size of 20– 30 nm. The synthesized ZnO nanoparticles proved to show case excellent antimicrobial activities and nanofertilizer potential, so that it could be recommended for agriculture applications at a commercial grade.

Marchiol et al. 2012 Synthesis of metal nanoparticles in living plants.

Nanotechnology could provide nano-fertilizers able to synchronize the release of nutrients with their crop uptake, thus avoiding nutrient losses and reducing the risks of groundwater pollution. Moreover, the nano-fertilizers should release the nutrients on-demand while preventing them from prematurely converting into chemical/gaseous forms that cannot be absorbed by plants. This can be achieved by preventing nutrients from interacting with soil, water and microorganisms, and releasing nutrients only when they can be directly internalized by the plant.

Kantabathini et al. 2018 The effect of Zinc Oxide nanoparticles (ZnO NPs) on *Vigna mungo* L. Seedling growth and antioxidant activity

Vigna mungo seeds treated with different concentrations of ZnO NPs showed decreased root growth and increased germination index, shoot and leaf growth. There was a significant change in Glutathione reductase, Guaiacol peroxidase and Catalase activity and ascorbic acid and hydrogen peroxide of *Vigna mungo* exposed to ZnO NPs. Aggregated nanoparticles penetration into the intracellular region of the seed was observed. A complete study on the toxic effects of ZnO NPs can help significantly in the safe disposal of ENPs for the reduction of adverse effects in both environmental and agricultural systems.

Pestovsky et al. 2017 The use of nanoparticles and nanoformulations in agriculture.

Nanoparticles have great potential to improve the agricultural productivity. Toxicity for humans must also be taken into account because in many cases the nanoparticles cannot be fully removed from human-consumed agricultural products.

Priya et al. 2020 TiO₂ nanoparticles can enhance germination and seedling growth of mung bean (*Vigna radiata* L.).

The uptake rate depends on the size and the surface properties of the nanoparticles. Nanoparticles could enter the xylem via the cortex and the central cylinder and may accumulate in the vacuole. The increase in seed germination along with seedling growth in specific concentration of nanoparticles suggested optimum dose limit on growth of seedlings.

Priyanka et al. 2019 Role of engineered zinc and copper oxide nanoparticles in promoting plant growth and yield: present status and future prospects.

Choosing a method for synthesizing Metallic nanoparticles is important that provides most compatible and preferably simple economical procedure to fabricate extremely fine-sized nanoparticles in order to synthesize metal oxide NPs in connection for a safer mode on living things. Crop plants can utilize nutrient-containing NPs as essential mineral nutrient sources through roots or leaves, thereby maintaining or enhancing their metabolism, antipathogen capacity, growth, and yield. Some of these NPs lead to better performance than associated conventional fertilizers.

Raja et al. 2018 Biogenic ZnO and Cu nanoparticles to improve seed germination quality in blackgram (*Vigna mungo*)

ZnO and Cu nanoparticles have significant impact in improving seed quality of black-gram (*Vigna mungo*). Seeds of *Vigna mungo* were soaked with different concentration of nanoparticles. And results were observed after 2-3 months.

Rajput et al. 2018 Effects of copper nanoparticles (CuO NPs) on crop plants: a mini review.

The toxicity of NPs depends on plant species, growth conditions, exposure time, concentration, type, and size of NPs. It is well noted that the Cu ions in small concentrations are able to stimulate the plant growth and play the role as microelement.

Rani, P et al. 2020. Impact of green synthesized metal oxide nanoparticles on seed germination and seedling growth of *Vigna radiata* (Mung Bean) and *Cajanus cajan* (Red Gram).

Similar to this paper, comparative analysis can also be made among copper oxide, iron oxide and zinc oxide nanoparticles, observing which nanoparticle has shown better results considering different parameters such as root and shoot length, biomass, antioxidant activity.

Rhaman et al. 2022 Seed priming with nanoparticles: An emerging technique for improving plant growth, development, and abiotic stress tolerance.

The excess usage of chemical stimulants are responsible for the reduction of crop yield and destruction of soil fertility and the ecosystem. Seed priming with NPs is a promising tool that can be employed to promote successful crop production. The literature showed that the use of NPs-priming has the latent to change the conventional agriculture based on the practice of agro-chemicals into a more maintainable agriculture, as these systems can improve plant growth and mitigate abiotic stressors, consequently, ensuring in increments of productivity and quality of foods.

Shnoudeh et al. 2019 Synthesis, characterization, and applications of metal nanoparticles.

Using their metallic characteristics scientists were able to synthesize metal NPs using top-down and bottom-up approaches including physical, chemical, and biological methods. Biological methods were found to be the most environmentally friendly and least toxic methods. Different analytical methods can be used to analyze and characterize the synthesized NPs, including, size, shape, the surface of the NP, functional groups, size distribution, aggregation, dispersion, structure, binding hydrodynamics, and confirmation to have enough information to find the proper application for these NPs or proper functionalization. The main methods that can be used to determine the characteristics of NPs have been explained and summarized; they include optical, spectroscopic, and

microscopic techniques such as UV spectroscopy, FTIR, SEM, ESEM, TEM, XRD, FCS, SERS, TERS, ZP, CD, MS, STM, DLS, and AFM.

Pal et al. 2019 Green synthesis, characterization and applications of nanoparticles.

Synthesized metal oxide nanoparticles were characterized using UV spectroscopy, SEM, FTIR and XRD. The peak range observed for zinc oxide nanoparticles ranges between 350-380nm and for copper oxide and iron oxide nanoparticles the observed range between 220-280nm.

N. Singh et al. 2021 Effects of metal nanoparticle-mediated treatment on seed quality parameters of different crops

It is concluded that the toxicity of the nanoparticles toward the environment as well as crops depends on the type, chemical composition, size, density, method of preparation, and application of nanoparticles. Further, the type and age of the crop as well as the duration of treatment of a specific nanoparticle toward a particular crop can also play a crucial role in their toxicity.

Sturikova et al. 2018 Zinc, zinc nanoparticles and plants.

Research should focus on plant mechanisms capable of controlling and mitigating the toxic effects of zinc nanoparticles. In addition, we should avoid the use of too high concentrations of zinc nanoparticles during the experiments of toxicity. Plants require low concentrations of zinc (and other micronutrients) for proper growth and development, so the effects of high concentrations cannot be considered representative.

Van Nguyen et al. 2022 Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions.

Copper oxide NPS positively affects plant biomass under both normal and drought conditions. Additionally, application of Copper oxide NPS retains leaf water status, as well as chlorophyll and carotenoid content under drought stress conditions. Copper oxide NPS priming has a major role in controlling ROS level in maize in response to water stress through increasing both ROS-scavenging enzyme activity and antioxidants.

Velsankar et al. 2022 Green synthesis and characterization of CuO using *Panicum sumatrense* grains extract for biological applications.

The plant growth analysis explained the efficient growth of *Vigna mungo* plant at 60 ppm concentration of CuO nanoparticles. As per the results, the synthesized CuO nanoparticles can be applied as a biocompatible, cost-effective insecticides, drugs and fertilizers.

Verma et al. 2020 Effects of Nanoparticles on Seed Germination, Growth, Phytotoxicity and Crop Improvement.

Various types of metallic NPs released in our natural environment have a great impact on plant species. Therefore, nanotechnology is becoming very essential today to further help promote ultra-modern farming systems with low environmental impact. Synthesized NPs were used in a wide variety of applications for crop growth and higher yield. It regenerates and accumulates in plant roots, stems, leaves and other parts of the plant and promotes plant growth, respiration, transpiration, biomass production, yield improvement and phytotoxicity. NPs have produced both positive and negative effects on plant growth and development, which depends on the size and shape of NPs.

Z. Khan et al. 2019 Impact of nanoparticles on abiotic stress responses in plants: an overview

It is essential to gain basic knowledge about the interaction of NPs with plants at various levels to exploit the best properties of NPs. NPs show their impact at very low concentrations, and the effect on plants is type and dose dependent. The scientific community has a major concern to overcome abiotic-stress-induced loss of crop productivity, and several NPs are being studied to assess their potential role in protecting plants from abiotic stresses, plant development, crop production, and modulating the various plant processes. Additionally, to prevent adverse effects of NPs on the environment, proper understanding of their role is essential: to reduce negative impacts of NPs such as ZnO NPs on the environment and crops, it is vital to engineer their properties and understand plant NP interactions better before their practical application in the field.

3. AIM AND SCOPE

- To study morphological and anatomical behavior of *Vigna mungo* before and after treatment of it with different concentrations of nanoparticles.
- To study toxicity of nanoparticles.
- To prepare zinc oxide, iron oxide and copper oxide nanoparticles by using chemical route method.
- To determine the size, optimum concentration of each nanoparticle to incubate *Vigna mungo* with nanoparticles.
- To check the stability of nanoparticles.

4. MATERIALS AND METHODS

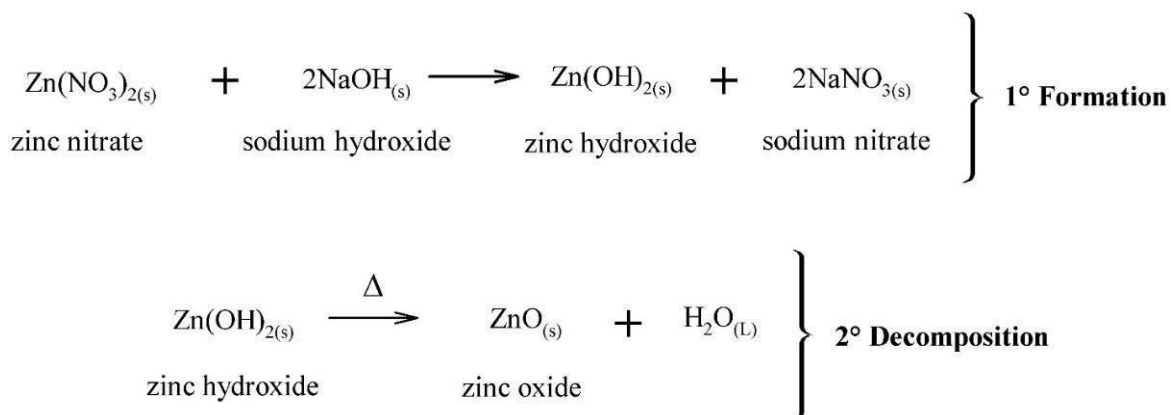
All chemicals used for the study were analytical grade purchased from Sigma-Aldrich himedia, Merck, India.

4.1. SYNTHESIS OF NANOPARTICLES

Synthesis of nanoparticles were done by chemical reduction method.

4.1.1 Zinc oxide nanoparticle

In this experiment 0.1 M zinc nitrate was dissolved in 100ml distilled water stirring on magnetic stirrer with heating at 70°C and 0.8 M Sodium hydroxide was dissolved in 50ml distilled water with stirring. Then Sodium hydroxide was added drop wise to the zinc nitrate solution with heating. A milky white solution was obtained and stirring continued for an hour. The white precipitate formed was centrifuged (5000rpm for 10 minutes) 3 times with distilled water and final wash with ethanol to remove any impurities in this solution (Ghorbani et al. 2015).



4.1.2 copper oxide nanoparticles

0.1M copper sulphate was dissolved in 50ml distilled water and was kept for stirring till CuSO₄ is completely dissolved. 0.5g PVP (polyvinylpyrrolidone) a stabilizing agent was added to solution. 0.1M NaBH₄ was dissolved in 20ml distilled water and was added dropwise till the solution color changes to black, till pH reaches to 6-7 (Dang et al. 2011). The precipitate formed was centrifuged (5000rpm for 10minutes) 3 times with distilled water and final wash with ethanol to remove any impurities in this solution.

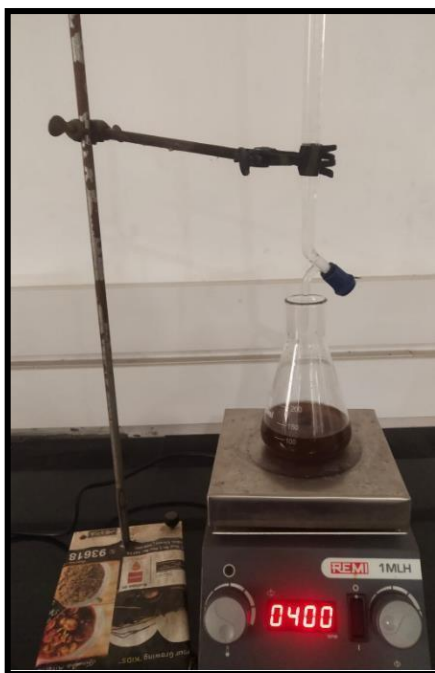


Fig 4.1: copper oxide synthesis

4.1.3 Iron oxide nanoparticles

Fe_3O_4 is prepared by adding a base to an aqueous mixture of Fe^{2+} and Fe^{3+} chloride at a 1:2 molar ratio. 250ml of 0.1M FeSO_4 was added to 250ml of 0.2M FeCl_3 in 1000ml conical flask and was kept for stirring at 70°C . 50ml of 1M NaOH was prepared and added dropwise to solution till the black precipitate was formed. The precipitate formed was centrifuged (5000rpm for 10minutes) 3 times with distilled water and final wash with ethanol to remove any impurities in this solution.



4.2 CALCINATION AND BALL MILLING

The obtained precipitate samples were dried overnight at room temperature and white powder was collected in ceramic crucible. The crucible placed in the furnace and heat-treated at 500°C for 3hrs. The calcined ZnO , CuO , Fe_3O_4 nanoparticles were ball milled to produce fine powder by reducing the size of nanoparticles.

4.3 CHARACTERIZATION OF METAL OXIDE NANOPARTICLE

4.3.1 UV- Visible Spectrometry

This method consists of a deuterium or tungsten lamp for the ultraviolet and visible region wavelengths, respectively, sample and reference beams, a detector, and a monochromator. Exposing the sample to UV light will give the UV spectrum. Cuvettes are used for sample holding and are kept inside the instrument for introducing samples to the light path. Glass, plastic, silica, or quartz cells can be used as cuvettes. Plastic and glass cuvettes absorb wavelengths below 310nm, so they cannot be used for absorbance studies below that wavelength. Therefore, quartz cuvettes are used for absorption measurements in the ultraviolet range as they are transparent to the wavelengths above 180 nm.

4.3.2 XRD

X-ray diffraction is used most often to identify crystalline phases by comparison to a known database, measure atomic spacings (with the use of appropriate standards), measure structural properties of the crystalline phases present (e.g., preferred orientation and particle size), and to probe atomic arrangements in amorphous materials. The identification of crystalline materials is often aided by chemical analysis in order to identify the elements present. The mass fractions of the phases present in a sample can be determined by comparison to known standards. Since XRD is relatively quick and is usually a nondestructive technique, it is often one of the first methods employed in the characterization of nanoparticles. The electron diffraction pattern is similar to an X-ray diffraction pattern (discussed below) in that diffraction occurs when the Bragg condition is satisfied. The Bragg equation ($n\lambda = 2d \sin \theta$) relates the wavelength (λ) of the electrons, the angle (θ) of the electron beam with respect to a set of diffracting planes, and the spacing (d) between those planes, which is determined by the crystal structure of the material examined. One challenge when working with electron diffraction is that electrons are often multiply scattered by the sample, which can complicate the interpretation of electron diffraction patterns (Burleson *et al.* 2014).

4.3.3 SEM

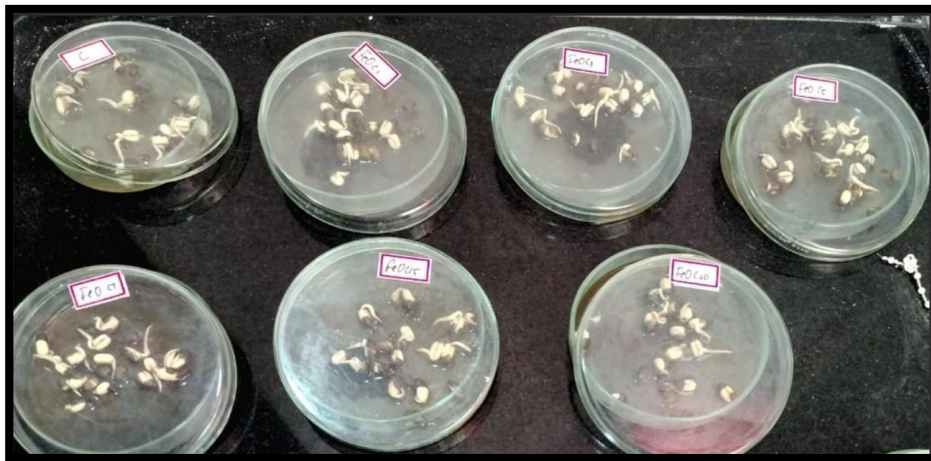
In scanning electron microscopy (SEM), an electron beam is directed toward the specimen instead of a light beam, as in the case of an optical microscope. A highly concentrated electron beam is shot from an electron gun located at the device's top. The two main electron gun types are field emission guns, which generate a strong electric field that rips electrons from the atom, and thermionic guns, in which the filament is heated until the electrons stream away. The SEM scans the surface of the sample with high-energy electron beams. Thus, SEM differs from conventional light microscopes as they uses light waves to create a magnified image. In SEM, when the electron beam strikes the specimen surface, it interacts with the surface (Titus *et al.* 2018).

4.4 SEEDLING EXPOSURE

The seeds were checked for their viability by suspending them in double distilled water. The seeds which are settled to the bottom were selected for further study. The seeds were rinsed in double distilled water thrice and then surface sterilization of seeds was done. The seeds were sonicated for 3hr in prepared nanoparticle suspensions (3, 5, 7, 15 and 20mg/l) using a sonicator instrument. The soaked seeds were put in prepared pots. Observe of the growth is studied.



(a)



(b)

Fig 4.2: seeds soaked in different concentrations of nanoparticles

4.5 SEED GERMINATION TEST

This test was conducted on black gram seeds. The seed germination rate (RSG) and relative root growth (RRG) were calculated using the equation and germination index (GI) (Jayarambabu et al. 2014).

$$\text{Relative seed germination Rate} = (SC \setminus SS) \times 100$$

$$\text{Relative root growth} = (RS \setminus RC) \times 100$$

$$\text{Germination index} = (RSG \setminus RRG) \times 100$$

Where SS is the number of seed germinated in sample, SC is the number seed germinated in control, RS is the average root length in sample and RC is the average root length in control

4.6 ROOT AND SHOOT LENGTH

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transission zone up

to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale. (Parisi et al. 2015)



(a)



(b)

Fig 4.3: shoot growth after 7 days (a) 7mg/l (b) different shoot length

4.7 POTTING SEEDS

Nanoparticle treated seeds were transferred to pots. Each pot were labelled with oxides of nanoparticles and their concentration. *Vigna mungo* seeds were allowed to grow for 60 days and morphological changes were noted.



Fig 4.4: germinated seeds were transferred to pot

5. RESULT AND DISCUSSION

5.1 CHARACTERIZATION

5.1.1 UV spectrophotometer

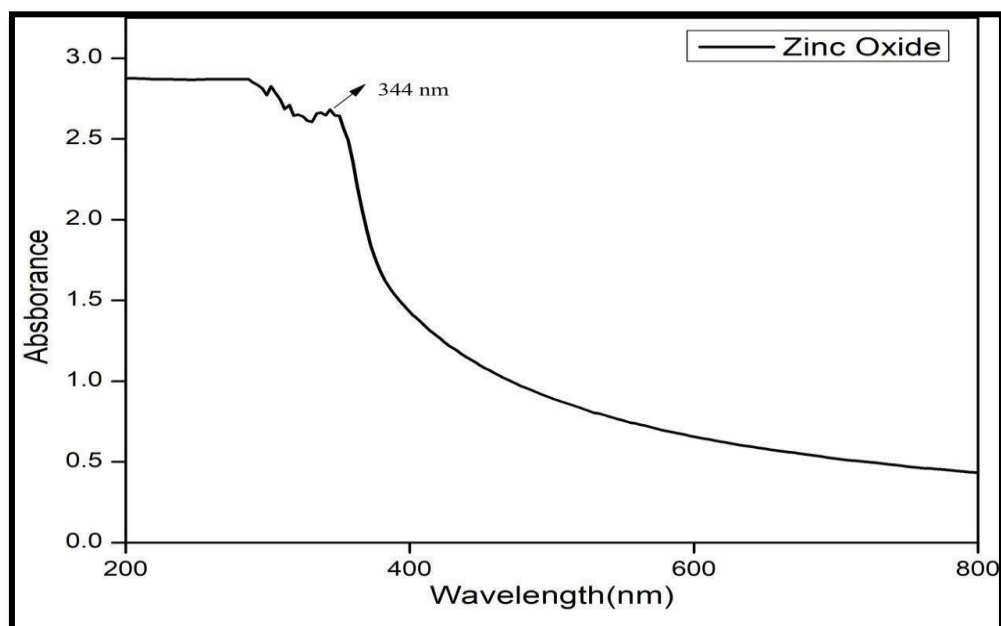


Fig 5.1: UV – vis of ZnO nanoparticle

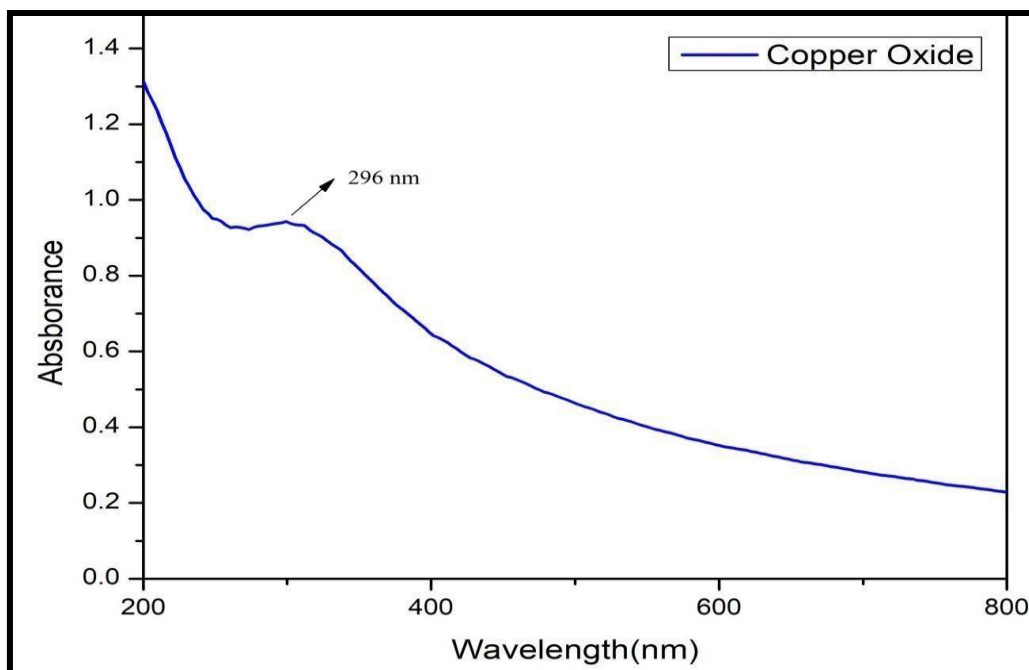


Fig 5.2: UV – vis of CuO nanoparticle

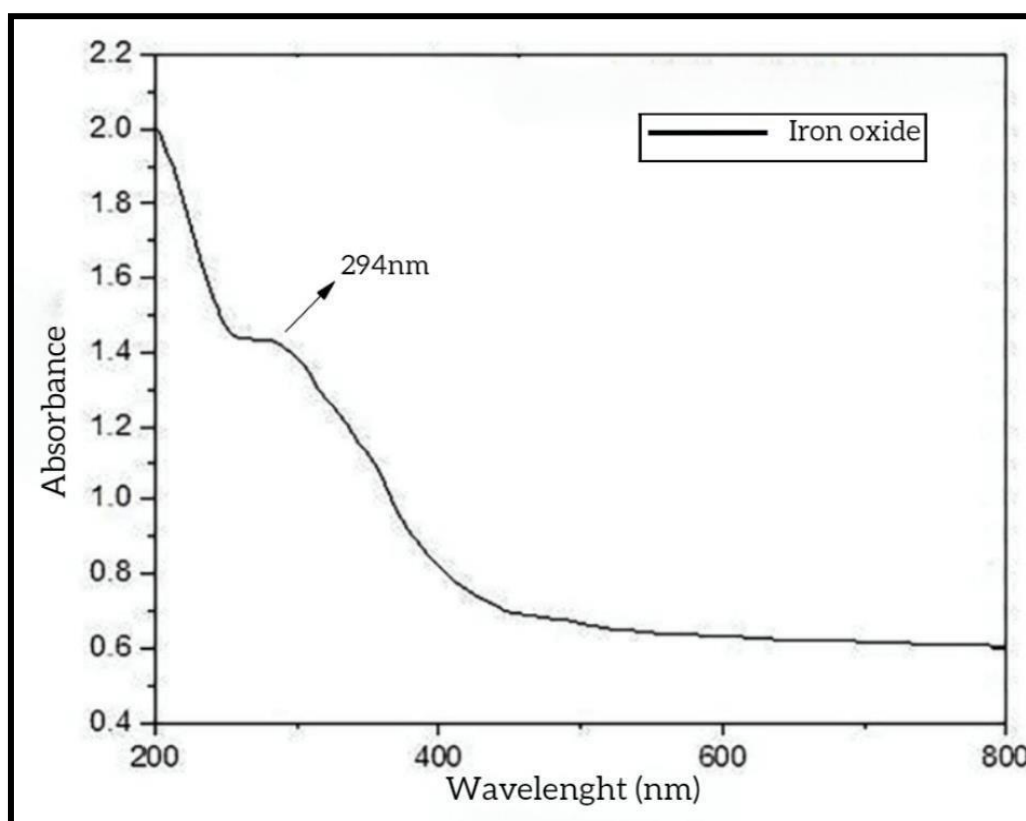


Fig 5.3: UV – vis of Fe_3O_4 nanoparticle

NPs with sizes between 2 and 100 nm are often characterized using wavelengths between 300 and 800 nm. Optical properties of ZnO, CuO and Fe₃O₄ nanoparticles were characterized based on UV absorption spectroscopy. The UV–vis absorption curves of ZnO, CuO and Fe₃O₄ nanoparticles calcination at 500° C for 3hrs presented and absorption spectra showed a broad band emission and a relatively narrow absorption band at 344nm, 296nm and 294nm respectively because of their surface plasmon resonance.

5.1.2 SEM analysis

SEM images of the ZnO nanoparticle prepared by chemical method. The SEM analysis has showed agglomeration of the particles and in the range of 10 µm. It is seen that ZnO nano-particles formed have an irregular planar structure with no distinct morphology.

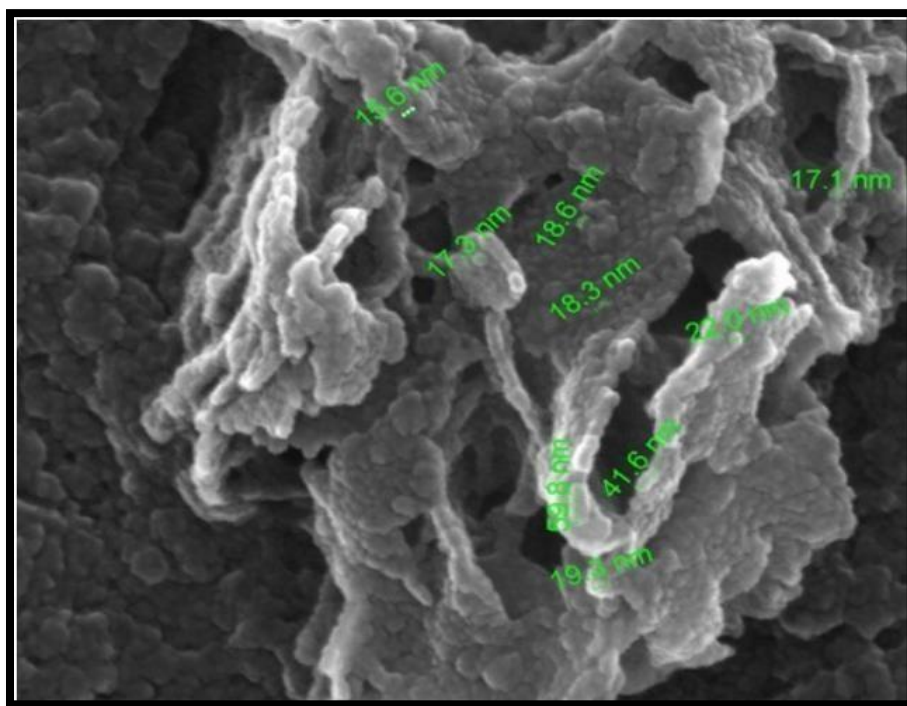


Fig 5.4: SEM image of ZnO nanoparticles

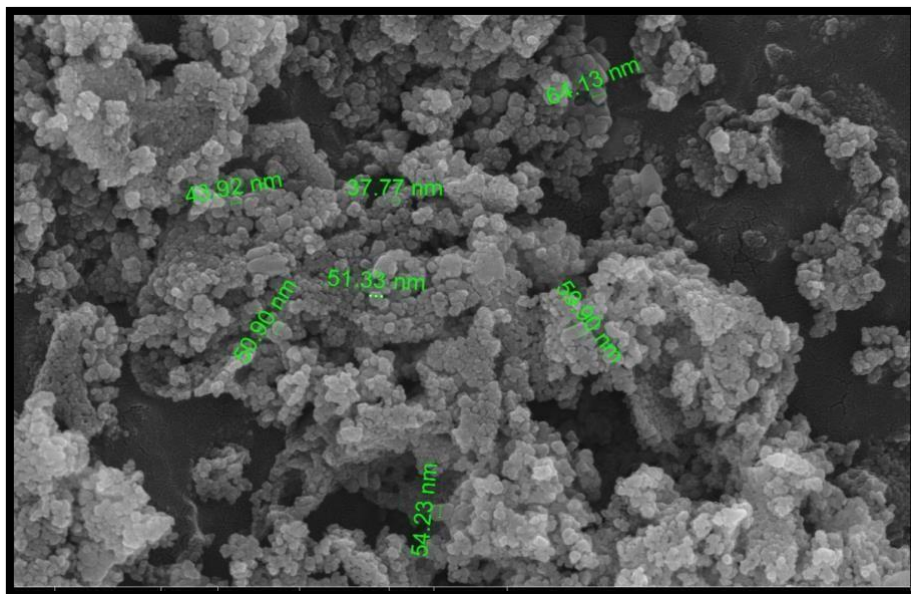


Fig 5.5: SEM image of CuO nanoparticles

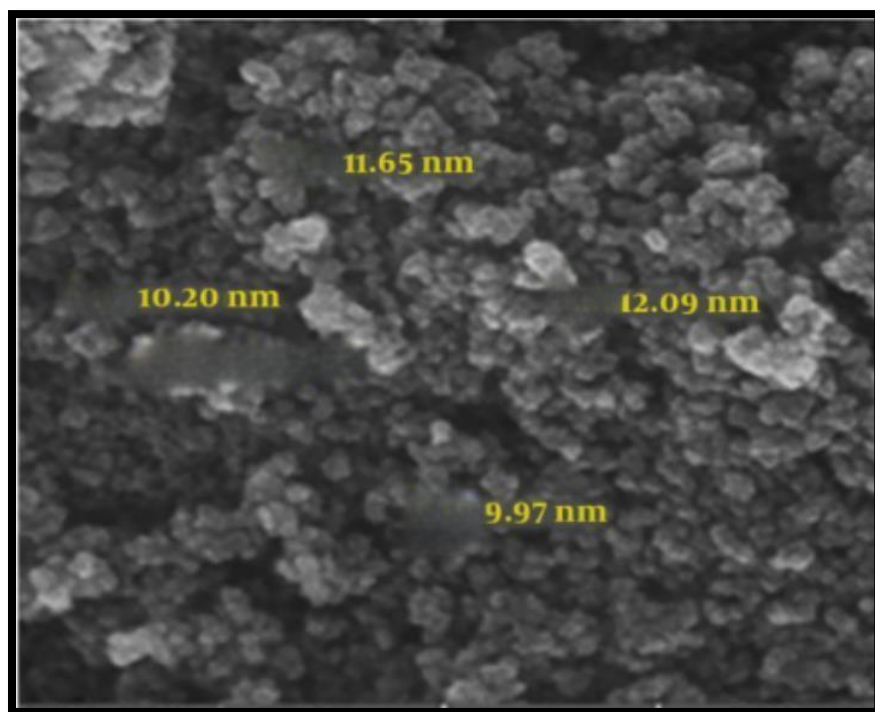


Fig 5.6: SEM image of Fe₃O₄ nanoparticles

5.1.3 XRD ANALYSIS

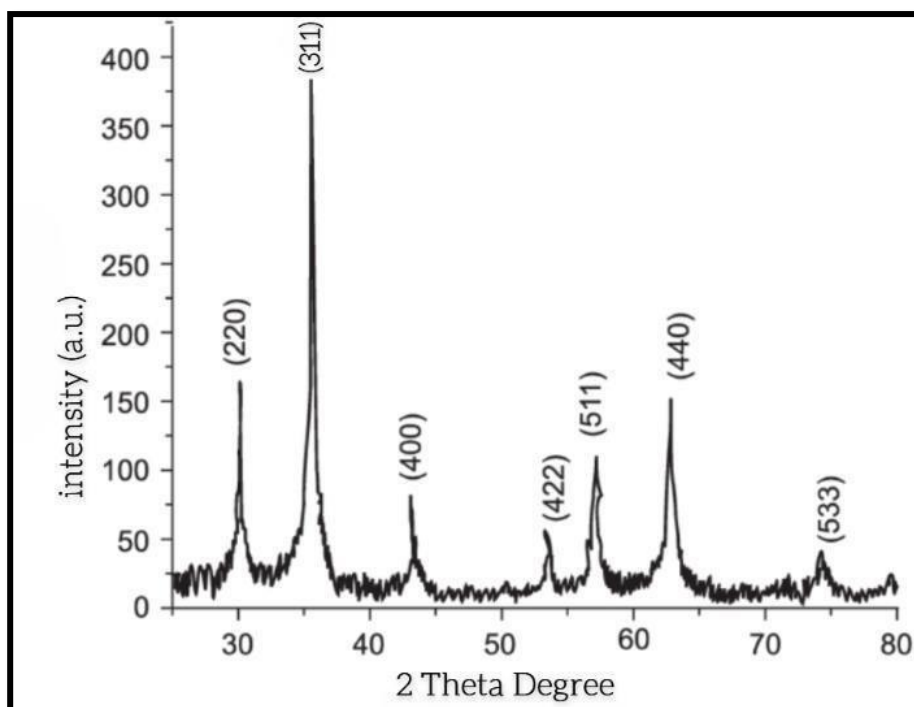


Fig 5.7: XRD Pattern of ZnO nanoparticles

Figure shows the X-ray diffraction pattern for sample calcinated at 500°C. Study of standard data JCPDS 76- 0704 confirmed that the synthesized material has a hexagonal ZnO phase. The pattern was indexed with hexagonal unit cell and the lattice parameters are given. The peak and relative intensities obtained for the ZnO match with the literature values (Jayarambabu, N., Kumari, B. S., Rao, K. V., & Prabhu 2014). The peaks at scattering angles of 31.64°, 36.85°, 43.65°, 57.1°, 63.12°, 63.2°, 75.9° which corresponds to (220), (311), (400), (422), (511), (440) and (533) crystal plane respectively.

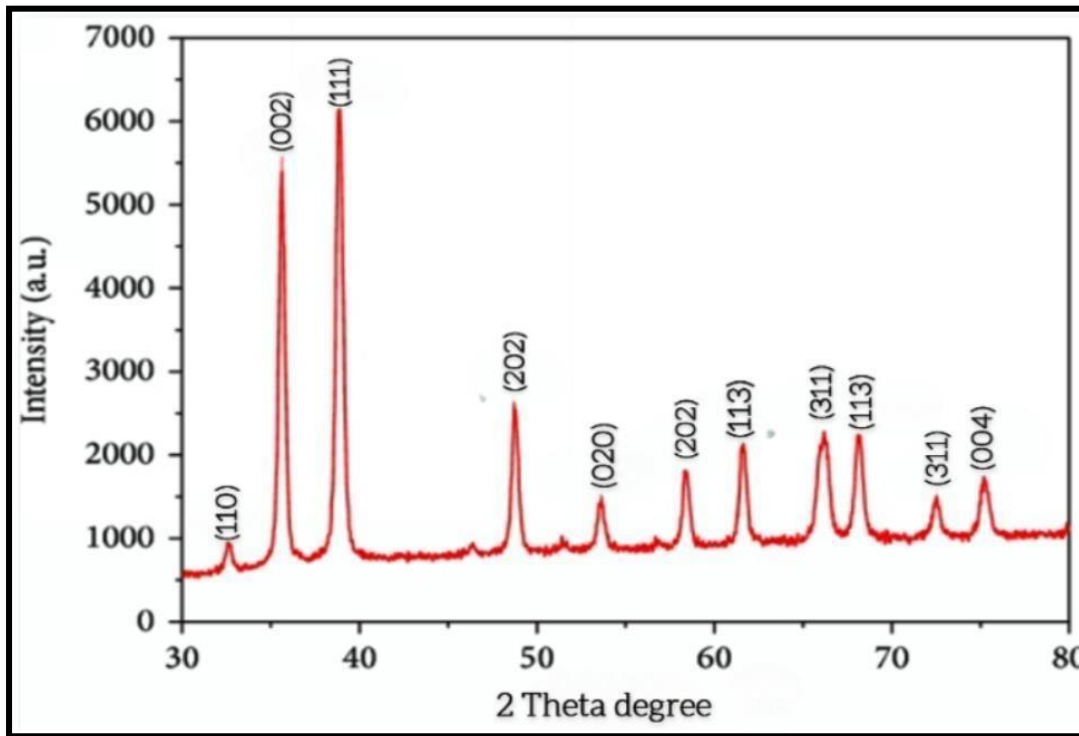


Fig 5.8: XRD Pattern of CuO nanoparticles

Figure shows the X-ray diffraction pattern for sample calcinated at 500°C. Study of standard data JCPDS 76- 0704 confirmed that the synthesized material has a hexagonal CuO phase. The pattern was indexed with hexagonal unit cell and the lattice parameters are given. The peak and relative intensities obtained for the ZnO match with the literature values (Jayarambabu, N., Kumari, B. S., Rao, K. V., & Prabhu 2014). The peaks at scattering angles of 31.64°, 36.85°, 43.65°, 57.1°, 63.12°, 63.2°, 75.9° which corresponds to (220), (311), (400), (422), (511), (440) and (533) crystal plane respectively.

5.2 ASSESSING THE EFFECT OF ZINC OXIDE, COPPER OXIDE AND IRON OXIDE NANOPARTICLES ON SEED GERMINATION, SHOOT AND ROOT DEVELOPMENT IN *Vigna mungo*

Table 5.1: Measurement (in cm) growth characteristics of black gram at different concentration of ZnO NPs (within 3 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	2	1	1	1	0.2	0.5
2	1	0.5	1	1	1	1
3	3	2	0.5	1	0.8	0.2
4	1.5	3	0.7	2		
5	1.8	2.5	2	2.5		
6	2.8	1	1	3		
7	1					
Average length	1.8	1.6	1.03	1.75	0.6	0.56

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.2: Measurement (in cm) growth characteristics of black gram at different concentration of CuO NPs (within 3 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	2	1	1	1	1	0.5
2	1	2.8	1	1	0.2	1.2
3	1.5	3	2.5	2.5	0.5	0.3
4	2.8	2	1.5	2	2	0.8
5	3	0.2	2	2.5		
6	1.5	0.1		3		
7						
Average length	1.9	1.5	1.6	2	0.9	0.7

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.3: Measurement (in cm) growth characteristics of black gram at different concentration of Fe₃O₄ NPs (within 3 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	3	2	1	1	1	0.5
2	1	2.8	2.5	1.5	0.4	1.2
3	0.5	2	1	2.5	0.5	0.6
4	2	2.5	0.5	3	2	0.8
5	3	3	2.5	2.5		
6	2.5	0.1		3		
7						
Average length	2	2.06	1.5	2.25	0.97	0.77

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.4: Measurement (in cm) growth characteristics of black gram at different concentration of ZnO NPs (within 5 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	11	10	5.5	8	11	4.5
2	10.5	7	6.5	10	3	3
3	12.5	4	4.5	6	4.5	7.5
4	8	6.5	5	4	5.5	5
5	7.5	3.5	8.5	8.5	4	6
6	6.8	5.5	1.5	5	8.5	7.5
7	9	7	3.2	1	8	0.5
8	5	7.3	4	5	6	0.8
9	6.5	7.6	4.7	2.5	5.5	1
10	3.5	4	2.5	11	2	2.5
11	1.5	8	6.5	3.5	7	3.5
12	3.5	9	10	6.5	9	5

13	6	5	1.5	7	6	0.8
14	4.5	3.5	1.5	5.4	4.5	
15	1	3	5.5	1.3		
16	13.5			3		
17						
Average length	7.1	5.71	5.05	5.48	5.03	3.66

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.5: Measurement (in cm) growth characteristics of black gram at different concentration of CuO NPs (within 5 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	7	5.5	10	3.5	9	4.5
2	2.2	6	4	6	5	3
3	3.5	2	4.5	6.5	4	4
4	5	7.8	8	4	2	1.5
5	1.5	5.5	7.5	4.2	4.5	3
6	1.5	6	2	4.4	5.5	3.5
7	1.8	5.8	1	5	4.5	5
8	2	8.8	2	7	2.5	2
9	7	6.5	6	7.8	2	1
10	3	1.2	1	5	1.5	1.2
11	3	2.8	3	2	3	2
12	1	5	.5	5.5	3.2	4
13	6.5	5.5	5.3	3	5	1.5
14	10	4.3	5.4	6	6.8	
15	8.5	1	2	5	4	
16		11.5		2	2	
17		11				

Average length	4.4	4.8	4.3	4.9	4.01	2.7
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C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.6: Measurement (in cm) growth characteristics of black gram at different concentration of Fe₃O₄ NPs (within 5 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	11	10	13.5	7.5	1.7	1
2	9.5	10.5	12	10	2.8	1
3	3	9.2	8	11.3	5.8	0.5
4	4.5	8.8	7	10.7	4	2
5	5	4	4.4	7.8	2	3.5
6	7.8	2	5	3.3	0.4	5.5
7	2.5	3.2	1	2	5.6	4
8	1.8	4.1	1	1	2	3.8
9	9.5	1	2	0.5	3.1	4.4
10	11.5	1.5	3	3	3	6
11	12	3	8	2.5	1	2.1
12	4	2.8	7	5	2.1	3
13	4.5	5.5	6.5	4.3	4	2
14	3.5	4.5	1	2.8	3.7	
15	2	3	2			
16	2	3	2.5			
17	2.5		1			
Average length	5.6	4.7	5.2	5.12	2.95	2.77

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.7: Measurement (in cm) growth characteristics of black gram at different concentration of ZnO NPs (within 8 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	8	8.3	1.5	3	9	5.5
2	10.5	9	2	13	8	1
3	10	1	2.3	9.5	11	1
4	13	4	1.5	11	11.5	2.2
5	11	9.5	3	14	10	3.2
6	15.5	11.2	4	3.7	7	3.5
7	5	12	2.5	8.3	7.8	10.5
8	9.5	11.5	9	2	6	3
9	5.5	11	7	7	5	4.5
10	11.5	12	12.2	11.5	5.8	7
11	2	12.6	9	7.5	11	3
12	5	8.5	8.8	9.5	5.3	1
13	10	10.8	11	14.5	4	0.5
14	10.5	15	8	9		
15	13	8	7.8	1		
16	10	5	10			
17	5.5	5.4	6			
18	6	4.5				
Average length	8.36	8.1	6.6	6.8	6.53	3.8

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.8: Measurement (in cm) growth characteristics of black gram at different concentration of CuO NPs (within 8 days)

No. of seeds	C1	C3	C5	C7	C15	C20

1	7	1	2	7	4	1
2	1	5	4	4	5.5	5
3	11	16.5	1.5	9	7.5	7
4	5	14.5	2.5	9.5	9.6	6
5	8	8	7.5	7	2	5.5
6	9.5	4	7.5	7.5	7.5	2
7	14	10	5	9.5	13.5	7.2
8	10	9	9.5	5	8.5	8
9	3.5	8	7	10	8.8	2
10	13.2	8	8.5	6.5	4	1
11	11	10.5	5.5	10	2	2.7
12	6	2	11.5	9	2.8	
13	10.5	11.5	14	5	4	
14	9.5	3	8.5	6	10	
15	6.5	2.5	11.2	1	4.5	
16	2	1	3	2	1	
17	2.8	1				
Average length	8.5	6.2	6.6	6.5	5.3	4.3

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.9: Measurement (in cm) growth characteristics of black gram at different concentration of Fe₃O₄ NPs (within 8 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	8.8	7.5	6.5	5.5	2.3	2.3
2	12	7.9	7	4.3	4	4
3	14.5	6	7.5	2.1	4.5	5.5
4	17	6.4	11	1	7	6
5	2	5.5	12.5	11.5	5.5	7

6	4.4	2	10.5	10	4	1.1
7	5	1	7	9.8	4.5	2.1
8	5.5	1.8	6	7.7	5.5	3.8
9	7	8.4	4.4	5.4	2	3.4
10	8.5	9.6	3	4.3	1	5.6
11	10.5	11	2.5	4.2	2	2.3
12	11	12.5	1.5	5.7	1	4.1
13	12	10.5	3.5	8.9	3.5	1
14	9.4	5.5	2.5	4.1	3	1.5
15	3.7	7.5	1	7.7		
16	6.6	7				
17	6.4	6				
18	3	5				
19	5.6					
Average length	8.04	6.7	5.76	5.76	3.5	3.4

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.10: Germination rate at day -3

Nanoparticle/ concentration	C1	C3	C5	C7	C15	C20
Zinc oxide	35%	30%	30%	20%	15%	15%
Iron oxide	35%	35%	30%	35%	30%	25%
copper oxide	30%	30%	25%	25%	20%	20%

Table 5.11: Germination rate at day -6

Nanoparticle/ concentration	C1	C3	C5	C7	C15	C20
Zinc oxide	85%	80%	75%	75%	70%	60%
Iron oxide	85%	80%	80%	75%	70%	70%

copper oxide	75%	85%	75%	80%	75%	60%
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Table 5.12: Germination rate at day -8

Nanoparticle/ concentration	C1	C3	C5	C7	C15	C20
Zinc oxide	90%	85%	80%	75%	75%	50%
Iron oxide	95%	90%	80%	75%	70%	70%
copper oxide	85%	85%	80%	80%	75%	65%

Table 5.13: Average root length (cm)

Nanoparticle/ concentration	C1	C3	C5	C7	C15	C20
Zinc oxide	4.75	3.01	3.4	4.1	3.76	2.8
Iron oxide	3.1	3.7	3.1	2.56	2.5	2.8
copper oxide	5.12	4.8	4.6	4.89	3.1	2.8

The highest percentage of seeds was germinated 90% at 1mg/l concentration of synthesized zinc oxide nanoparticles, 95% at 1mg/l of synthesized iron oxide nanoparticles and 85% at 3mg/l of synthesized copper oxide nanoparticles. The shoot and root length were low when the concentration of the nanoparticles increased. The effects of various concentrations of each nanoparticle on seed germination was assessed and concluded that the lowest concentration of zinc, iron and copper oxide nanoparticles was enhancing the seed germination, shoot and root formation (Hatami *et al.* 2014).

5.3 GERMINATION RATE OF POTTED SEEDS



Fig 5.9: growth of vigna mungo after 30days

Table 5.14: Maximum germination rate of *Vigna mungo*

Nanoparticle	Concentration	Germination rate
Control	0	70%
ZnO	5mg/l	80%
CuO	5mg/l	95%
Fe ₃ O ₄	7mg/l	90%

Comparing to control the seed germination rate was higher in seeds treated with copper oxide nanoparticles at concentration of 5mg/l.

$$\text{CuO} > \text{Fe}_3\text{O}_4 > \text{ZnO}$$



(a)



(b)



(c)



(d)

Fig 5.10: germination of *Vigna mungo* in different concentrations of nanoparticles (a) control (b) Fe_3O_4 5mg/l (c) ZnO 5mg/l (d) CuO 7mg/l

6. CONCLUSION

Zinc oxide, iron oxide and copper oxides were synthesized by chemical reduction method and was characterized by UV Vis spectroscopy, FTIR, SEM and XRD. The different concentrations (1,3,5,7,15,20mg/l) of each nanoparticles effect on black gram seed germination and the length of root and shoot were studied. Nanoparticles of metal oxide are quickly transported through the plant and included in the metabolic processes. It was observed that in black gram seeds germination 5mg/l concentration of ZnO, 7mg/l of Fe₃O₄, and 5mg/l of CuO suspension solution shown good shoot, root growth results compare other concentrations and control in this experiment. The nanoparticle up taken seeds were place in pot and let it grow for 1-2 months. Maximum growth was found in 5mg/l and 7mg/l concentrations. The shoot and root length were low when the concentration of the nanoparticles increased. ZnO, Fe₃O₄ and CuO nanoparticles have significant impact in improving seed quality of black-gram (*Vigna mungo*).

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8. ANNEXURE

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1 message

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