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# Biosynthesis of CuO and MgO Nanoparticles using *Cucumis sativus* (Cucumber) Fruit Extract and their effects on the Seeds Germination in *Arachis hypogaea*.

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***Abstract:*** *The objective of the present study were to synthesize copper oxide and magnesium oxide nanoparticles, using aqueous fruit extracts of Cucumis sativus as a reducing agent with copper nitrate and magnesium nitrate serving as a precursors. The synthesized nanoparticles were characterized using Uv-vis, FTIR, XRD, and SEM techniques. The UV-Vis spectral of CuO-NPs displayed an absorption peak at 228 nm while MgO-NPs showed a specific absorption at 266.7 nm which is in the range of 260-280 nm. X-ray diffraction analysis identified the CuONPs possess monoclinic structure while MgO-NPs possess highly crystalline structure. Fourier transform Infrared analysis identified some biomolecules and functional groups in the aqueous fruit extract of Cucumis sativus as responsible for the reducing copper nitrate and magnesium nitrate to CuO and MgO nanoparticles respectfully. After the characterization, the effect of CuO and MgO nanoparticles on germination of Arachis hypogaea seeds was investigated. The parameters such germination percentage, root length and shoot length were analyzed and the result compared. The result indicated that germination percentage in CuO-NPs treated seed of Arachis hypogaea decreases with increase in concentration of nanoparticles while in MgO-NPs treatment showed increase in germination percentage with increase in concentration. In root length, CuO-NPs decreases root length and MgO nanoparticles increases root length. Both CuONPs and MgO nanoparticles shoot length with increase in concentration of nanoparticles.*

**Keywords:** *Cucumis sativus, Arachis hypogaea,* CuO nanoparticles, MgO nanoparticles and germination percentage.

## 1. Introduction

The agricultural sector is dealing with enormous challenges such as rapid climatic changes, a decrease in soil fertility, macro and micronutrient deficiency, overuse of chemical fertilizers and pesticides in the soil. However, the global population increase has subsequently escalated food demand (Bose, 2021). By 2050, the projected global population is estimated to be 9 billion. With the projected increase in population, there will have to be great changes in the agricultural world. To supply the world with healthy nutrition, the production of agriculture and food will have to increase by about 60% to accommodate for the increased demand. With conventional farming practices, nutrients are striped from the soil and supplemented with chemical fertilizers that are harmful to the environment (Vassel, 2019). The green revolution resulted in increased production of agriculture products worldwide, but sustainable food and agriculture is still a challenge especially in developing countries which lack advanced technology and mechanized agriculture. Today's agriculture needs less/minimum resources with maximum production like water-efficient crops and drought-tolerant crops. Moreover, different technologies have been in practice nowadays for improvement of agriculture such as biotechnology, nanotechnology and especially molecular biology with genome editing are latest in this list (Conway, 2019). Today, nanotechnology is working as technological advancement to solve problems related to food security and agriculture. Nanotechnology is providing efficient alternatives to increase the crop production by managing the insect/pests in agriculture in an eco-friendly manner. It also promotes plant efficiency to absorb nutrients (Ghouri, *et al*. 2020). Nanotechnology plays an important role in the agriculture sector and its use in agriculturally based products can monitor the growth of plants and can also detect diseases. Nanomaterials in agriculture have been used to replace conventional plant protection methods. In addition, nanotechnology has also been applied to increase yield in plants by controlled release and reduced loss of nutrients (Burhan *et al*. 2019).

To meet the food demands of the growing population the agriculture sector is being pressurized to assure food security. Hence chemical fertilizers are being considered as an inevitable source of plant nutrition for improving the crop production. This lead to a notion in farmers that using higher quantities of chemical fertilizers gains higher crop yields. However, only less than half of the amount of applied fertilizers will be utilized by the crop whereas the remaining amount of fertilizer which is intended to be taken up by plant may get lost through leaching, become fixed in soil or contribute to water pollution which is even worse. According to recent statistical reports it has been observed that the key macronutrient elements Nitrogen, Phosphorous and Potassium applied to the soil are lost at a rate of 40-70%, 80-90% and 50-90%, respectively, thus causing a considerable loss of applied resources.

Additionally, farmers tend to apply fertilizers repeatedly in order to achieve yields at desired levels, this overdose of chemicals counter acts and lead to decreased soil fertility and increased salt concentrations thereby causing future crop losses. Furthermore, irregular use of fertilizers without control on nutrient release patterns causes deterioration of product quality. Therefore, developing slow or controlled release fertilizers plays a crucial role not only in enhancing the crop production, productivity and quality, but also helps towards up gradation of sustainability in agricultural production. Given the unique properties of nanomaterial as such as high surface-to-volume ratio, controlled-release kinetics of nutrients to the targeted sites and sorption capacity, nanotechnology has a high importance for designing and using of new fertilizers. Nanofertilizers are nutrients encapsulated/coated with different types of nanomaterials for the control and slow delivery of one or more nutrients in order to satisfy the imperative nutrient requirements of plants. These "smart fertilizers" are now being regarded as a promising alternative, to an extent that they are to be considered as preferred form of fertilizers over the conventional ones in several cases (Meghana, *et al* 2021).

Plant emergence is significantly based on seed germination, so, the time from sowing to the emergence of seedling has substantial value in crop production. For a crop subjected to salt stress, one of the most significant steps is seed germination. In soil with a high concentration of salt in the planting zone, seed germination fails. Increased germination can lead to strong early growth and better plant establishment (Ghouri, *et al*. 2020). Good quality of seed is required for maintaining optimum plant stand which ultimately increases grain yield. Ensuring a good germination is one of the key steps to ensure proper plant stand and moisture assurance during seed germination may help to speed up the germination. The tiny size of nanoparticles may help in entry of water into seed without disturbing their internal structure (Rawat, *et al.* 2018). Water molecules are an important factor for seed germination, a process by which a plant grows which originates from the radical and plumule. The NPs present in the plant growth media and controls the water imbalance by the seed coat and thus affect the germination of seeds (Verma, *et al*. 2020).

Accordingly, several nanotechnology applications have been developed and tested as potential agrochemicals such as bactericides, fungicides, growth regulators and fertilizers. Nanofertilizers are defined as materials in the nanometer scale, usually in the form of nanoparticles, containing macro and micronutrients that are delivered to crops in a controlled mode. According to the type of formulation, nanofertilizers are classified into three categories: 1) nanoscale fertilizer, which corresponds to the conventional fertilizer reduced in size typically in the form of nanoparticles; 2) nanoscale additive fertilizer, is a traditional fertilizer containing a supplement nanomaterial; and 3) nanoscale coating fertilizer, refers to nutrients encapsulated by nanofilms or intercalated into nanoscale pores of a host material. Encapsulated nutrients by films or held in nanopores within a carrier material such as clays have been used to form nanocomposite structures for controlling the nutrient release. Nanotechnology applications in agriculture appear to be a promising approach, fostering the transformation of conventional production systems into upgraded agricultural practices with a clear emphasis on the development of more efficient and environmentally friendly methodologies. Nanofertilizers could be a crucial development in the protection of the environment because they can be applied in smaller quantities compared to traditional fertilizers, hence reducing leaching, runoff, and gas emissions to the atmosphere. At present, uncertainty exists about the production costs of nanofertilizers compared to conventional fertilizers, as well as the magnitude of the possible disruption in the existing conventional fertilizer industry (Mejias, *et al*. 2021). Application of nanoparticles in the field of agriculture can increase crop production. It can also decrease mineral loss and can lessen the fertilizer use (Awan, *et al*. 2020).

Nanoparticles (NPs) have diverse properties due to the nanoscale composition of atomic structures. This property makes them attractive for pharmaceutical, energy, electronics, cosmetic, textile, and agriculture industries (Zafar, *et al*. 2020).

Among the available large number of nanoparticles, metal oxide nanoparticles are considered to be more promising as they exhibit unique physical, chemical, and biological properties. In the synthesis process of nanoparticles, the use of toxic chemicals for the reduction and as a capping agent leads to various side effects and toxicity. As a result, the synthesis of metal oxide nanoparticles through plant extracts has gained importance. This approach is ecofriendly and has a higher reaction rate compared to conventional chemicals. Plant extracts contain various active biomolecules that help in the reduction and stability of nanoparticles (Demissie, *et al*. 2020). The synthesis, characterization, and application of metal oxide nanoparticles are very essential because the properties of nanomaterials can be used as optical, electronic, catalytic, and magnetic. The properties of nanomaterials are affected by the shape, size, and chemical surroundings (Fatoni, *et al* 2021). Different physical and chemical methods are used for the synthesis of metal oxide nanoparticles; however, the conventionally used methods such as sol-gel, chemical reduction, and hydrothermal are costly methods and non-eco-friendly by producing toxic chemicals as end products (Amin, *et al*. 2021). Biological methods of synthesis of NPs using microorganisms such as algae, fungi, bacteria, and plant leaves extracts have been suggested as possible ecofriendly alternatives to chemical methods as these methods are of low cost, energy efficient, and nontoxic. Use of plant extracts for synthesis of NPs could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Furthermore, the advantage of using plants for the synthesis of NPs is that they are easily available, safe to handle, and possess a broad variability of metabolites that may aid in reduction (Andualem, *et al*. 2020).

In plants, copper (Cu) is a micronutrient necessary for the protein components of enzymes. Photosynthetic electron transport, mitochondrial respiration (OMR), oxidative stress response (OSR), cell wall metabolism (CM), and hormone signaling all rely on Cu as a structural metal. In addition, Cu is an essential transition element involved in many physiological activities (Ibrahim, *et al*.

2022) Copper atoms or ions are generally known to be toxic to plants and pose a threat to both agriculture and the environment. Some sources of contamination from copper are a result of industrial, urban, and agricultural wastes in the form of agrochemicals. Copper is readily available in soils, and the bioavailability can be measured by the free 2+ 14 Cu ions. Most of the copper in soil is not free, more than 98% of Cu is bound to soluble organic matter, thus, the concentration of Cu in the soil is usually extremely low. Because Cu has a high affinity for organic matter, it is not easily leached from the soil. As a result, it accumulates in the surface soil. Although Cu is one of the essential micronutrients, if there is high exposure, it tends to inhibit the plant growth, specifically in the roots and morphology (external structure). Due to the tendency of Cu to accumulate in the roots, roots are usually affected more strongly by copper compared to shoots (Vassel, *et al*. 2019).

Magnesium plays a key role in manipulating important biological polyphosphate compounds like ATP, DNA and RNA. It is actively involved in photosynthesis as a component of chlorophyll with significant role in plant respiration and energy metabolism. It is also an essential nutrient for photophosphorylation, such as ATP formation in chloroplasts, carbon dioxide ($CO_2$) fixation, protein synthesis, phloem loading, partitioning and utilization of photo assimilates, generation of reactive oxygen species, and photo-oxidation in leaf tissues (Shinde et al. 2018). MgO nanoparticles have interesting applications in microelectronics, diagnostics, and biomolecular detection (Ali, et al 2020). Magnesium oxide (MgO) nanoparticles are currently popular among researchers, due to their exclusive biological, electronical and thermal properties. This has led to the search of various synthesis approaches to yield smaller sized MgO nanoparticles with distinct morphologies. Chemical approaches such as sol-gel, hydrothermal, co-precipitation and solvothermal methods are under extensive research to yield smaller sized nanoparticles (Jeevanandam, *et al*. 2020). Magnesium oxide (MgO) is an attractive and basic metal oxide material. It's generally used as a catalyst, electrochemical biosensor, and pharmaceutical industry and paints. The highly crystalline MgO nanoparticles exhibit low electrical conductivity and higher thermal stability. Conventionally, MgO nanoparticles are synthesized by different methods such as, hydrothermal, sol gel, chemical gas phase deposition and wet precipitation methods. MgO nanoparticles produced from conventional methods are toxic and not used in medical application. In other hand green synthesized nanoparticles are very efficient and nontoxic (Palanisamy and Pazhanivel 2017). Magnesium Oxide is an interesting basic oxide that has many applications in catalysis, adsorption and in synthesis of refractory ceramics. It is a unique solid of high ionic character, simple stoichiometry and crystal structure and it can also be prepared widely in variable particle sizes and shapes. It has been reported that the shape and size of nanocrystalline magnesium oxide particles have high specific surface and reactivity because of the high concentration of edge/ corner sites and structural defects on their surface (Varghese and Vishal 2018).

There are a number of reports on the preparation of MgONPs via plant-mediated route. Galal *et al* 2022, synthesized MgO-NPs using Hyoscyamus muticus leaf extract, Kaur, *et al*. 2022, compared chemo-bio synthesized MgO NPs on the Maize seed germination, they observed green synthesize MgO nanoparticles shown the good shoot, root growth results compare to the chemical and the control during this experiment.

Nowadays, green method using plants extract is being widely utilized for the synthesis metals and metal oxide nanoparticles as it is simple, cheap and ecofriendly. The objective of this work is to synthesize Cu and Mg oxides nanoparticles using aqueous fruit extract of cucumber and compare their effects on germination and seedling growth in peanuts (Arachis hypogaea)

## 2. Materials and Methods

Fruits of *Cucumis sativus* (Cucumber) and *Arachis hypogaea* (Peanut). Copper (ii) nitrate trihydrate (Cu(NO)2.3H2O) and Magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$) are of analytical grade, 3.5% sodium hypochlorite and double distilled water were used in all the experiments. X-ray diffractometer (XRD) Goniometer miniflex 300/600 and Scanning electron microscopy

### 2.1. Sample collection

*Cucumis sativus* (Cucumber) fruits and Seeds of *Arachis hypogea*e (groundnut) were all purchased from Talata Mafara local market and it was identified by the Botanist of the Department of Agricultural Technology, College of Agriculture and Technology, Bakura. Zamfara state Nigeria.

### 2.2. **Preparation of *Cucumis sativus* extract.**

*Cucumis sativus* (cucumber) fruit was thoroughly washed several times with distilled water to remove dirt and particles. The *Cucumis sativus* was chopped into pieces and pulverized using Sony blender. About 100ml of distilled water was added to the paste and heated for 10min. The mixture was then cooled at room temperature then filtered with Whatmann's No 1 filter paper and stored in refrigerator for further experiment. The extract served as reducing and stabilizing agents for the synthesis of the different nanoparticles.

### 2.3. Synthesis of CuO Nanoparticles.

The synthesis of CuO nanoparticles was in accordance with the reported method by Sharma *et al* (2016) with minor modification. About 30g of copper (II) nitrate trihydrate precursor salt were added to 100ml of *Cucumis sativus* fruit extract. The mixture was stirred continuously using magnetic stirrer at $80^0C$ until the colour changes from deep green to black precipitate was observed. The precipitate was then centrifuged at 1000rpm for 30min. and then washed three times with distilled water to removed impurities and then centrifuged again for 20min. Finally the precipitate was dried in an oven at $80^0C$ for 4 hrs then grounded to a fine powder using Pestle and Mortars. The powder was then calcinated at $300^0C$ for 3hrs in muffle furnace. The synthesized CuO nanoparticles were then kept for characterization and then for germination studies.

### 2.4. Synthesis of MgO Nanoparticles

Magnesium oxide nanoparticles were prepared in accordance to the method of Prasanth *et al* (2019) with minor modification. About 20g of magnesium nitrate were added to 50ml of aqueous solution of *Cucumis sativus* in 250ml beaker with continuous stirring using a magnetic stirrer for 2hrs at $80^0C$ until colour change from colourless to deep brown was observed confirming the formation of magnesium oxide nanoparticles. The solution was then centrifuged at 1000rpm for 30 min. the precipitate formed was washed three times with distilled water. The precipitate was dried in an oven for 3hrs at $150^0C$. The powder was then calcined at $300^0C$ for 4hrs in a muffle furnace. The synthesized MgO nanoparticles were then kept for characterization and then for germination studies.

### 2.5. Characterization of the Nanoparticles

All the synthesized CuO and MgO nanoparticles were characterized by different techniques. The functional groups on the surface of nanoparticles were determined by Fourier-transform infrared spectroscopy (FT-IR) by using KBr pellet method recorded using Agilent Technologies Cary630 in the range of 4000—400$cm^{-1}$ with resolution of 4cm-1. The UV/Vis absorption spectrum of the two nanoparticles was recorded using Shimadzu UV/Vis spectrophotometer 1650. The Phase identification was performed by the X-ray diffraction (XRD) technique, using an X—Ray Diffractometer (XRD) Goniometer miniflex 300/600, using CuKa as a radiation source with a wavelengths of 1.5406A. The XRD technique was performed in the 2θ range of 30-70 to examine the crystalline structure and phase of the nanoparticles and the morphology and particle size was recorded by scanning electron microscope. (SEM) using phenon: model NO: 721.20000.00.0478.

### 2.6. Seeds germination test

The seed of *Arachis hypogaea* were soaked in 3.5% sodium hypochlorite solution (hypo, without further preparation) for 10min to ensure surface sterility, and then washed three times with distilled water. Different concentration of the biosynthesized nanoparticles were suspended in distilled water to make (Afrayeem and Chaurasia 2017) 0.00, 0.25, 050, 0.75 and1.00g/L followed by sonication for 30min for uniform dispersion. The seeds of *Arachis hypogaea* were then soaked in the different nanoparticles for 2hrs. A total of 20 seeds were then planted on sterilized loamy soil by autoclaving in polythene bags. The seeds were irrigated with 10ml of distilled water (Khalaki *et-al*. 2016) under labouratory conditions. Each treatment was replicated three times. Seed germination was observed on the $5^{th}$ day after planting, and the germinated seeds were counted and recorded. On the $10^{th}$ day, the experiment was halted and the number of germinated seeds was counted, root and shoot length of the seedlings were measured using ruler.

### 2.7. Germination percentage (GP)

The germination percent (GP) was calculated using the equation (1):

GP = Xi/N x100………………………………….. (1).

Where Xi is the total number of germinated seeds on the $10^{th}$day, and N is total number of seeds planted.

### 2.8. Root and shoot length

The root and shoot length of the germinated seeds were measured in centimeter using ruler. The shoot length was taken from the base of the seed to the tip of the shoot, while the root length was measured from the point of root emergence from the seed to the root tip.

### 2.9. Statistical analysis

For statistical analysis each treatment were conducted in triplicates, and the results were presented as standard deviation (SD) of the mean using Minitab 17 software.

## 3.0. RESULTS AND DISCUSSION.

### 3.1. UV/VIS Analysis

The UV/Vis spectra of biosynthesized CuO, and MgO, nanoparticles are shown in Fig. 1a**,** and b. The synthesized CuO nanoparticles displayed an absorption peak at 228nm due surface plasmon absorption of copper oxide particles. The result is in closed agreement with study of (Nasrollazadeh *et al* 2015 and Sukumar *et al* 2020) all reported 250nm.

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The formation of MgO nanoparticles was confirmed by UV/Vis spectrum as indicated in figure 1b with specific absorption at 266.7 nm which is specific for MgO nanoparticles which is in the range of 260-280 nm. The UV/Vis result indicates that the biomolecules in the leaf extract of *Cucumis sativus* were reduced resulting to the formation of MgO nanoparticles (Essien *et al*. 2020, Abdulla *et al*. 2019 and Moorthy *et al*. 2015) reported 260 nm, 250 nm and 273.5 nm respectively.

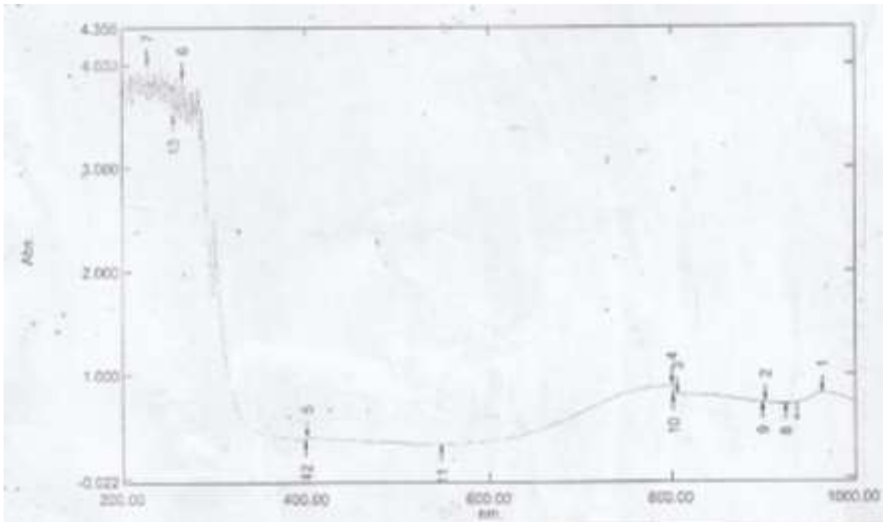


**Fig. 1a:** UV/VIS of spectrum of CuO Nano Particles

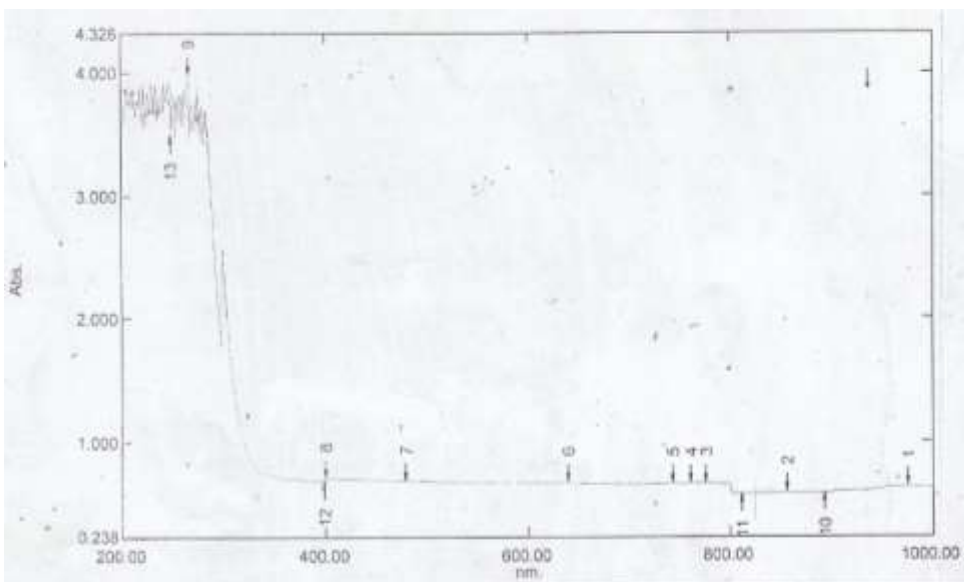


**Fig. 1b:** UV/VIS spectrum of MgO Nano Particles

### 3.2. FT-IR Analysis

#### 3.2.1. Copper oxides (CuO) nanoparticles.

Fourier transform infrared (FTIR) spectroscopy is based on the vibration of chemical bonds in a molecule at characteristic frequencies depending on the elements in the molecule and types of bonds. Figure 2a shows the spectrum of CuO nanoparticles in the range of 650-4000 $cm^{-1}$. The peak at 1079 $cm^{-1}$ represent C—O stretching of ether. The band at 961 $cm^{-1}$ represent C—C stretching of alkane. The Cu—O stretching vibration is represented at 697 $cm^{-1}$ (Anwaar et al 2016, Sukumar *et al*. 2020 and Shi *et al*. 2016).

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### 3.2.2. Magnesium Oxide (MgO) nanoparticles.

The FTIR spectrum of MgO nanoparticles is shown in figure 2b. There were 5 main bands in the FTIR profle of the MgO nanoparticles synthesized in this study. These characteristic bands of FTIR can be assigned to various biologically active functional groups. The peaks are at 3613.7 $cm^{-1}$, 1709 $cm^{-1}$, 1517 $cm^{-1}$, 1330 $cm^{-1}$ and 1000.8 $cm^{-1}$. The absorption peak at 1709 $cm^{-1}$ is due to C=C stretching of ketone. The peak at 1517 $cm^{-1}$ is for amide in protein (Mohammadian *et al* 2018). The peak at 1330 $cm^{-1}$ is assigned to Mg—O vibration (Essien *et al*. 2019 reported 1382 $cm^{-1}$) and the peak at 1000.8 $cm^{-1}$ is associated with C—C stretching in alkane

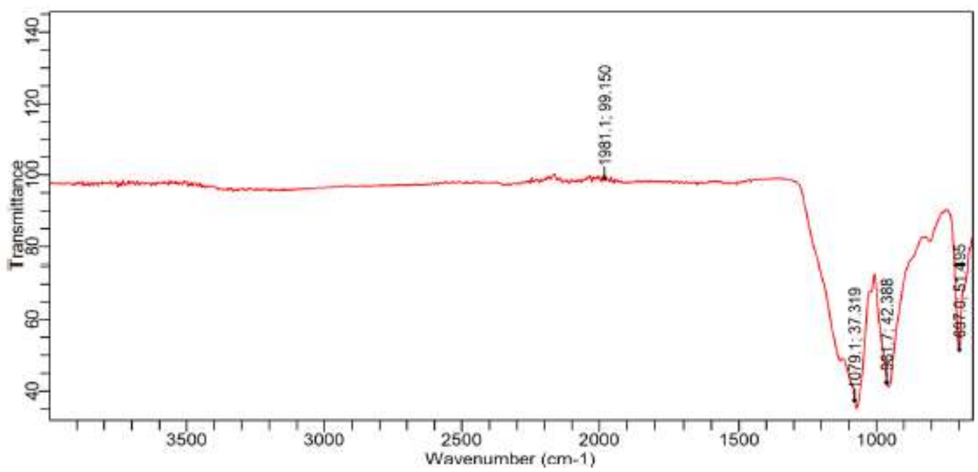


**Fig. 2A:** FTIR of CuO Nano Particles

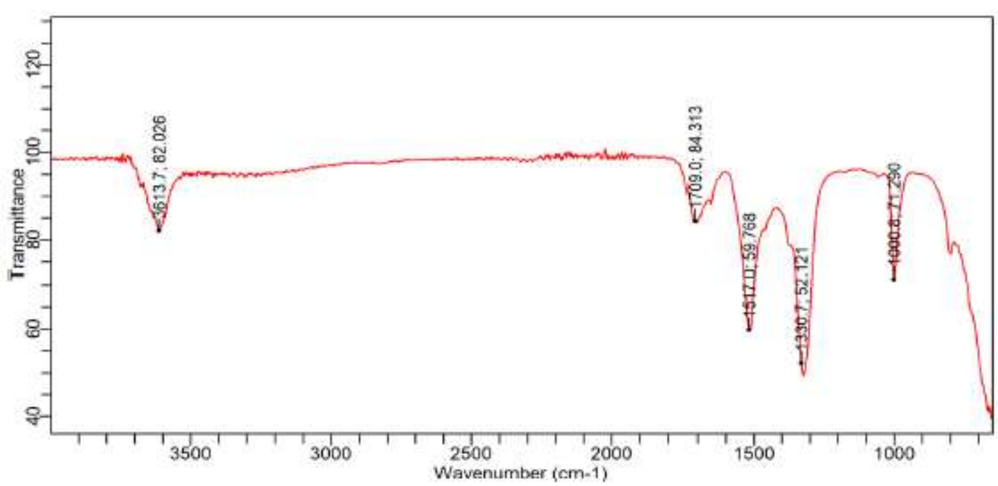


Fig. 2b. FTIR of MgO Nano Particles

### 3.3. XRD Analysis

### 3.3.1. Copper Oxide (CuO) nanoparticles

The XRD pattern of synthesized CuO nanoparticles is represented in figure 3a. The XRD pattern reveals two intense peaks at 25.76$^{o}$ and 29$^{o}$ corresponds to (110) and (111) reflection respectively (Anwar *et al* 2016 and Nadaf and Venkatesh 2015). This indicates

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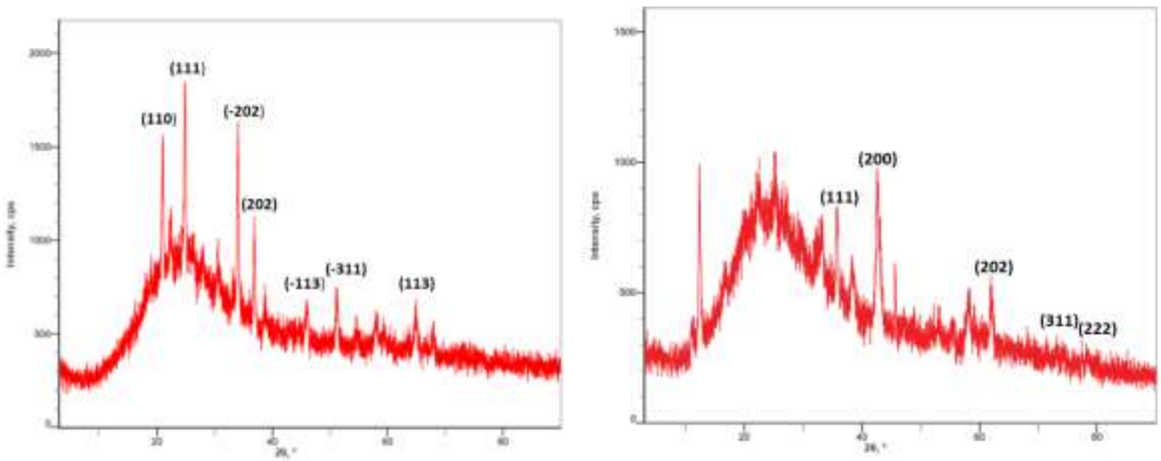
that the CuO nanoparticles possess the monoclinic structure. Other weak peaks reflections observed at (−202),(202),(−113),(−311),and(113) are due to the structural packing of the CuO nanoparticles.

### 3.3.2. MgO Nanoparticles

The XRD of the MgO nanoparticles presented in Fig. 3b showed that the sample contains some amount of impurities and the structure was found to be cubic in nature (Anwaar *et al* 2016 and Prado *et al* 2020).The diffractogram showed that the synthesized sample is highly crystalline, with peaks located at $2\theta = 37.15^{\circ}$(planes (111)), $43^{\circ}$(planes (200)), $62^{\circ}$(planes (202)) and two peaks of lower intensity in the range $75^{\circ}<2\theta<80^{\circ}$ corresponding to planes with Miller indices (311) and (222)(Anwaar *et al* 2016 and Prado *et al* 2020). The XRD diffractogram suggest that the synthesized nanoparticles is crystalline in nature




**(a)** **(b)**

**Fig. 3**. X-Ray Diffraction (XRD) patterns of (a) CuO and (b) MgO nanoparticles

### 3.4. Scanning electron microscopy (SEM)

Fig. 4a shows the SEM micrograph of CuO nanoparticles. From this image, the SEM confirmed that morphology of the biosynthesized CuO NPs has a spherical morphology and not homogenously distributed. All CuO nanoparticles have mean particles sizes of 20-35 nm (Veisi *et al*. 2021). The morphological and structural properties of MgO nanoparticles as observed in scanning electron microscopy (SEM) is depicted in fig. 4b. The agglomeration observed is due to the electrostatic attraction of MgO nanoparticles. The porous nature of the MgO nanoparticles is very useful in seed germination study (Ashok *et al* 2016).

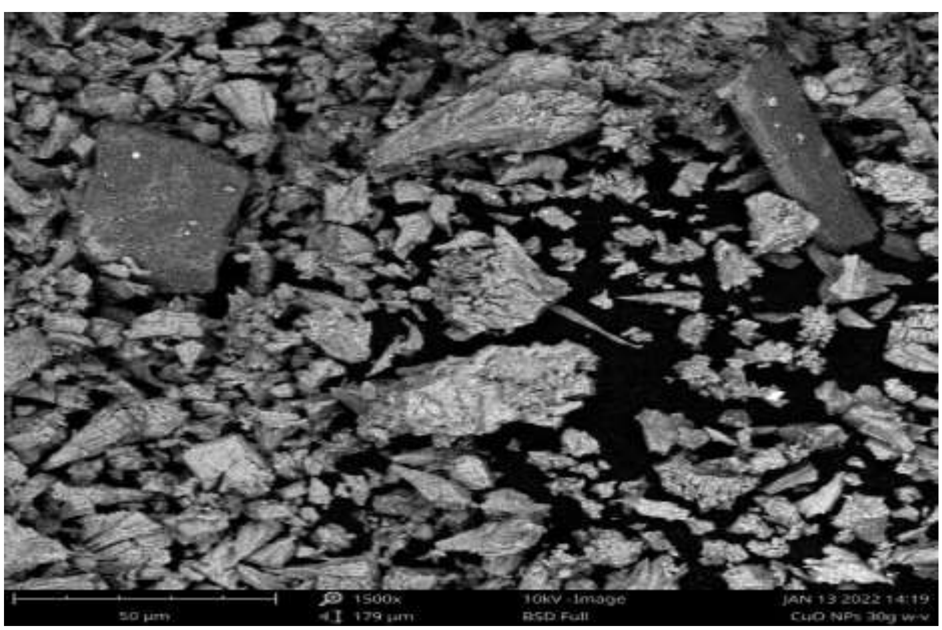



Fig. 4 a SEM images of CuO nanoparticle

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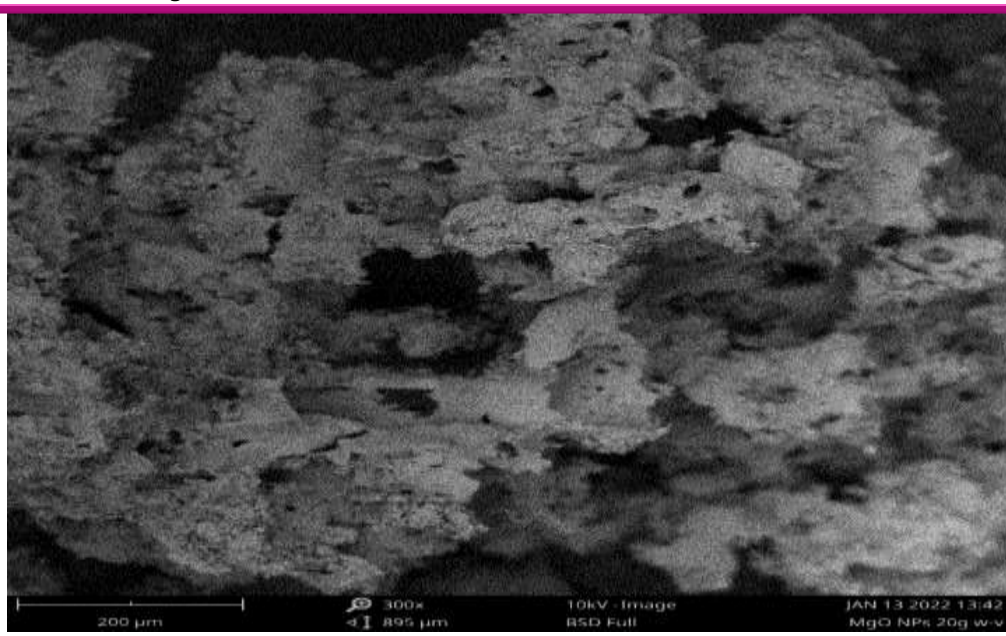


Fig 4b, The SEM micrograph of MgO nanoparticles

### 3.5. **Effect of synthesized nanoparticles on germination of arachis hypogaea**

The analysis of CuO and MgONPs impact on seeds germination has shown at higher concentrations of nanoparticles, CuO NPs reduced germination of Arachis hypogaea, while MgONPs improved germination as concentration increases as shown in figure 5a below. Both CuO and MgO nanoparticles decreases root and shoot length as depicted in the figures 5b and 5c below.

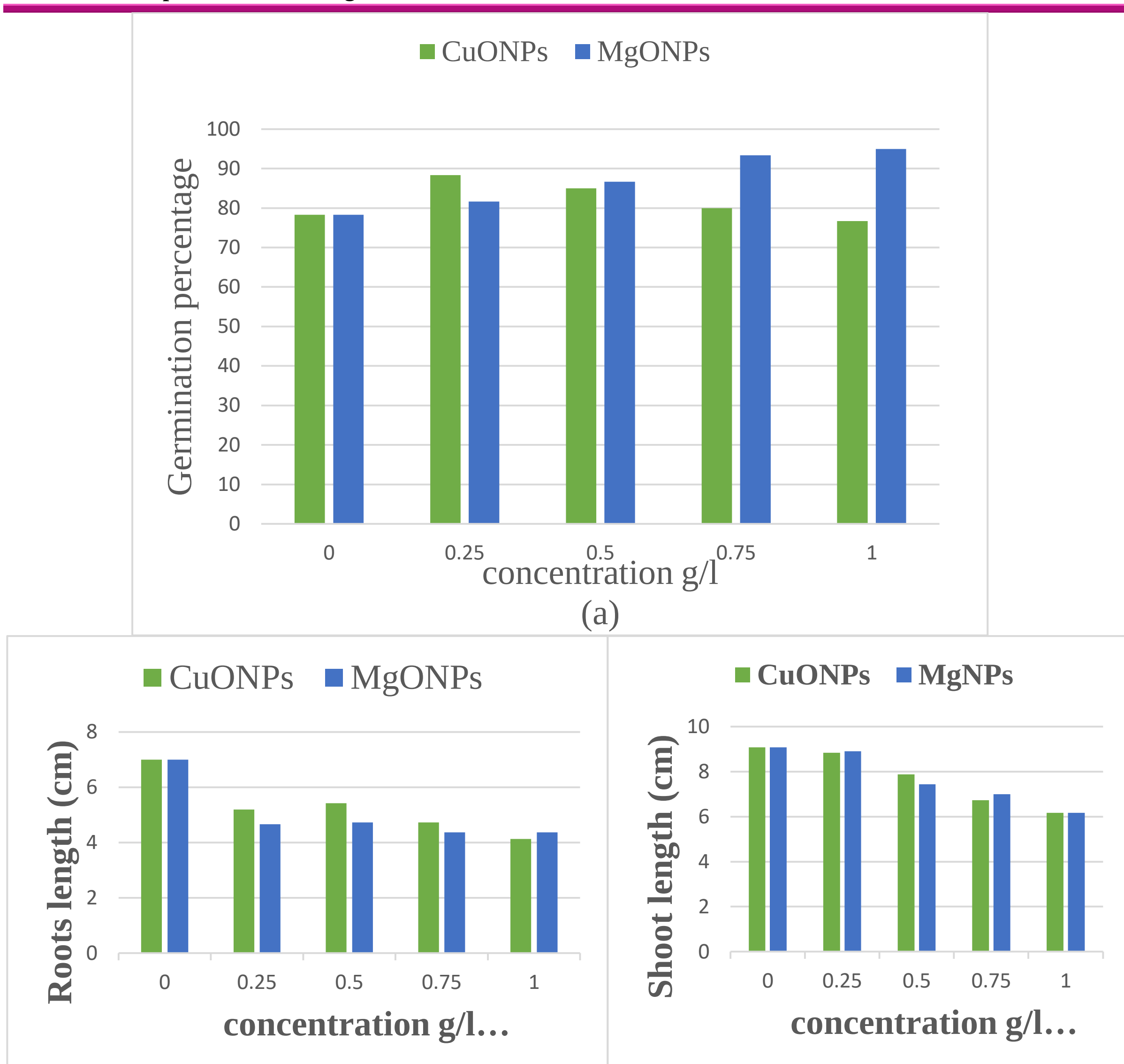


Figure 5.effects of various concentration of nanoparticles on percentage germination (a), root length (b) and shoot length (c)

## 4. Conclusion

Biosynthesis of CuO and MgO nanoparticles were achieved by simple, nontoxic and eco-friendly green procedure using aqueous fruits extract of Cucumis sativus. The biosynthesized nanoparticles were found to be suitable in the germination and seedling growth in Arachis hypogaea. The result demonstrated that MgONPs enhanced germination of Arachis hypogaea as the concentration increases while for CuONPs, there are decrease in germination. Root and shoot length in nanoparticles decreases with increase in concentration. Thus, both CuO and MgO nanoparticles have positive effect on the seed germination of Arachis hypogaea and have significant importance in agriculture and hence can be used as nanonutrients for efficient plant growth.

## Conflict of interest

The authors declared that there is no conflict of interest.

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