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EFFECT OF CuO NANOPARTICLES SYNTHESIZED USING *Cucumis sativus* EXTRACT ON GERMINATION OF *Arachis hypogaea* SEEDS.

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Abstract

The objective of the present study was to synthesize CuONPs using aqueous extract of Cucumis sativus as reducing agent with copper nitrate as precursor. The CuO nanoparticles have been synthesized by eco-friendly, simple and rapid method. The synthesized nanoparticles were characterized using UV-vis spectroscopy, Fourier Transform infrared (FT-IR) spectroscopy, Scanning electron microscopy (SEM) and X-ray diffraction (XRD) techniques. The existence of CuONPs as revealed by UV-Vis spectroscopy showed the absorption maximum at 3.991 with a wavelength of 228 nm. The FT-IR spectra identified the functional groups of the active component in Cucumis sativus. The XRD and SEM revealing crystallinity and spherical morphology of the synthesized CuONPs. The synthesized CuONPs were used to investigate its effect on the germination and seedling growth of Arachis hypogaea seed at different concentration of 0.00, 0.25, 0.50, 0.75 and 1.00g/l. The result showed a positive effect on both germination, roots and shoots length of seed of Arachis hypogaea at lower concentration. The results confirmed the feasibility of using aqueous extract of Cucumis sativus for the synthesis of CuONPs.

Keywords: *Arachis hypogaea*, copper oxide, *Cucumis sativus*, germination percentage, nanoparticles.

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INTRODUCTION

Nanotechnology is a novel, innovative interdisciplinary scientific approach that involved designing, development and application of materials and devices at molecular level in nanometer scales. i.e. at least one-dimension ranges in size from 1-100 nanometer, a billionth of meter. It is a broad-spectrum emerging field of science which has brilliant applications in basic and applied sciences. It will leave no field untouched by its captivating scientific applications and agriculture sector in no exception (Muhammad *et al*. 2014). Agriculture is always most important and stable sector because it produces and provides raw materials for food and feed industries: The limit of natural resources and the growth of population in the world claim the agricultural development to be economically further viable, environmentally and efficiently. Agricultural nutrient balances are differed noticeably with economic growth, and especially from the surmise, the development of soil fertility is very much significant in developing countries (Ram *et al*. 2017).

Among much scientific advancement, nanotechnology has been identified as one of the potential technologies that can revive the agriculture and food industry and can improved livelihood of poor people. Thus nanotechnology can provides the much needed green revolution in agricultural sector with emphasis on sustainable production (Pramanik *et al*. 2020). Most of the nanotechnology research in agriculture is related to sensing the soil condition, growth of plant parts, and provide adequate amount of fertilizer, pesticides, herbicides as per requirement (Kulkarnik, 2015), reducing pollution caused by chemical or even protect crops against environmental stress (Ghidan, & Tawfiq, 2019 and Paramo *et al*. 2020). Nanotechnology plays a decisive role in agricultural sector and its used in agriculturally based products can monitor growth of plants and can also detects diseases. Plant emergence is significantly based on seed germination, so the time from sowing to emergence of seedling has substantial value in crop production (Bakht *et al*. 2020).

Nanotechnology proceeds by three processes- separation, consolidation and deformation of materials by one atom or molecule. It is divided into three types: Wet nanotechnology which deals with biological system such as enzymes, membranes, cellular component. It is the study of biological systems that exist primarily in water environment. The functional nanometer scale structures of interest here are genetic materials.Dry nanotechnology deals with surface sciences, physical, chemistry and gives importance on fabrication of structure in carbon, silicon inorganic materials. Dry nanotechnology unlike wet technology technique; admit the use of metals and semiconductor (Pradhan 2013, and Singh*et al*. 2008). Computational nanotechnology which deals with modeling and stimulating the complex nanometer scale these fields are inter dependent to each other (Pradhan, 2013)

Nanomaterial are cornerstones of Nano science and nanotechnology, they are defined as set substances where at least one dimension is less than approximately 100 nanometers (Alagarasi, 2009). A nanoparticle is the most fundamental component in the fabrication of nanomaterial and is far smaller than the world of everyday objects that are described by Newton's law of motion but bigger than an atom or simply molecule that are governed by quantum mechanics (Horikashi, and Serpone, 2015). Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, and manufactured nanoparticles may find practical applications in a variety of areas (Javie, n.d).

Several techniques are developed day by day for the synthesis of nanoparticles. There are three basic categories of these techniques: Biological method, physical method and chemical method (Ijaz *et al*. 2020). Innumerable physical and chemical synthesis approaches require

high radiation, high toxic reductant and stabilizing agents, which can cause pernicious effects to both humans and marine life. In contrast, green (biological) synthesis of nanoparticles is one pot or single step eco-friendly bio-reduction method that requires low energy and is cost effective (Singh, *et al* 2018). In biosynthetic techniques, biomolecules replaces the conventional reducing and stabilizing agents (Haider, and Inn-kyu, 2015). Green sources include a wide range of natural precursors that includes plant extracts, bacteria, algae, fungi, microbes (Vega-Baudril, *et al.* 2019). The main mechanism considered for the synthesis of nanoparticles mediated by plant is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes etc. Different plants can be used to reduce and stabilized the nanoparticles. Many researchers have used the green synthesis method for the synthesis of metals or metal oxides nanoparticles by using different plant parts like leafs, stems, root and fruits (Ijaz, *et al*. 2020).Numerous researches have been done on the synthesis of nanoparticles from biological (green) system for their application in the field of biomedical, pharmaceutical, cosmetics, environmental and agriculture.

A number of plants had been investigated for their role in the synthesis of nanoparticles such as: *Imperata cylindrical L* (Saputra, and Yulizar, 2017), *Ficus microcarpa* leaf extract (Praba, *et al* 2015), *Cinnamomun zylinicum* (Hamsa, *et al* 2019), *Lysiloma acapalcesis* (Garibo, *et al* 2020), aqueous leaf extract of *Eupactorium odoratum* (Elemike, *et al* 2017), leaf extract of *Catha edulis* (Andualen, *et al*. 2020), *Passiflora caeruea* leaf extract (Santhoshkumar, *et al*. 2017), leaf extract of *Lippia adoensis* (Demissie, *et al*. 2020), flower of *Hibiscus rosa-sinensis*(Kumar, *et al*. 2020), *Ipomeapes-caprae* (Vankateasan, *et al*. 2017), leaf extract of *Origanum vulgare* L (Shaik, *et al*. 2018) and *Pongamia pinnata* (Sundrajan, *et al*. 2015).

Copper (Cu) is known as an essential component which functions in the regulating plant growth and development, including chlorophyll formation and seed germination. Copper is one of micronutrients needed in very small quantity by plants (Nguyen, *et al*. 2020), in addition to its many important function such as cell wall metabolism and protein regulation, it also act as secondary signaling molecules in plant cells. It take part in mitochondrial respiration, photosynthetic electron transport, ions mobilization, hormones signaling, oxidative stress response and also acts as cofactor for many enzymes (Sarkary, *et al* 2020). Copper nanoparticles, due their unique physical and chemical properties and low cost of preparation, have been of great interest recently. Copper nanoparticles can easily oxidized to form copper oxide (Honary, *et al* 2012). Copper oxide (CuO) nanoparticles are one of the major and frequently used engineered oxide nanoparticles with significant industrial applications like catalyst, superconducting materials, thermoelectric material, sensing material, glass, ceramics (Adhikary *et al* 2012), antioxidant drug delivery agents and imaging agent in field of biomedicine (Ijaz, *et al* 2017 and Dormer, *et al* 2019).

Various synthesis methods are used for CuO nanoparticles synthesis such as chemical precipitation, electrochemical reduction, microwave irradiation and thermal decomposition plants, which attracted many researchers in green routes for the synthesis of copper, but in the chemical method, the use of toxic chemical limited its application. Thus, the synthesis of CuO nanoparticles using biological methods gains more attention due to its easy availability, easy handling, elimination cell culture and being ecofriendly (Amin, et al 2021).

Many research works had been carried out on the synthesis of CuO nanoparticles from plant extract and their impact on plant growth and germination which includes: *Ocimumbasalicum* extract (Altikatoglu, *et al* 2017), *Cassia auriculata* leaf extract (Shi, *et al*. 2017), *Terminalia phanerphilebia* leaf extract (Keabadile, *et al* 2020), *Caesalpinia bonducella* seed extract

(Sukumar, *et al*. 2020), *Stachys lavandulifolia* flowers (Veisi, *et al* 2021), *Gloriosa superba* L extract (Naika, *et al* 2015), *Abutilon indicum* leaf extract (Ijaz, *et al*. 2017).

In this research work, CuO were synthesized from aqueous solution of *cucumis sativus*. The effects of this nanoparticle on germination of *Arachis hypogaea* seeds were investigated.

Materials and Method

Fruits of *cucumis sativus* (Cucumber) and *Arachis hypogaea*(Peanut), copper (ii) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$) 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

Sample collection

Fruits of *Cucumis sativus* (Cucumber) and Seeds of *Arachis hypogeae* (groundnut) were all purchased from Talata Mafara local market and it was identified by the Botanist of department of Agricultural Technology, College of Agriculture and Animal Science, Bakura. Zamfara state Nigeria.

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 then 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 Cucumis *sativus*extract served as reducing and stabilizing agents for the synthesis of the nanoparticles.

Synthesis of CuO Nanoparticles.

About 30g of copper (II) nitrate trihydrate precursor salt were added to 100ml of Cucumis *sativus* 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 a muffle furnace. The synthesized CuO nanoparticles were then kept for characterization and then for germination studies.

Characterization of nanoparticles

Uv-Visible spectroscopy was used to confirm the formation of nanoparticles using Shimadzu UV-vis spectrophotometer 1650 in the range of 200-1000 nm. Frourier transform infrared (FTIR) spectrum was recorded using Agilent Technologies Cary630 in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.XRD technique was performed in the 2θ range of 30–70, so as to determine the phase and crystallinity of the synthesized using X-ray diffractometer (XRD) Goniometer miniflex 300/600.SEM analysis was performed by (SEM) phenon: model No: 72120000.00.0478to provide information about the sample topology and morphology

Seeds germination test

The seed of *Arachis hypogaea* were soaked in 3.5% sodium hypochlorite solution for 10min to ensure surface sterility, and then washed three times with distilled water. Different concentration of the synthesized nanoparticles was suspended in distilled water to make 0.0,

0.25, 050, 0.75 and1.00g/l in accordance with Afrayeem and Chaurasia 2017 followed by sonication for 30min for uniform dispersion. The seeds of *Arachis hypogaea* were then soaked in the different concentration of the nanoparticles for 2hrs.about 20 seeds were then planted on sterilized clay soil by autoclaving in polythene bags. The seeds were irrigated with 10ml of distilled water (Khalaki *et al*. 2016). 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 germination was halted and the number of germinated seeds was counted, root and shoot length of the seedlings were measured in centimeter using ruler.

Germination percentage (GP)

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

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.

Root and shoot length

The root and shoot length of the germinated seeds were measured in centimeter. 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.

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.

RESULTS AND DISCUSSIONS

Uv-visible absorption spectroscopy

The Uv-vis spectra of synthesized CuO nanoparticles is depicted in fig.3.1 below. The synthesized CuO nanoparticles displayed an absorption peak at 228mm due to **s**urface 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 250mm.

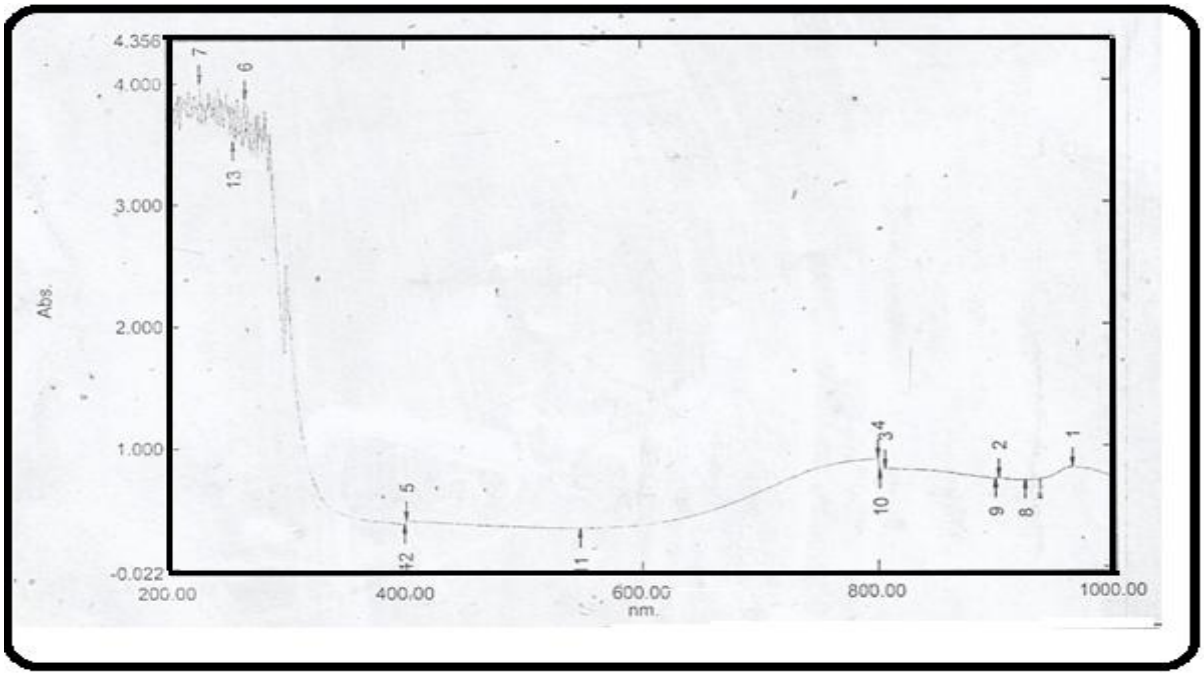


Fig. 1. UV–vis spectra of CuO nanoparticles

Fourier Transform Infrared (FT-IR) Spectroscopy.

This technique is used to detect the biomolecules responsible for the reduction and capping agent for the formation of nanoparticles. Figure 3.2 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).

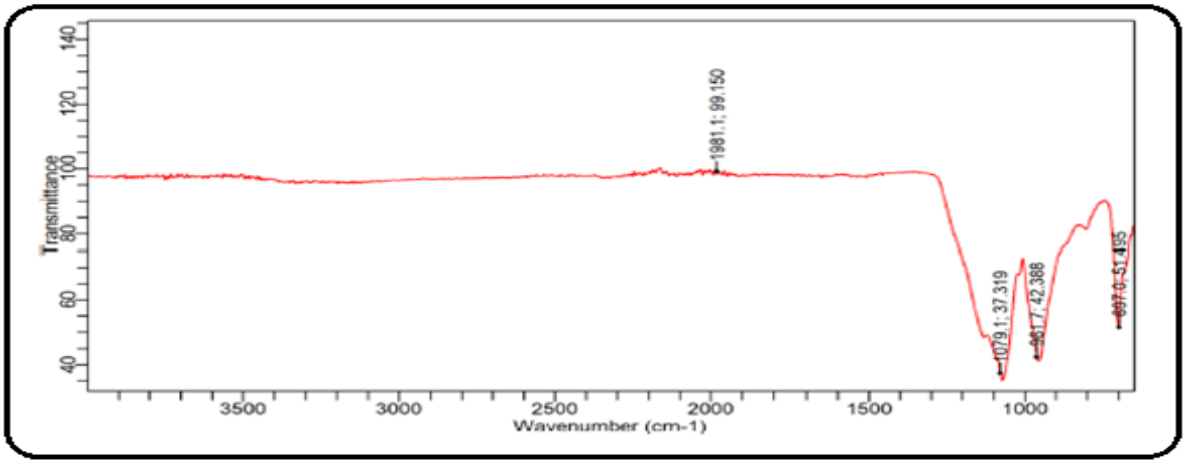


Fig. 2: FTIR spectrum of CuO Nano Particle

X-ray diffraction (XRD) analysis

The XRD pattern of synthesized CuO nanoparticles is represented in figure 3.3. 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 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

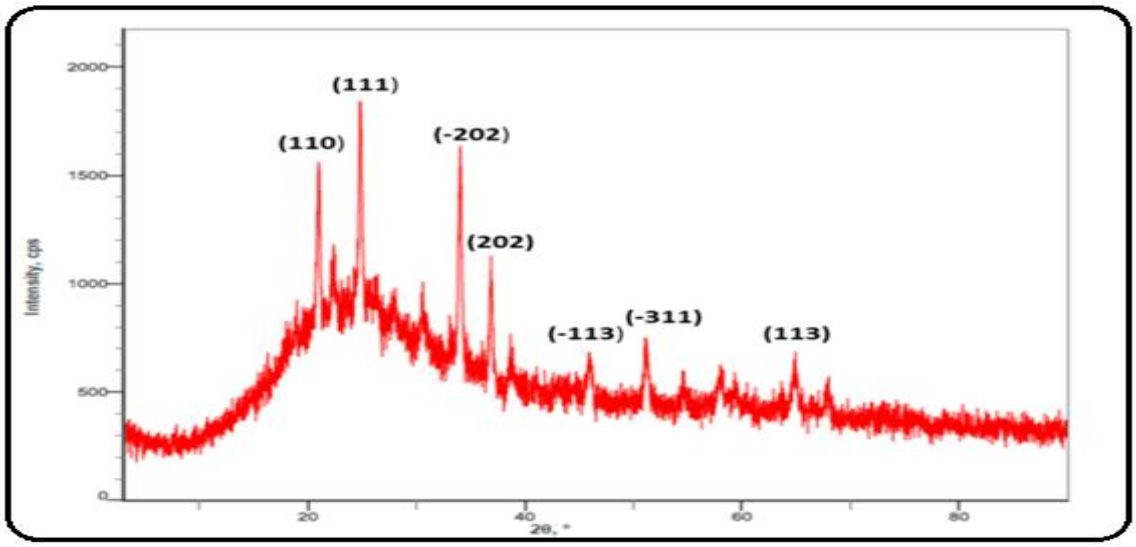


Fig. 3 XRD pattern of CuO nanoparticles

Scanning electron microscopy (SEM)

Figure 3.4.show the SEM micrograph of CuO nanoparticles. From this image, the SEM confirmed that morphology of the synthesized 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).

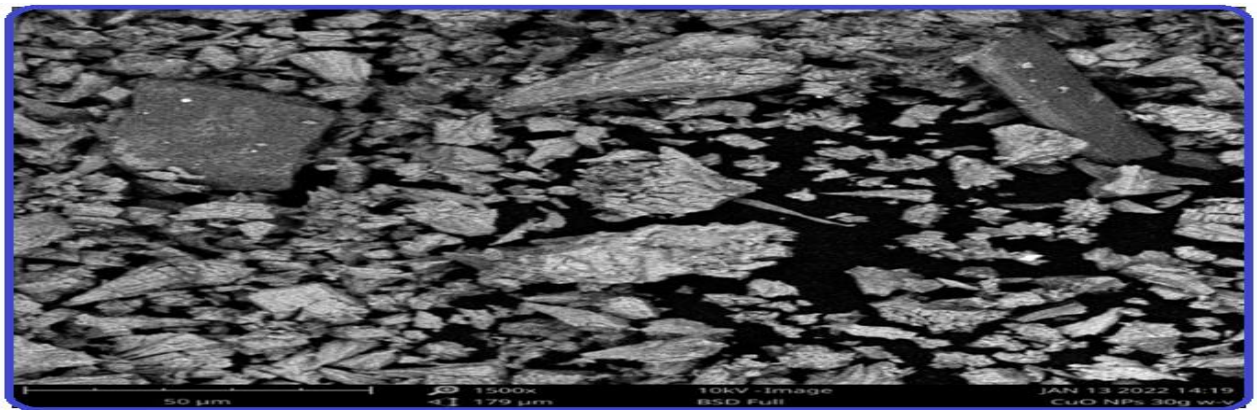


Fig. 4. SEM micrograph of CuO nanoparticle

Germination of *Arachis hypogaea*

Result pertaining seeds germination, root and shoot length of *Arachis hypogaea* under the influence of CuO nanoparticles is depicted in the table 1 below:

Table 1. Germination percentage, root and shoot lengths of *Arachishypogaea*.

	Concentration (g/L) of nanoparticles	Germination %	Root length (cm)	Shoot length (cm)
CuO	Control	78.33±2.89	7.00±1.32	9.07±1.56
	0.25	88.33±2.89	5.20±0.60	8.83±1.54
	0.50	85.00±5.00	5.43±0.15	7.87±2.10
	0.75	80.00±0.00	4.73±0.60	6.73±1.67
	1.00	76.67±2.89	4.13±0.55	6.17±1.33

Conclusion

In summary, CuO nanoparticles were synthesized using aqueous solution of *Cucumis sativus* in a laboratory condition. The FT-IR analysis result confirmed the different functional group in aqueous solution of *Cucumis sativus* which act as reducing and stabilizing agents in the reduction of copper nitrate salt to CuO nanoparticles. XRD analysis confirmed the crystalline monoclinic structure of CuONPs, whole SEM analysis confirmed the spherical morphology of CuONPs. The result obtained from germination study showed that the synthesized CuONP's possess a positive effect on the germination of *Arachis hypogaea* at a low concentration (0.25) while the root and shoot length decreases with increase in concentration.

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