JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EFFECT OF CuO AND FeO NANOPARTICLES ON SEED GERMINATION, MICROPROPAGATION, AND CALLUS CULTURE.

[1]Sawant R., [2]Kamat S., [3]Salvi P., [4]Vagare R., [5]Dalvi R.

Department of Biotechnology, Chikitsak Samuha's Sir Sitaram and Lady Shantabai Patkar College of Arts & Science and V. P. Varde College of Commerce & Economics, Goregaon (W), Mumbai, Maharashtra 400061.

Abstract: The tissue culture involves the growth of tissues or cells in an artificial medium. It is generally performed on MS medium and gives more plantlets in a short time. This medium is however prone to microbial contamination therefore, modification in MS medium is needed. Current research highlights the use of FeO and CuO NPs at two different concentrations (5 and 50 mM) in the MS media to check the effect of Nps on plant growth promotion as well as prevention of Microbial contamination. The modified MS medium was used to check its effect on seed germination, micropropagation and callus induction. This study showed the effect of CuO and FeO Nps on plant growth using Tissue culture techniques. UV spectroscopy, FITR, and FEG-SEM techniques were used to check the qualitative and quantitative nature of nanoparticles. The modified MS medium supplemented with suitable Np concentration was used to check its effect on seed germination, micropropagation, and callus culture. Out of two nanoparticles FeO Nps showed better result for seed germination whereas CuO Nps were found to be best for plant micropropagation. Supplementation of only Nps were not found to be suitable for Callus induction in given plant. Cu and Fe are vital trace elements for plant growth and they play important role in plant metabolism. Effect of these ions on plant growth as well as their importance to control microbial contamination was confirmed by modifying MS medium. There was an increase in growth percentage was observed. This finding is very important as higher number of plantlets can be obtained as well as microbial contamination also can be prevented. The present investigation also promises reduction in problems which can arise due to depletion of fertile soil and its inconvenience.

Keywords – Modified MS medium, CuO NPs, FeO NPs, micropropagation.

I. INTRODUCTION

The area of nanotechnology is one of the most dynamic areas of exploration in present-day materials science. Nanostructured materials are drawing in a lot of consideration in light of their capability of accomplishing explicit cycles and selectivity, particularly in organic and drug applications (Gomez and Fernandez et.al, 2002). Copper oxide (CuO) have attracted attention mostly because of their antimicrobial and biocide properties and they may be used in many biomedical applications. The major disadvantage for their use on the medical field is due to their potentially toxic effects. The mechanism of NP uptake by plant roots is not clearly understood. Studies have shown that, depending on the size, NPs may enter the plant cell through carrier proteins, aquaporins, 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 (N.

Thakur, S. Rana, and S. Kaur, et.al, 2021).

Copper is an essential micronutrient required for the normal growth of plants. It acts as a structural component in regulatory proteins and those involved in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism, and hormone signaling (Marschner 1995, Raven et al. 1999, Solymosi and Bertrand 2012). Copper nanoparticles are easily synthesized in the laboratory and appear as black solid substances. Iron (Fe) is a key determinant of the biological functions for a large number of cellular enzymes in organelles, for plant photosynthesis, respiration and for plant product quality. The fate of the Fe-NPs in plants is not fully understood. Some literature reported that the Fe-NPs can be internalized, transported and utilized in vascular tissues, stems, and leaves (Yuan J. *et. al.*, 2018). Iron oxides display extraordinary potential in the fields of life sciences

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 plant growth. Cu at a high concentration is extremely toxic to plants. The commonest Cu toxicity symptoms are poorly developed roots stunted and inhibited growth, necrosis, and leaf chlorosis. Classic symptoms of Fe toxicity are leaf discoloration (bronzing) and a stunted root system(Li et al., 2015).

The green and chemical synthesis of CuO NPs and FeO NPs may be beneficial for agriculture and medical uses. Their green synthesis using neem plant extract was an eco-friendly technique and chemical synthesis was a rapid and time-saving method. Plant Tissue Culture has evolved as an essential tool for basic and applied plant research. In vitro, culture techniques have various applications in the production of disease-free plants, micropropagation of rare plant genotypes, plant genome transformation, germplasm conservation of elite plant varieties, and production of plant-derived secondary metabolites of commercial importance (Claudia A. *et. al.*, 2018). Using Tissue Culture, it has become possible to mass-produce plantlets with desired genetic makeup. In Tissue Culture techniques, one of the biggest challenges faced by many researchers is microbial contamination and to overcome this hurdle research on the use of Nanoparticles like Silver, Titanium oxide as an antimicrobial agent has been carried out by some researchers (Anwaar S. *et. al.*, 2016).

Okra (Abelmoschus esculentus L.) is a popular, easily available, low-cost vegetable crop with various nutritional values and potential health benefits. Different parts such as mucilage, seed, and pods contain certain important bioactive components. The phytochemicals of okra have been studied for their potential therapeutic activities on various chronic diseases, such as type- 2 diabetes, cardiovascular, and digestive diseases, anti-fatigue effect, liver detoxification, antibacterial, and chemo-preventive activities. Moreover, okra mucilage has been widely used in medicinal applications such as plasma replacement or blood volume expanders. (Abd Elmoneim O. Elkhalifa *et. al.*, 2021)

II. RESEARCH METHODOLOGY

2.1Green Synthesis of CuO NPs and FeO NPs using A. indica Leaf Extract: Authenticated A. indica leaves were collected near shops of the Jain temple in Goregaon. 20 grams of dried A. indica leaves were finely ground with a Mortar and pestle in 200 ml distilled water. This mixture was placed in a shaking incubator for 2.5 hours at 40 ^{0}C at speed of 50 rpm. Then the mixture was filtered using Whatman filter paper no 1. $Cu(Co_2CH_3)_2.H_20$ salt and $FeCl_3$ salt in 4 molar concentrations (0.001M, 0.005M, 0.01M, and 0.1M) was added to the mixture, and were kept in 4 different conditions ie- RT, 37^{0}C, 60^{0}C and 100^{0}C. The mixture was then centrifuged at 5,000 rpm for 20 minutes and the black powdered extract was collected from the supernatant. The supernatant was discarded and the powdered pellets were washed with distilled water thrice. The CuO NPs and FeO NPs were dried at 60^{0}C overnight in a hot air oven.

2.2Chemical Synthesis of CuO NPs and FeO NPs:

CuO NPs: 0.9g of copper chloride dihydrate and 0.54 g sodium hydroxide flakes were dissolved in ethanol separately. Dropwise addition of sodium hydroxide solution to copper (II) chloride dihydrate solution was carried with constant stirring at R.T. Colour of the solution turned from green to bluish green and finally to black as the reaction proceeded. The precipitate was separated using a centrifuge at 5000 rpm for 10 mins and washed with ethanol and deionized water. The precipitate was dried at 50°C in Hot Air Oven and grounded to get the powdered nanoparticle particles. (luna, Hilary, et.al, 2015).

FeO NPs: The co-precipitation method was used to obtain the nanoparticles. 5.4 gm of Iron (III) Chloride was dissolved in 20 ml of deionized water. Iron (II) chloride was dissolved in 5ml of 2M HCl. Both solutions were added to 100ml of deionized water and mixed thoroughly. 2M ammonia was then added to the reaction mixture and kept for 10 mins at R.T at 300 rpm using magnetic stirrer. The NPs are washed using deionized water till the solution is neutral and were suspended in toluene.

2.3Characterization of Bio and Chemically synthesized CuO and FeO NPs:

To confirm the presence of CuO and FeO NPs, to check changes in the optical properties, and check the presence of impurities in the neem extract according to their spectrum and absorbance, UV analysis was carried out using a UV-Visible Spectrophotometer using a wavelength range of 200 – 800nm. The stabilization mechanism of nanostructures was carried out using a Fourier transform infrared (FTIR) spectrophotometer in the transmittance mode of the range of 400–4000 cm^{-1}. The morphology of CuO NPs in the nano-meter scale was checked using Field Emission Gun Scanning Electron Microscopy (FEG-SEM).

2.4Procurement and Surface sterilization of Seeds:

Hybrid variety of okra seeds was collected from a shop called Bombay Seeds in Malad. The surface sterilization method was carried out for the following seeds. The seeds were soaked in water for 30 mins and washed with dilute liquid detergent (Teepol) for 5mins, 1% v/v solution of antiseptic (Savlon) for 60s, and 0.1% w/v solution of Mercuric chloride (HgCl2) for 90s. The seeds were then rinsed thoroughly with sterile double-distilled water 5 times. Surface sterilized seeds were germinated in half-strength Murashige and Skoog's medium with 1.5% w/v sucrose.

2.5Seed Germination:

1L of Half strength MS media was prepared with 1.5% w/v of sucrose. The pH of all the media was adjusted between 5.5 to 5.7 using 1N NaOH or 1N HCl. The media were then solidified sing 8gm/L Agar (Himedia, India), prior to autoclaving at 121° for 3 hours. After autoclaving the media, CuO and FeO-NPs were added at 5 mg/L and 50 mg/L final concentrations. To avoid cluster formation of NPs at the base, the medium was allowed to cool up to 45^0C and after this, 20 ml of the media was poured into each of 10 Borosil glass fat tubes (150 x 25mm) and was kept at 4^0C till the media solidified. The surface-sterilized seeds were inoculated in 20 ml of half-strength MS media in Borosil glass fat tubes (150 x 25mm) for seed germination and further for shoot initiation and root induction. The explants were cultured in Plant Tissue Culture laboratory conditions of cool white fluorescent light (40.5 µmol/sq.m/s^2) with a photoperiod of 16hrs light and 8hrs dark at 22° ± 2.

2.6Micropropagation of okra plantlets:

The apical nodal explants of the plantlets were inoculated in full-strength MS medium supplemented with a standard concentration of IAA as control. The shoots obtained in 14 days were transferred for root initiation and development in MS + 0.55µM IAA+ CuO and FeO NPs medium in 10 Fat tubes. CuO and FeO NPs were added at 5 mg/L and 50 mg/L final concentrations.

2.7Callus of okra plant:

To induce callus, okra leaf segments were taken and cultured in MS (Murashige and Skoog, 1962) medium supplemented with 0.5 mg/l BAP and 2.0 mg/l NAA and incubated in the light of 25±2o C for 3- 4 weeks. The results were observed and noted accordingly.

III. RESULTS

3.1 Synthesis of CuO and FeO NPs:

Copper oxide and Iron oxide nanoparticles were biologically synthesized using neem extract. The two molar concentrations of 0.001M and 0.005 M obtained a large number of Nanoparticles when the mixture was centrifuged and dried. The pellets obtained after centrifugation were black-colored in powder form. For chemically synthesized copper oxide nanoparticles, two molar concentrations of 1:10 and 1:100 of salt were taken. A color change was observed from bluish-green to black under constant stirring at 1:10 molar concentration. The chemical FeO nanoparticles were precipitated using the co-precipitation method. The FeONps were extracted using a magnet which confirms the magnetic nature of the chemical NPs which were black in colour.

CuO:

Fig 1: Biologically synthesized Nanoparticles

FeO:

Fig 2: Biologically synthesized nanoparticles

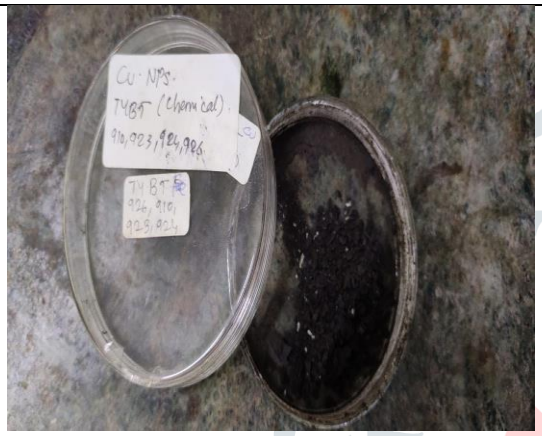


Fig 3: Chemically synthesized Nanoparticles

Fig 4: Chemically synthesized Nanoparticles

3.2 Characterization of NPs:

UV -Visible Spectrophotometer:

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. The standard range of maximum absorption of copper oxide nanoparticles is 200-800nm and of iron oxide nanoparticles is 250-350nm. The maximum absorption of the extracted iron and copper oxide nanoparticles is mentioned below.

Nanoparticles	**Maximum Absorption**
CuO Biological NPs	273nm,282nm,241nm,312nm,244nm,288nm
CuO Chemical NPs	431nm,353nm,258nm
FeO Biological NPs	321nm,255nm,350nm,255nm,303nm,238nm
FeO Chemical NPs	290nm

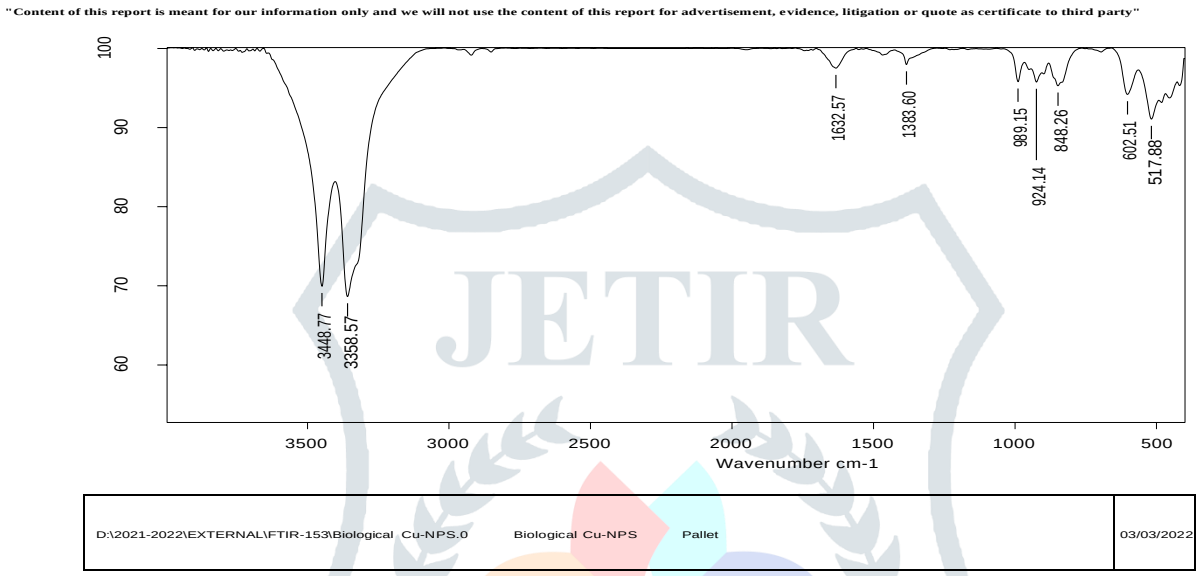
Table 1: UV Absorption table

Fourier transform infrared spectroscopy (FTIR):

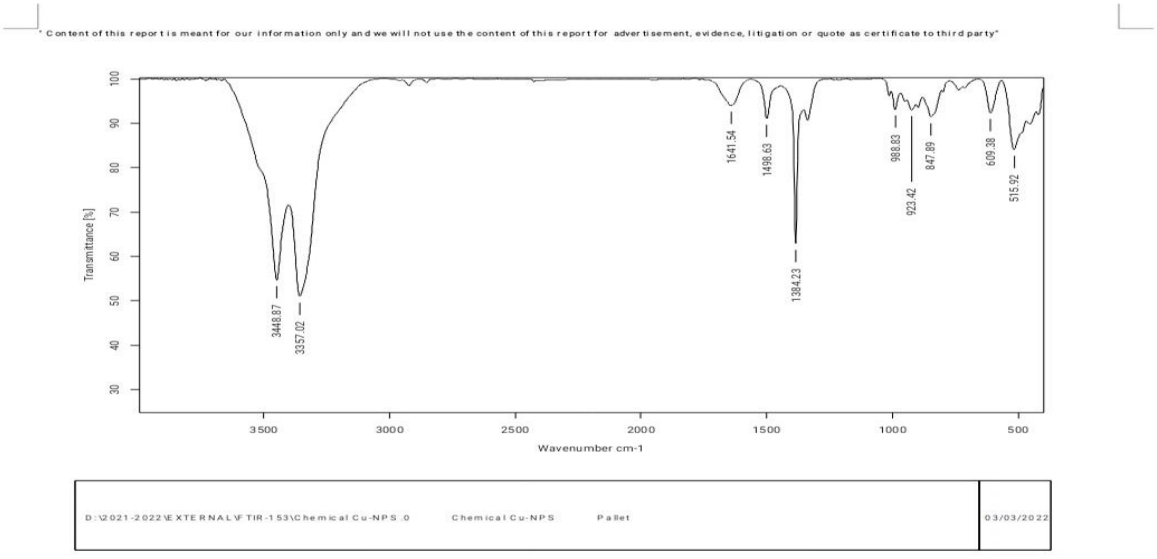
FTIR spectroscopy is a useful tool to identify functional groups in a molecule, because each specific chemical bond often has a unique energy absorption band, and can be used to obtain structural and bond information of a complex to study the strength and type of bonding. Biological CuO NPs shows bands at 517.88 cm^{-1}, 602.51cm^{-1}, 989.15cm^{-1}, 1632.57cm^{-1}, and 3448.77cm^{-1} wavelengths. The absorption spectrum obtained at 602.51cm^{-1} indicate different mode of bending vibration of the Cu–O bond and confirms the presence of CuO NPs. The Absorption spectrum band obtained at 3448.77cm^{-1} belongs to the symmetric and

asymmetric stretching vibration of the O–H bond respectively. The appearance of bands at 1632.5 cm^{-1} and 1383.60cm^{-1} indicates stretching vibration of the Cu–O bond of copper oxide nanoparticles (). Chemical CuO NPs show bonds at 515.92 cm-^{1}, 609.38 cm^{-1}, 847.89 cm^{-1}, 923.42 cm^{-1},
988.83 cm^{-1} and 3448.87 cm^{-1} wavelengths. The absorbance spectrum obtained at 515.92 cm- 1 indicates different modes of bending vibration of Cu-O bond and confirm the presence of chemical Cuo-Nps. The absorbance spectrum band obtained at 3448.87 cm-1 belongs to the symmetric and asymmetric stretching vibration of the O-H bond respectively.

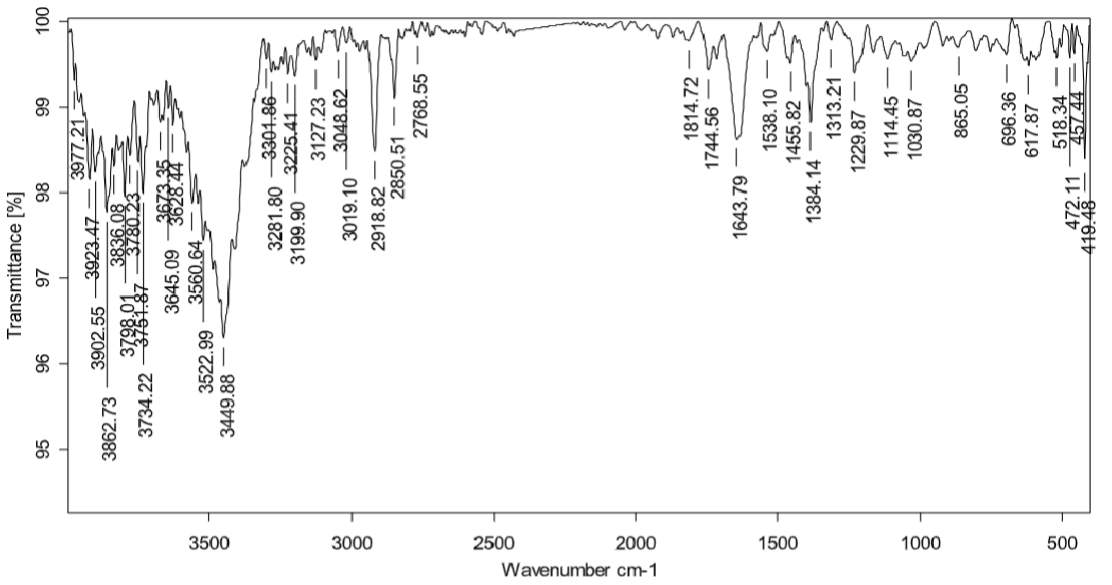
Biological FeO-NPs shows bands at 518.34 cm^{-1}, 617 cm^{-1}, 687cm^{-1}, 1229.87cm^{-1}, 1384.14cm^{-1}, 1643.79cm^{-1}, 2918.82cm^{-1}, and 3449.88cm^{-1} wavelengths. The wavelengths 617 cm^{-1} & 687 cm^{-1} indicate presence of FeO NPs.. The Absorption spectrum band obtained at 3449.88 cm^{-1} belongs to the symmetric and asymmetric stretching vibration of the O–H bond respectively. Chemical FeO NPs shows absorption peak for 1623 cm^{-1} and 1628 cm^{-1}, and for 3407 cm^{-1} and 3401 cm^{-1} confirming its presence.



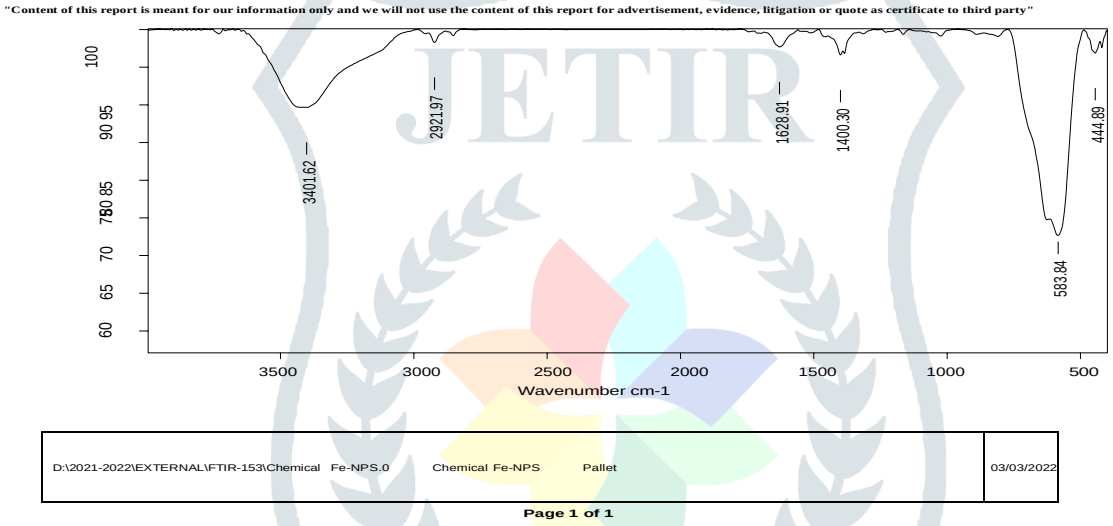
Graph 1: FTIR spectrum graph of Biological CuO NPs.



Graph 2: FTIR spectrum graph of Chemical CuO-NPs



Graph 3: FTIR spectrum graph of Biological FeO NPs.



Graph 4: FTIR spectrum graph of Chemical FeO NPs.

Field Emission Gun Scanning Electron Microscopy (FEG-SEM):

A FEG-SEM is an indispensable analytical tool for research and solving industrial problems where optical microscopes will not provide the required resolution. The surface morphology the copper oxide NPs synthesized are shown. FEG-SEM spectra of green synthesis of copper nanoparticles show nearly mono dispersed distribution of particle sizes. It shows the presence of nanoparticles with more or less uniform shape but varying in sizes and also no aggregation is seen. The particle size is about 2.7nm. The chemically synthesized copper nanoparticles have size around less than 50 nm. The SEM graph shows that the copper oxide nanoparticles are sheetlike or rodlike in shape and have particle size of 0.66nm. For Iron oxide nanoparticles, the SEM micrograph of the nanoscales are magnetic particles. Biologically synthesized iron oxide nanoparticles shows the appearance to be spherical and agglomerated. The agglomeration of FeONPs was observed might be due to the electrostatic interaction between layers of nanoparticles surface. The size of these biologically synthesized iron oxide nanoparticles was observed to be 3 nm. The chemically synthesized FeO nanoparticles have a spherical shape, they are crystalline in nature and have a particle size of 0.68 nm.

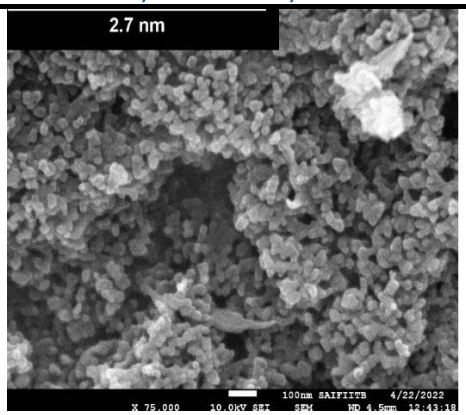


Fig 5: Biological CuO-NPs

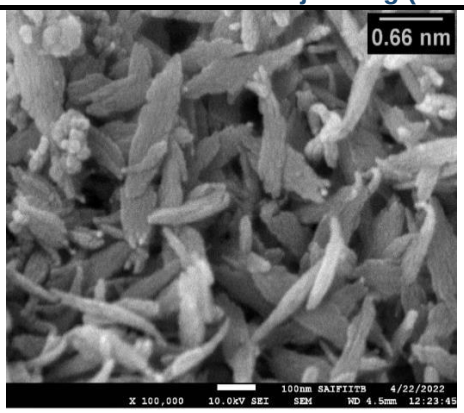


Fig 6: Chemical CuO-NPs.

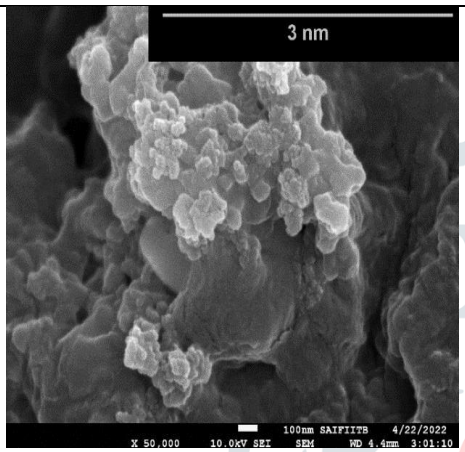


Fig 7: Biological FeO-NPs

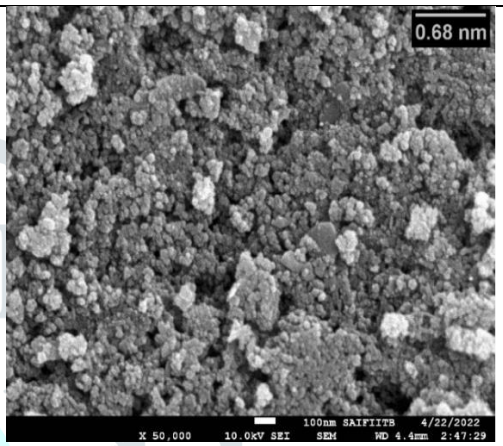


Fig 8: Chemical FeO NPs

3.3 Effect of NPs on Plant tissue culture on modified MS medium:

The comparative analysis of seed germination, micropropagation and callus culture was carried out. The following parameters taken into consideration were growth percentage, number of days required for growth of the plant and height of the plant. The analysis shows that the modified MS medium showed better results than the control. Copper oxide nanoparticles showed better growth results for 2 concentrations than iron oxide nanoparticles. Seed germination showed comparatively faster growth than micropropagation. A complete absence of growth for callus culture was observed in the modified MS medium than control. The comparative efficiency of the three parameters is seen in the table below:

Types of NPs	Seed Germination			Micropropagation			Callus Culture		
Comparative efficiency of NPs on PTC	Growth%	No of days	Height (cm)	Growth%	No of days	Height (cm)	Growth%	No of days	Height (cm)
Control Tubes	3.33	30	14	100	20	10	100	20	-
1.Biological: CuO(5mg/L)	6.66	12	4	100	16	20		-	
CuO(50mg/L)	16.66	15	9	100	16	20		-	
2.Chemical: CuO(5mg/L)	20	12	8.3		-			-	
CuO(50mg/L)	16.66	12	5.5	80	16	20		-	
3.Biological: FeO(5mg/ml)	16.66	15	8		-			-	

FeO(50mg/ml)	16.66	15	8	-			-
4.Chemical: FeO(5mg/ml)	0	0	0	40	16	5	-
FeO(50mg/ml)	10	15	9	-			-

Table 2: Comparative analysis of NPs on Plant tissue culture

Seed Germination:

Okra seed growth was observed.

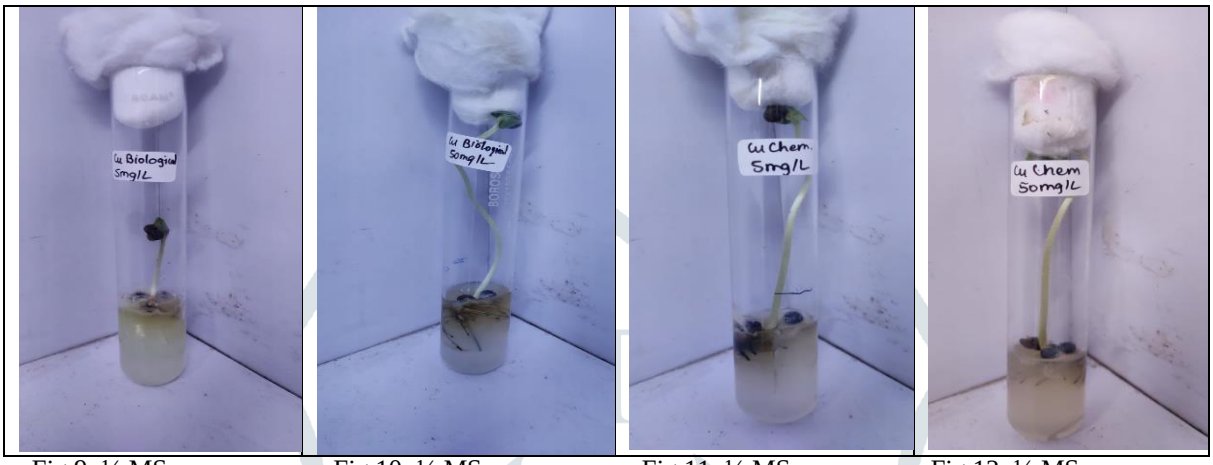


Fig 9: ½ MS +CuO NPs(5mg/L)

Fig 10: ½ MS + CuO NPs(50mg/L)

Fig 11: ½ MS +CuO NPs(5mg/L)

Fig 12: ½ MS +CuO NPs(50mg/L)

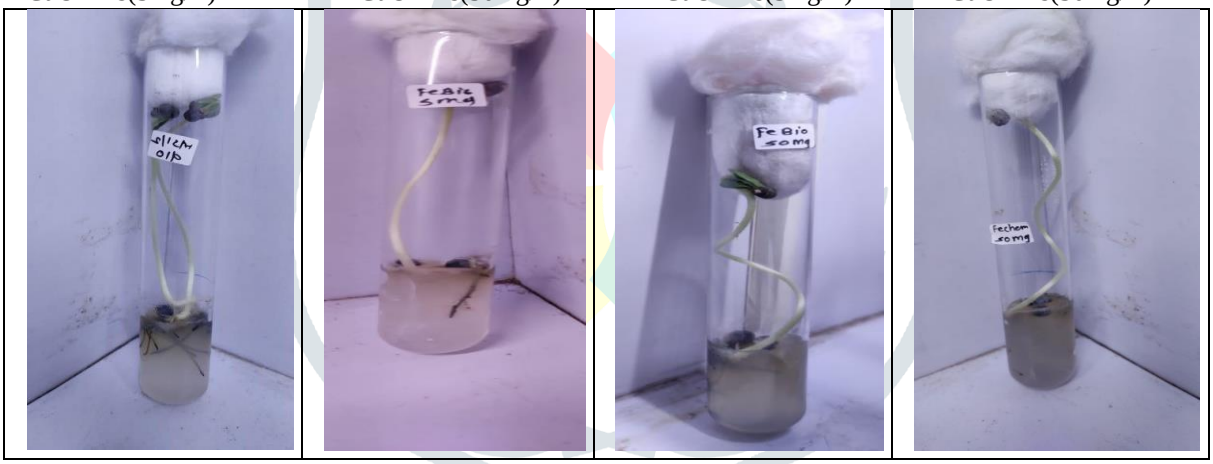


Fig 13: Control

Fig 14: ½ MS+FeO NPs (5mg/ml)

Fig 15: ½ MS+FeO NPs (50mg/ml)

Fig 16: ½ MS+FeO NPs (50mg/ml)

Micropropagation:

A dense network of roots was observed with 2-3 internodes.

Fig 17: Control

Fig 18: Full MS + CuO NPs(5 and 50 mg/L)

Callus Induction:

A presence of brown patchy growth of callus in the control tubes was seen whereas the tubes containing nanoparticles showed no growth and the leaves faded their color as they died.

IV. CONCLUSION

In this study, we investigated the effect of copper oxide and iron oxide nanoparticles on okra seed germination, shoot length, micropropagation and callus induction. The Nanoparticles were synthesized biologically and chemically. These nanoparticles were analyzed and characterized using a U.V Visible spectrophotometer, FTIR and their morphology was checked using FEG-SEM. Copper oxide nanoparticles is the key nutrient for plant growth and development whereas iron oxide nanoparticles showed no major effect. During seed germination, 16.66% growth of seeds were observed when copper oxide nanoparticles were added in the media. A growth percentage of 10% was seen when iron oxide nanoparticles were added to the media. Micropropagation showed greater results when using copper oxide nanoparticles. There was a complete absence of growth for callus induction after the addition of nanoparticles which tells us that nanoparticles do not support the growth of callus induction. Comparatively, it can be stated that copper oxide nanoparticles showed more effect on growth than iron oxide nanoparticles.

Micropropagation and callus may show better results after the addition of the thidiazuron (TDZ) hormone. The different unitary concentrations of NPs, and combinations of type growth hormones can be added after digestion of modified MS media are some of the parameters which can be followed for result of callus proliferation.

V. BIBLIOGRAPHY

- Nair S., 2017, UV-Visible and FTIR Spectroscopic studies of fine powders of Azadirachta indica leaves, *International Journal of Advanced Research in Science and Engineering*.
- Navgire A, George I., 2010, Micropropagation from Axenic Seedlings of Abelmoschus Moschatus Medic (Kasturi Bhendi), *Biosci Biotech Res Asia*.
- Concepción G Gómez, M Fernández., 2019, Impacts of metal oxide nanoparticles on seed germination, plant growth and development, *Instituto Nacional de Investigaciones y Tecnologı´a Agraria y Alimentaria (INIA), vol: 84, 75-124.*
- Da costa M.V,J., Sharma P.K., 2016, Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa, *PHOTOSYNTHETICA 54 (1): 110-119*.
- Zakharova O., Kolesnikov E., Shatrova N., Gusev A, 2019, The effects of CuO nanoparticles on wheat seeds and seedlings and Alternaria solani fungi: in vitro study, *IOP Conf. Series: Earth and Environmental Science 226*.
- Adhikari T, Biswas Kumar A, Kundu S, Tarafdar A.C, June 2012, Effect of Copper Oxide Nano Particle on Seed Germination of Selected Crops, *Journal of Agricultural Science and Technology*.
- ELIENE S., DOS SANTOS., DANIELA E., GRACIANO., WILLIAM F., FALCO., ANDERSON R., LIMA CAIRES & EDUARDO J. DE ARRUDA., 2021, Effects of copper oxide nanoparticles on germination of Sesbania virgata (FABACEAE) plants, *An Acad Bras Cienc*.
- Hanaa L., Essa., Mohamed S., Abdelfattah., Alaa S., Marzouk., Hania A., Guirguis, and Mayyada M. H. El-Sayed., 2020, Nano-Formulations of Copper Species Coated with Sulfated Polysaccharide Extracts and Assessment of Their Phytotoxicity on Wheat (Triticum aestivum L.) Seedlings in Seed Germination, Foliar and Soil Applications, *Appl. Sci*.
- Thakur N., Chrungoo S., Rana S., Kaur S., Kaur S., Pathak A., 2021, EFFECT OF COPPER NANOPARTICLES ON IN-VITRO SEED GERMINATION OF WHEAT(TRITICUM AESTIVUM L.) VARIETIES, *International Journal of Pharmaceutical Sciences and Research*.
- Safavi K., 2014, Effect of Titanium Dioxide Nanoparticles in Plant Tissue Culture Media for Enhance Resistance to Bacterial Activity, Bulletin of Environment, *Pharmacology and Life Sciences*.
- Safavi K., 2012, Evaluation of Using Nanomaterial in Tissue Culture Media and Biological activity, *2nd International Conference on Ecological, Environmental and Biological Sciences (EEBS'2012)* Oct. 13-14.
- Pande N., Jaspal D., Malviya A., Jayachandran P, 2015, Green Route Synthesis of Iron Nanoparticles and Antibacterial Studies, *International Journal of Advances in Science Engineering and Technology*, ISSN: 2321-9009 Volume- 3.

- Anwaar S., Maqboo Q., Jabeen N., Nazar M., Abbas F., Nawaz B., Hussain T., Hussain S.Z, 2016. The Effect of Green Synthesized CuO Nanoparticles on Callogenesis and Regeneration of Oryza sativa L. *Front. Plant Sci. 7:1330*.
- Ugwu S., Enweremad C., 2019, Enhancing anaerobic digestion of okra waste with the addition of iron nanocomposite (Fe_3O_4), *Taylor and Francis*.
- Kombaiah, K., Vijaya J., Kennedy L., Bououdina M., Ramalingam R., Al-Lohedan H., 2017, Okra extract-assisted green synthesis of $CoFe_2O_4$ nanoparticles and their optical, magnetic, and antimicrobial properties, *Materials Chemistry and Physics*,
- Ghazal S., Khandannasab N., Hosseini H., Sabouri Z., Rangrazi A., Darroudi M., 2021, Green synthesis of copper-doped nickel oxide nanoparticles using okra plant extract for the evaluation of their cytotoxicity and photocatalytic properties, *Ceramics International*.
- Zafar S., Hasnain Z., Aslam N., Mumtaz S., Jaafar H.Z., Wahab P.E.M., Qayum M., Ormenisan A.N., 2021, Impact of Zn Nanoparticles Synthesized via Green and Chemical Approach on Okra (Abelmoschus esculentus L.) Growth under Salt Stress, *Sustainability (MDPI)*, 13, 3694.
- Alsalhi et al., 2017, Synthesis of Silver Nanoparticles from Abelmoschus esculentus extract, *United States Patent.*
- Plaksenkova I., Jermaļonoka M., Bankovska L., Gavarāne I., Gerbreders V., Sledevskis E., Sniķeris, J., Kokina I., 2019, Effects of Fe3O4 Nanoparticle Stress on the Growth and Development of Rocket Eruca sativa, *Hindawi Journal of Nanomaterials*, Article ID 2678247, 10 pages.
- Lau E., Carvalho L., Pereira A., Montanha G., Corrêa C., Carvalho H., Ganin A., Fraceto L., You H., 2020, Localization of Coated Iron Oxide (Fe3O4) Nanoparticles on Tomato Seeds and Their Effects on Growth. *Applied Biomaterials 3* (7), 4109-4117.
- Asadi-Kavan Z., Khavari-Nejad R., Iranbakhsh A., Najafi F., 2020, Cooperative effects of iron oxide nanoparticle (α-Fe2O3) and citrate on germination and oxidative system of evening primrose (Oentherabiennis L.), *Journal of Plant Interactions*, 15:1, 166-179.
- Rui M., Ma C., Hao Y., Guo J., Rui Y., Tang X., Zhao Q., Fan X., Zhang Z., Hou T., and Zhu S., 2016, Iron Oxide Nanoparticles as a Potential Iron Fertilizer for Peanut (Arachis hypogaea). 7:815, *Frontiers in Plant Science*.
- Alam MD., Sultana F Md. Toufiq I., 2015, Potential of Iron Nanoparticles to Increase Germination and Growth of Wheat Seedling. *Journal of Nanoscience in Advanced Technology 1(3): 14-20*.
- Kornarzyński K., Sujak A., Czernel G., Wiącek D., 2020, Effect of Fe_3O_4 nanoparticles on germination of seeds and concentration of elements in *Helianthus annuus* L. under constant magnetic field**,** *Scientific Researches*, 10: 8068.
- El-Temsah YS., Joner EJ., 2012, *Environ Toxicology*. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil, *Environ Toxicology*, Jan;27(1):42-9.
- VARIETIES, 2021, *International Journal of Pharmaceutical Sciences and Research.*
- Hayat, Khizar, Ali, Shahid, Ullah, Saif, Fu, Yujie and Hussain, Mubashir, Green synthesized silver and copper nanoparticles induced changes in biomass parameters, secondary metabolites production, and antioxidant activity in callus cultures of *Artemisia absinthium* L, *Green Processing and Synthesis*, vol. 10, no. 1, 2021, pp. 61-72.