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Synthesis and Characterization of Copper Oxide and Iron Oxide Nanoparticles and its Impact on Physiological and Biochemical Parameters of *Vigna radiata*

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ABSTRACT

Nanotechnology is an enabling technology that allows us to develop materials with improved or totally new properties. Nanotechnology has the ability to completely transform the way seeds germinate by providing improved development, faster germination rates and greater resistance to environmental stressors. The present study focuses on synthesis and characterization of copper oxide and iron oxide nanoparticles using sol- gel method and chemical precipitation method, further these nanoparticles were subjected to characterization techniques like energy dispersive X-ray microanalysis (EDAX) and transmission electron microscopy (TEM) for evaluating its size, composition and surface morphology. Moreover, the influence of these nanoparticles were observed on physiological and biochemical parameters of *Vigna radiata* seeds. The results indicated that synthesized nanoparticles were copper oxide and iron oxide and were of size 20-50 nm having squarish to spherical morphology for copper oxide nanoparticles and hexagonal to spherical morphology for iron oxide nanoparticles. The *Vigna radiata* seeds exhibited that iron oxide and copper oxide nanoparticles considerably improved physiological characteristics at lower concentrations, however they have phytotoxic effects at greater doses, which impede the growth of seedlings and decrease seed germination. The biochemical indices, such as proteins, phenols, and carbohydrates, were shown to be highest at lower concentrations and to decrease with increasing dosages of nanoparticles.

Keywords Copper oxide, Characterization, Iron oxide, *Vigna radiata.*

INTRODUCTION

Nanotechnology holds significant potential to revolutionize seed germination processes, offering enhanced growth, higher germination rates, and increased resilience to environmental stresses. Due to their distinctive innovative qualities, such as high reaction activities and huge surface area, nanotechnology and nanomaterials have garnered a lot of attention (Jayarambabu *et al.* 2014). Among different types of

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nanomaterials metal and metal oxide nanoparticles play a significant role in agriculture. Metal and metal oxide nanoparticles like titanium dioxide (TiO_2), silver, zinc oxide, cerium dioxide, copper, copper oxide, aluminium, nickel, and iron are primarily investigated for their effects on various plants. Copper oxide nanoparticles (CuO NPs) and iron oxide nanoparticles (Fe_2O_3 NPs) are two of the most widely studied metal oxide nanomaterials due to their distinctive properties. Copper oxide nanoparticles have been investigated for possible use in agriculture, where they may affect plants in both positive and negative ways, contingent on exposure length and concentration. It has been demonstrated that copper oxide NPs enhance photosynthesis, nitrogen uptake, and root growth, hence improving plant growth and development (Feigl 2023).

Iron oxide nanoparticles also have drawn interest in agricultural research because of their special qualities, which include high surface area, biocompatibility, and magnetic activity. These characteristics can have a big impact on plant development, seed germination, and crop performance as a whole. For plants, iron is a vital micronutrient that is involved in metabolic activities such as the production of chlorophyll, photosynthesis, and enzyme activity. In addition to improving seed germination and seedling growth, iron oxide nanoparticles can offer protection from environmental stressors (Shirsat and Suthindhiran 2024, Imran *et al.* 2015). In addition, both CuO and Fe_2O_3 NPs are considered eco-friendly alternatives for improving crop productivity and combating soil-borne diseases in sustainable agricultural practices. In the context of agriculture, the application of CuO and Fe_2O_3 NPs has shown promising results in enhancing seed germination, promoting plant growth, and improving resistance to various stresses such as drought, salinity, and pathogens. The use of CuO and Fe_2O_3 nanoparticles as it offers multiple advantages, including the plant's economic significance, rapid growth cycle, and responsiveness to environmental stresses.

Vigna radiata (moong bean) seeds were selected for the study purpose. *Vigna radiata* has been grown in India and thought to be a native crop of this country. Globally, moong beans (*Vigna radiata*), which belong to the Fabaceae family and is regarded as the most practical and extensively used pulse crops (Kanatt and Sharma 2011). Proteins, fibers, amino acids, lipids, and carbohydrates are all abundant present in moong beans, making them a highly nutritious food. Additionally, its calcium, phosphorus and polyphenol concentrations are balanced (Imran *et al.* 2015). Due to its many beneficial qualities, including its ability to reduce the risk of stroke, minimize stomach problems and have anti-diabetic, anti-inflammatory, and anti-cancer effects, it has been a staple diet for the Chinese for 2000 years (Nair *et al.* 2018).

Various studies have been conducted to study the effect of different types of Metal oxide nanoparticles on seed germination, growth and development and have been reported for several plants (Tripathi *et al.* 2017, Lee *et al.* 2010). However, only few investigations have been conducted to understand the effect of copper oxide and iron oxide nanoparticles at the physiological and biochemical levels in major food crops (Dimkpa *et al*. 2012, Shaw and Hossain 2013).

MATERIALS AND METHODS

Synthesis of copper oxide & iron oxide nanoparticles

The Fig. 1 represents the flow chart for synthesis of copper oxide and iron oxide nanoparticles. The copper oxide nanoparticles were synthesized by Sol- gel method (Thanoon *et al.* 2017) and iron oxide nanoparticles by precipitation method (Lassoued *et al.* 2017).

Characterization of synthesized nanoparticles

A variety of advanced scientific tools, like energy dispersive X-ray microanalysis (EDAX) and transmission electron microscopy (TEM).

A transmission electron microscope (TEM), model Philips Tecnai 20, Holland, with an accelerating voltage of 100 kV, was used to evaluate the particles' size and shape. For TEM examination, the material was placed on copper grids coated with carbon. The samples were made by dispersing the particles in 2-propanol. EDAX was used at a voltage of 30 kV and a magnification of up to 2, 50,000X for elemental

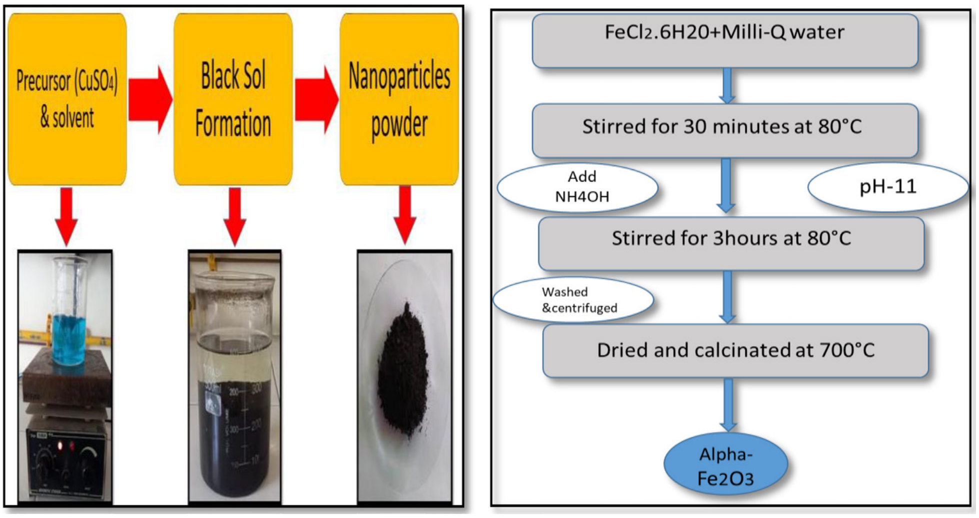

Fig. 1. Synthesis of copper oxide and iron oxide nanoparticles.

analysis. The Sophisticated Instrumentation Center for Applied Research and Technology (SICART) was the center for all characterization activities.

Preparation of seeds and seed priming in nano solutions

For the study purpose two nanoparticles i.e. copper oxide and iron oxide nanoparticles were taken into consideration. Different concentration of nanoparticles were dissolved in double distilled water, further these solutions of different concentration were sonicated thoroughly for even mixing and thus different concentration of nano solutions were prepared as shown in Fig. 2.

As shown in Fig. 2, for the study purpose *Vigna radiata* seeds were selected, these seeds were thoroughly washed with water, then after these seeds were suspended in 2.5% sodium hypochlorite solution for surface sterilization, washed with distilled water and primed in a suspension of different concentration of prepared nano solutions for 12 hours. Then after the primed seeds were kept on double folds of filter paper in a petri plate and then transferred to germination sheets. Each treatment was replicated three times, with ten seeds in each concentration.

Measurement of physiological parameters

Different parameters like germination percentage, seedling length, seedling vigour index and mean root length and shoot length were recorded on different period of growth. The study was done for the period of week.

Germination percentage

By creating ideal circumstances for every experimental set, seed germination was observed. Every experimental set's germination was noted, and the total germination was computed and reported as a percentage (Raskar and Laware 2013).

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Seedling length (cm)

The length of roots and shoots of the germinated seeds were measured for every experimental set. The average shoot length was measured from the base of the primary leaf to the base of the hypocotyl and was stated in centimeters. The mean root length was measured from the primary root's tip to the hypocotyl base and was recorded in centimeters. The seedling length was computed and represented in centimeters by summing the root and shoot lengths (Dash 2012).

Seedling length (cm)= Shoot length + Root length

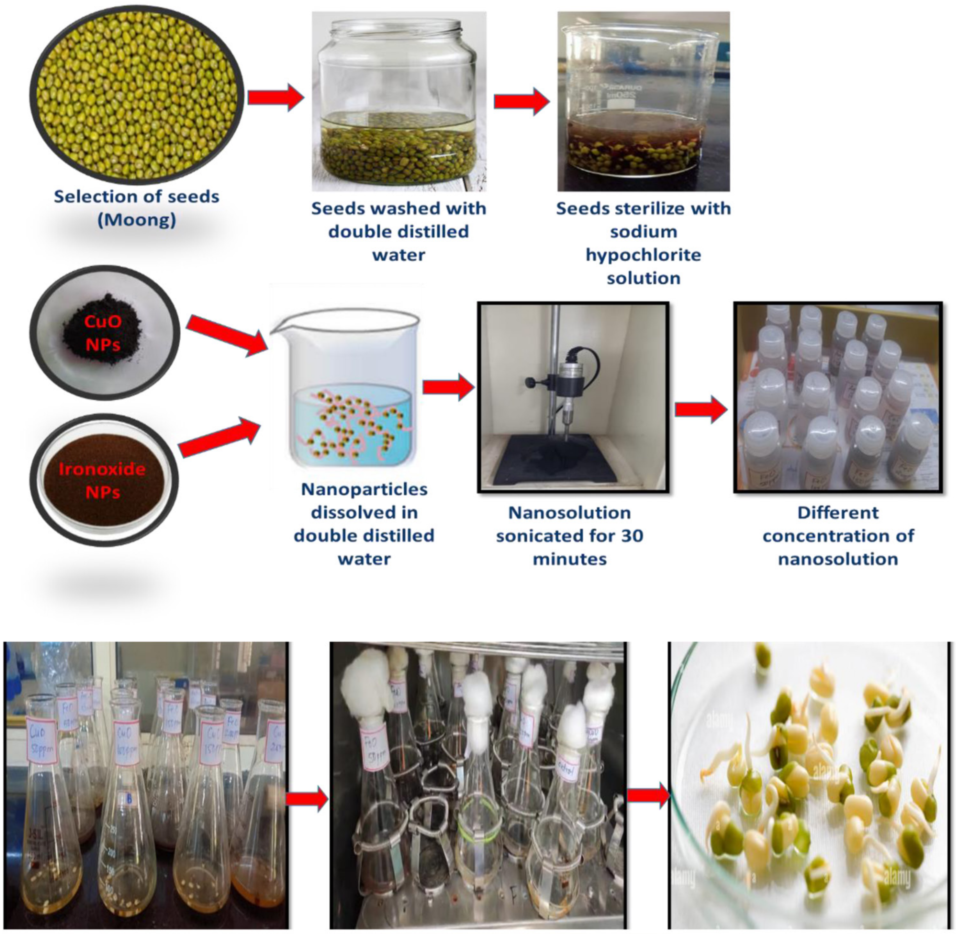

Fig. 2. Methodology for seed priming.

Seed vigor index

The seed vigor index was calculated and given as a number using the formula proposed by (Abul-Baki and Anderson 1973).

SVI= Germination (%) X Seedling length

Measurement of biochemical parameters

Estimation of carbohydrates

The total carbohydrate content was ascertained spectrophotometrically using the Anthrone technique. Carbohydrates were dehydrated using concentrated H_2SO_4, which then interacts with anthrone to produce a furfural blue-green complex that can be detected spectrophotometrically at 630 nm. The glucose standard curve was used to determine the amount of total carbohydrates (Buckan 2015).

Estimation of proteins

Estimation of protein was done by Folin Lowry's Method. The seedlings were thrice washed with double-distilled water before being crushed in five milliliters of 80% ethanol. After centrifuging at 7000 g for 10 minutes at 4 degrees Celsius, the pellet was suspended in 5.0 milliliters of 10% TCA, and the supernatant was disposed of the centrifuged tubes were kept in a water bath set 30 minutes at 60 degrees Celsius. After cooling, the samples were again cen-

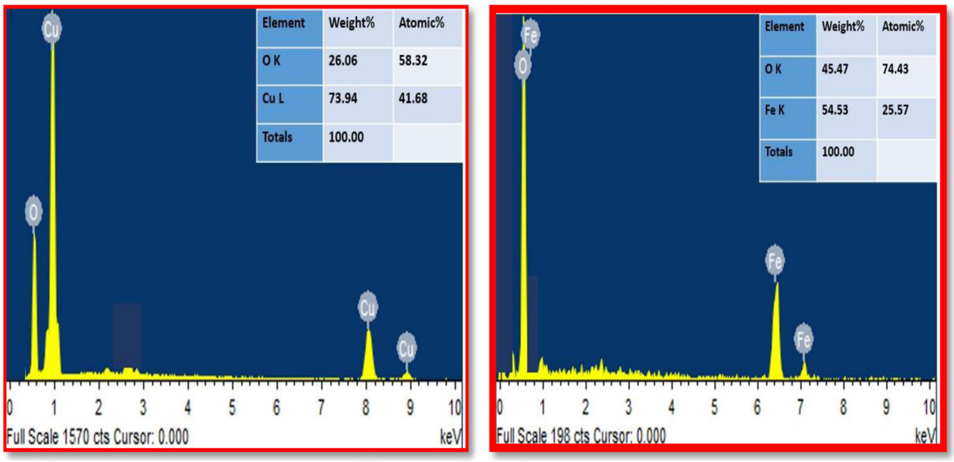

Fig. 3. EDAX Spectra of synthesized copper oxide and iron oxide nanoparticles.

trifuged at 7000 rpm for 10 min at 4 degree Celsius and the pellet was suspended in 1.0 ml of IN NaOH. The samples were centrifuged at 7000 rpm for 10 min at 4 degree Celsius to collect supernatant. To test tubes containing 1.0 ml of the protein extract, addition of 5 ml of the lowry reagent was done and incubated at room temperature for 10 min. Then 0.5 ml of folin-ciocalteau reagent was added rapidly and mixed thoroughly. It was allowed to incubate at room temperature for 30 minutes; the absorbance of the blue solution was read at 660 nm in a spectrophotometer. The amount of protein was estimated using a standard curve of bovine serum albumin (Waterborg 2009).

Estimation of total phenols

The culture was centrifuged at 7000 rpm for 10 min at 4 degree Celsius the supernatant that obtained was discarded and the pellet was suspended in 5.0 ml of 80% ethanol. The pellet was then carefully crushed in a pre-chilled mortar and pestle with glass powder. The suspension was again subjected to centrifugation at 7000 rpm for 10 min (4°C) and the supernatant was collected to estimate the phenol content. One ml of supernatant was taken into clean test tube and subjected to evaporation till dryness. The deposits were allowed to get dissolved in 2.0 ml of distilled water and 0.5 ml of FCR reagent. After incubating the tubes for about 3 min, 2.0 ml of 20% $Na2CO_3$ solution was added into each tube, mixed properly and was placed on boiling water bath for exact 1 min. The blue-colored complex was produced which was measured at 650 nm and the amount of total phenol was calculated against a calibration curve prepared by catechol (Mundhe *et al.* 2011).

RESULTS AND DISCUSSION

Characterization of nanoparticles

Energy dispersive X-ray microanalysis (EDAX)

The elemental composition of the nanoparticles can be determined through EDAX analysis. The copper oxide nanoparticle has 73.94% copper and 26.06 % oxygen by weight, which indicates the successful synthesis of copper oxide nanoparticles, similar composition was observed by(Amna *et al.* 2013), correspondingly the iron oxide nanoparticle has 54.53 % iron and 45.47 % oxygen as shown in Fig. 3 (Abhilash *et al.* 2019).

TEM (Transmission electron microscopy) analysis

TEM analysis pictures of copper oxide and iron oxide nanoparticles were generated at room temperature indicating that the copper oxide nano catalyst were of agglomerated spherical to squarish shape with an average size about 20-50 nm. On contrary iron oxide nano catalyst exhibited hexagonal to spherical shape (Fig. 4) (Pertiwi *et al.* 2021). The particle size of each nano catalyst was found to be consistent with

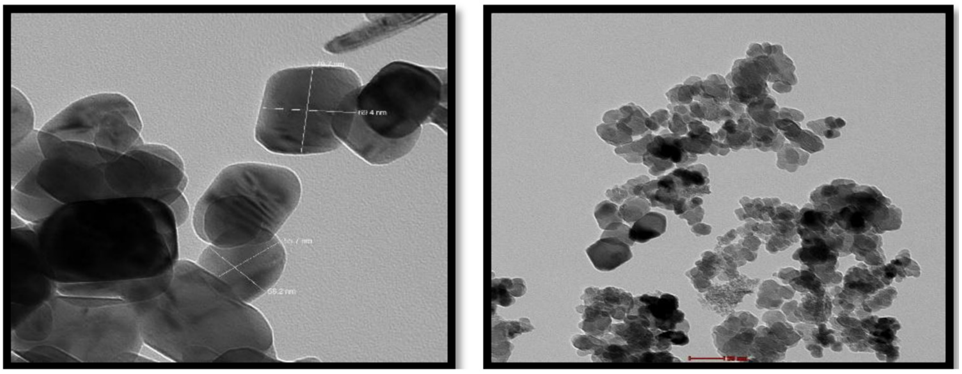

Fig. 4. TEM images of synthesized copper oxide and iron oxide nanoparticles.

the particle size estimated using XRD examination.

Measurement of physiological parameters

Effect on seed germination, seedling length, vigor index and mean root length and shoot length of Vigna radiata

The germination percentage of the *Vigna radiata* decreased with an increase in the copper oxide and iron oxide nanoparticles. As shown in Fig. 5(A) at lower concentrations (50 ppm) the germination percentage was maximum i.e. 90% and 80% for copper oxide and iron oxide nanoparticles, but as the concentration increases, the germination percentage shows a drop, with only 50% and 45% at 200 ppm concentration for both the nanoparticles. The results revealed that an increasing concentration of nanoparticles results in decreases germination percentage. Enhanced seed germination in lower concentration of NPs may be attributed with the findings of (Raja *et al.* 2019) as stated that NPs have tendency to penetrate plant seed coats and enhance seed germination and growth.

The seed *Vigour index* of *Vigna radiata* decreased with increasing copper oxide and iron oxide nanoparticle concentrations. As shown in Fig. 5(B), the seed vigor index at 50 ppm concentration was 859.5 and 701.6 for copper oxide and iron oxide nanoparticle respectively. However, the seed vigour index was reduced to 286.5 and 234.45 at 200 ppm concentration was observed for *Vigna radiata.*

The moong beans were germinated on germination sheets for period of 7days root and shoot length were measured on alternate day up to 6th day by using ruler. The increasing trend was observed as the number of days increased. As shown in Fig. 5(C) the shoot length of copper oxide treated seeds increased from 3.97 cm to 10.05 cm from day 2 to day 6 at 50 ppm concentration, whereas root length increased 5.58 cm to 13.65 cm at 50 ppm concentration. For iron oxide nanoparticles the shoot length of treated seeds increased from 3.74 cm to 9.72 cm at 50 ppm concentration from day 2 to day 6 and root length increased from 5.03 cm to 12.05 cm at 50 ppm concentration this may be due to combination of improved nutritional availability, hormonal stimulation, increased stress tolerance, and the plant's normal development cycle causes moong beans treated with copper oxide and iron oxide nanoparticles to gradually lengthen their roots and shoots. The apparent increase in length is the result of several variables working together to support continuous growth as the days pass.

Seedling length is an important indicator of plant growth. It is an essential measure in assessing a plant's growth, health, and potential for successful establishment. As shown in Fig. 5(D) at lower concentration of 50 ppm the seedling length for both nanoparticles was highest i.e. 9.55 cm and 8.77 cm for copper oxide and iron oxide nanoparticles respectively and as concentration increased the seedling length decreased this is supported by fact that the seedlings may grow more quickly at lower concentrations of copper oxide and iron oxide nanoparticles because of the nutritional advantages and stress-reduction strategies, which will result in longer seedlings. However, at higher con-

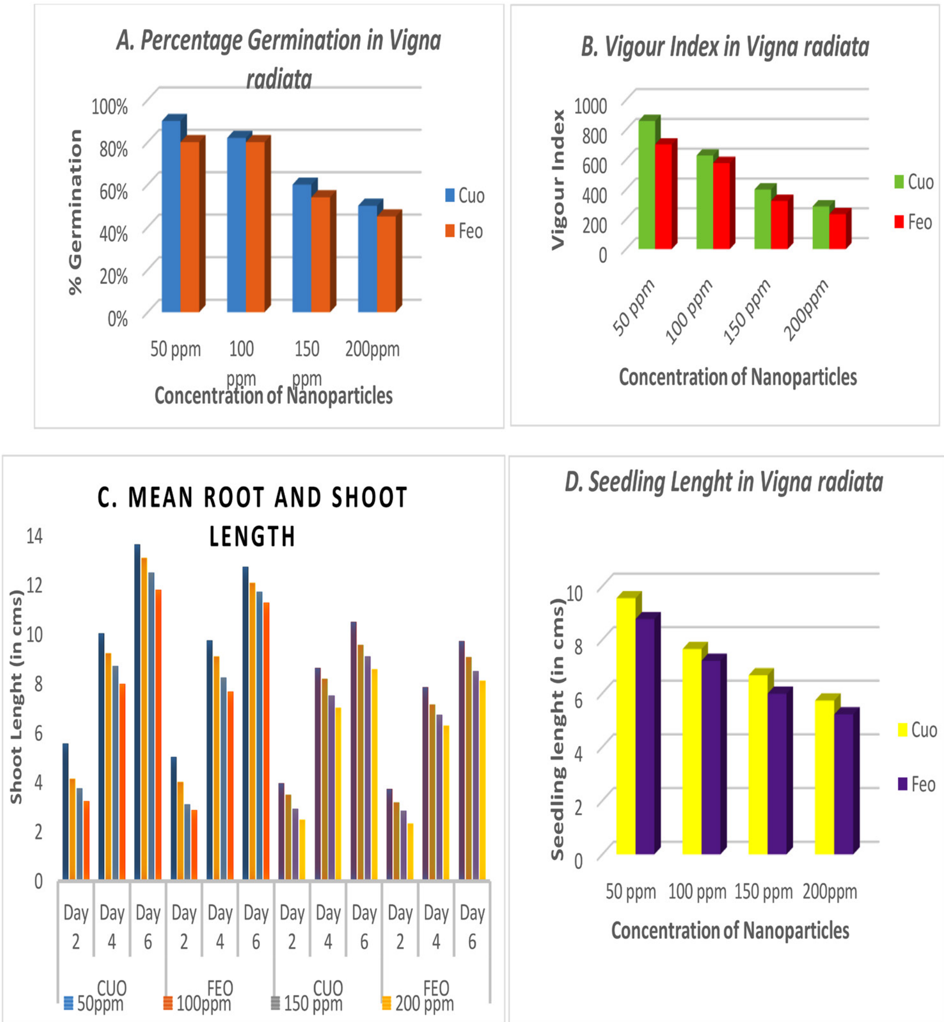

Fig 5. Graph showing percentage (A) seed germination, (B) vigour index, (C) mean root and shoot length and (D) seedling length of *Vigna radiata.*

centrations, the toxicity and harmful effects of these nanoparticles can overwhelm the plant's protective mechanisms, resulting in stunted growth, oxidative stress, and reduced seedling length (Sarkhosh *et al.* 2022).

Measurement of biochemical parameters

Carbohydrates content

As shown in Fig. 6 (A) the copper oxide nanoparticles at day 2 at 50 ppm concentration the Carbohydrate content was 5.6 mg/g followed by 5.2 mg/g and 4.8 mg/g at 100ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 4mg/g. At day 4 at 50 ppm concentration the carbohydrate content was 7.2 mg/g followed by 6.7 mg/g and 6mg/g at 100ppm and 150 ppm and lowest was recorded at 200 ppm which was around 5.4 mg/g. At day 6 at 50 ppm concentration the carbohydrate content was 8.1 mg/g followed by 7.4 mg/g and 6.8 mg/g and lowest was recorded at 200 ppm which was around 6 mg/g.

For Iron oxide nanoparticles at day 2 at 50 ppm

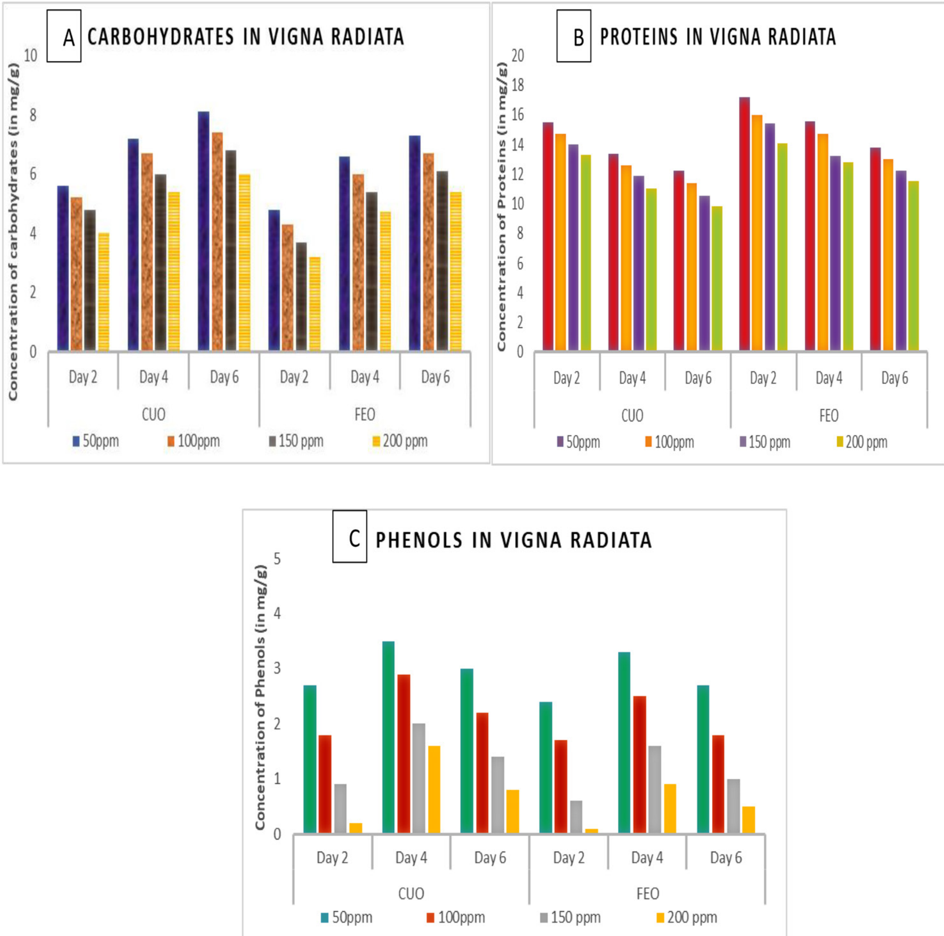

Fig. 6. Graph showing different biochemical parameters (A) carbohydrates, (B) proteins, (C) phenols for *Vigna radiata.*

concentration the carbohydrate content was 4.8 mg/g followed by 4.3 mg/g and 3.7 mg/g at 100 ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 3.2 mg/g. At day 4 at 50 ppm concentration the carbohydrate content was 6.6 mg/g followed by 6 mg/g and 5.4 mg/g at 100ppm and 150 ppm and lowest was recorded at 200 ppm which was around 4.7 mg/g. At day 6 at 50 ppm concentration the carbohydrate content was 7.3 mg/g followed by 6.7 mg/g and 6.1 mg/g and lowest was recorded at 200 ppm which was around 5.4 mg/g.

The initial increase could be a part of a stress response (enhanced synthesis of soluble sugars), especially at lower concentrations. This could be due to the plant's attempt to store more energy as a result of oxidative stress. As number of days passed at lower concentrations, carbohydrates might stabilize or decrease depending on the recovery ability of the plants. At higher concentrations there could be a decrease in carbohydrate content. This is likely due to the plants' inability to effectively synthesize carbohydrates due to stress-induced damage or inhibition of key metabolic pathways stress. It has been reported in various studies that metal oxide NPs at their high concentration alters the physiological processes of the plants (Singh *et al.* 2017).

Protein content

As shown in Fig. 6 (B) for copper oxide nanoparticles at day 2 at 50 ppm concentration the protein content was 15.5 mg/g followed by 14.7 mg/g and 14mg/g at 100 ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 13.3mg/g. At day 4 at 50 ppm concentration the protein content was 13.4 mg/g followed by 12.6 mg/g and 11.9 mg/g at 100 ppm and 150 ppm and lowest was recorded at 200 ppm which was around 11 mg/g. At day 6 at 50 ppm concentration the protein content was 12.2 mg/g followed by 11.4 mg/g and 10.5 mg/g and lowest was recorded at 200 ppm which was around 9.8 mg/g.

For iron oxide nanoparticles at day 2 at 50 ppm concentration the protein content was 17.2 mg/g followed by 16 mg/g and 15.4 mg/g at 100 ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 14.1mg/g. At day 4 at 50 ppm concentration the protein content was 15.6 mg/g followed by 14.7 mg/g and 13.2 mg/g at 100 ppm and 150 ppm and lowest was recorded at 200 ppm which was around 12.8 mg/g. At day 6 at 50 ppm concentration the protein content was 13.8 mg/g followed by 13 mg/g and 12.2 mg/g and lowest was recorded at 200 ppm which was around 11.5 mg/g.

The decrease in protein content in moong beans treated with copper oxide and iron oxide nanoparticles over time is a result of oxidative stress, inhibition of protein synthesis, and increased protein degradation. The severity of these effects is more pronounced at higher nanoparticle concentrations and increases over time as the plant experiences prolonged stress. The balance between stress-induced protein production and degradation shifts toward protein loss, leading to the observed decrease in protein content from day 2 to day 6 (Singh *et al.* 2017).

Phenol content

As shown in Fig. 6(C) for copper oxide nanoparticles at day 2 at 50 ppm concentration the phenol content was 2.7 mg/g followed by 1.8 mg/g and 0.9 mg/g at 100ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 0.2 mg/g. At day 4 at 50 ppm concentration the phenol content was 3.5 mg/g followed by 2.9 mg/g and 2 mg/g at 100 ppm and 150 ppm and lowest was recorded at 200 ppm which was around 1.6mg/g. At day 6 at 50 ppm concentration the phenol content was 3 mg/g followed by 2.2 mg/g and 1.4 mg/g and lowest was recorded at 200 ppm which was around 0.8 mg/g.

For iron oxide nanoparticles at day 2 at 50 ppm concentration the phenol content was 2.4 mg/g followed by 1.7 mg/g and 0.6 mg/g at 100 ppm and 150 ppm concentration and least was recorded at 200 ppm concentration which was around 0.1 mg/g. At day 4 at 50 ppm concentration the phenol content was 3.3 mg/g followed by 2.5 mg/g and 1.6 mg/g at 100 ppm and 150 ppm and lowest was recorded at 200 ppm which was around 0.9 mg/g. At day 6 at 50 ppm concentration the phenol content was 2.7 mg/g followed by 1.8 mg/g and 1 mg/g and lowest was recorded at 200 ppm which was around 0.5 mg/g. Thus, the increase in phenolic content over time at lower nanoparticle concentrations is due to the plant's antioxidant response, while the decrease at higher concentrations reflects the plant's inability to maintain this response under intense stress (Singh *et al.* 2017).

CONCLUSION

The study indicates successful synthesis of copper oxide and iron oxide nanoparticles. The characterization technique helped in confirming the shape, size and composition of nanoparticles. The results proved successful synthesis of both the nano catalyst. Furthermore the influence of synthesized NPs on different physiological parameters like seed germination, seedling length, seed vigor index and mean root and shoot length was studied as well as different biochemical parameters like Carbohydrates, proteins and phenol content of *Vigna radiata* were also investigated. The results indicated that at lower concentrations of copper oxide and iron oxide nanoparticles significantly enhanced physiological properties. However, at higher concentrations, all nanoparticles exhibit phytotoxic effects, reducing seed germination and inhibiting seedling growth. The biochemical parameters like carbohydrates, proteins and phenols were found highest at lower concentration and declined at higher concentration of nanoparticle dosage.

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