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Impact of copper and iron nanoparticles on photosynthesis and stomatal frequency in wheat plant

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Abstract
Wheat (*Triticum aestivum*), known as one of the most crucial cereal grains on a global scale, occupies a significant role in the assurance of food security internationally, thereby rendering the augmentation of its productivity an essential aim within agricultural research endeavors. This investigation aims to elucidate the impacts of CuO and FeO nanoparticles on the mechanisms of photosynthesis and stomatal frequency in wheat specimens, highlighting a thorough comprehension of their physiological and morphological consequences. Copper and iron nanoparticles were administered at two distinct concentrations: FeO at 25 µM and 500 µM, and CuO at 15 µM and 100 µM.The results revealed a concentration-dependent effect on plant performance. At lower concentrations, both nanoparticles significantly improved plant morphology, as reflected by increased root and shoot length, enhanced photosynthetic rates, higher stomatal frequency, and elevated protein content. These enhancements suggest that appropriately dosed nanoparticles can positively modulate key physiological and biochemical processes. On the other hand, higher concentrations of CuO and FeO nanoparticles induced oxidatice stress, resulting in stunted growth, reduced photosynthetic efficiency, and decreased stomatal frequency. These outcomes tell us the dual nature of nanoparticles at different concentrations and the importance of proper dosage of nanoparticles to maximize the benefits.

Keywords: Copper nanoparticle, iron nanoparticle, wheat crop, photosynthesis, stomatal frequency and oxidative stress

1. Introduction
Wheat (*Triticum aestivum* L.) is a primary crop, globally consumed as staple food by most of the population (Snape and Pánková, 2013) [25]. When combined, these crops offer vital nutrients, constitute a staple diet in many nations, and sustain the livelihoods of millions of farmers. Their output has a direct effect on socioeconomic stability, especially in developing countries like China and India where population growth is fuelling the demand for food. Improving the resilience and productivity of rice and wheat crops is a crucial task for contemporary agriculture, particularly in light of resource constraints, climate change, and rising biotic and abiotic stresses (Noya *et al*., 2018; Godfray *et al*., 2010) [18, 6].

Nanotechnology presents a paradigm shift in agricultural methodologies by offering the ability to manipulate materials at the nanoscale, potentially revolutionizing plant nutrition, stress management, and overall crop yield (Tripathi *et al*., 2017). The escalating integration of nanotechnology into agricultural research is driven by its potential to develop advanced tools, such as nanosensors for precise detection of biotic and abiotic stresses, nano pesticides designed for enhanced targeting and reduced environmental impact, and nano fertilizers formulated to optimize nutrient delivery and minimize waste (Pérez-Velasco *et al*., 2020) [19]. Nano fertilizers, specifically engineered to improve nutrient use efficiency, hold promise for increasing crop yields while reducing the dependence on conventional chemical fertilizers, thereby mitigating ecological imbalances and promoting sustainable agricultural practices (Nongbet *et al*., 2022) [17]. Nanomaterials have been used in increasing numbers in agriculture in recent years, especially to improve grain nutritional content, boost yield, and improve crop development and stress tolerance. These materials go through a number of intricate processes in the soil, including migration, transformation, dissolution, precipitation, dispersion, aggregation, and redox reactions, because of their small size, huge surface area, and high reactivity (Nair, 2016) [15].

Through formulations based on nanomaterials, like nano-fertilizers used in agriculture, nanotechnology has the potential to support the agricultural sector (Prasad *et al*., 2017). Because it minimises nutrient losses, fertiliser application in the form of nanoparticles may be beneficial for plant fertilisation (Ahmad *et al*., 2019) [1].

The introduction of metal-based nanoparticles into agricultural systems prompts an imperative to scrutinize their potential impacts on plant physiology, particularly concerning photosynthetic efficiency and stomatal regulation, as these processes are fundamentally linked to plant productivity and overall ecosystem health (Zhao *et al*., 2020) [31]. The presence of nanoparticles can influence stomatal behaviour, which is crucial for gas exchange and photosynthesis. While specific studies on stomatal frequency are limited, the general impact of nanoparticles on plant physiology suggests potential alterations in stomatal function due to changes in water and nutrient uptake (Francis *et al*., 2024) [5]. Nanoparticles can induce oxidative stress, which affects photosynthesis and stomatal function. Plants may respond by activating antioxidant enzymes, although excessive nanoparticle exposure can overwhelm these defences, leading to impaired photosynthetic performance (Jampilek & Kráľová, 2019) [8].

Copper is not only a micronutrient, but a vital catalytic component in various enzymatic processes that is necessary for thr plant growth (Mydy *et al*., 2021) [14]. Copper oxide nanoparticles (CuO NPs) are viewed as some of the most essential engineered nanoparticles attributable to their remarkable optical, electrical, and catalytic attributes. They find extensive applications in various fields including electronics, ceramics, films, polymers, inks, metallics, and coatings (Richard and Kauppinen, 2000) [16]. Copper nanoparticles exhibit a dual nature, being beneficial at low concentrations by enhancing photosynthesis and chlorophyll content, but potentially harmful at higher concentrations due to oxidative stress (Rai *et al*., 2021) [20]. On the other hand, Iron plays a vital role in various physiological processes in plants, including chlorophyll synthesis, respiration, and DNA synthesis (Zhang *et al*., 2023) [30] whereas, Iron-based nanoparticles have been shown to enhance photosynthesis in Gramineae plants, by promoting chlorophyll synthesis and improving plant growth. Specifically, iron nanoparticles have been observed to increase leaf length and chlorophyll content in wheat, although they can also reduce light absorption at certain wavelengths, affecting chlorophyll a and b content (Feng *et al*., 2022) [4]. Through in situ immobilization, iron nanoparticles have been demonstrated via experiments to successfully reduce the potential leachability of heavy metals and stop their movement into deeper soil layers, rivers, and groundwater (Fajardo *et al*., 2015) [3].

Use of many nanoparticles can negatively affect the plant growth when used at certain concentrations, often leading to changes in seed germination and overall development of the plant (Lin and Xing, 2008; Lee *et al*., 2010; Singh *et al*., 2018) [12, 10, 23]. However, when it comes to major agricultural crops, only a few studies have explored how copper oxide nanoparticles (CuO NPs) and iron oxide nanoparticles (FeO NPs) can affect plants at the biochemical, physiological, and molecular levels, as well as their potential toxicity (Dimkpa *et al*., 2012; Shaw and Hossain, 2013) [2, 22]. Thus, the goal of the current study was to examine how iron and copper nanoparticles affect photosynthesis, stomatal frequency, and important leaf, root, and other structures. Since over half of the world's population depends on wheat as their staple food.

2. Materials and Methods

2.1. Plant growth conditions

Wheat seeds (*Triticum aestivum*) were procured from the Indian Agricultural Research Institute (IARI). The seeds were then washed properly and sterilization process was done using 10% (v/v) sodium hypochlorite solution. After that the seeds were rinsed many four to five times with distilled water and with distilled water then it was soaked in distilled water for 4 hours. Following this, the seeds were placed in the petriplates (150 mm, Riviera TM) while keeping Whatman No.1 filter paper on it, which had been moistened with a half-strength Hoagland solution. The seeds were then kept in darkness overnight at a temperature of 25 ± 2 °C to facilitate germination after which the seeds were transferred to a plant growth chamber (Biotechnologies Inc.) where they were subjected to a 12-hour dark photoperiod, a photon flux density of 300 µmol photons m^2 s^{-1}, with relative humidity of 65-70% and a temperature at 25 ± 2 °C. After eight days, uniformly sized seedlings were given different treatments and then grown in distinct pots.

2.2. Treatments provided to the wheat seedlings

Uniform sized wheat seedlings were subjected to different treatments, i.e., 25 µM FeO (Iron oxide nanoparticle), 500 µM FeO (Iron oxide nanoparticle), 15 µM CuO (Copper oxide nanoparticle) and 100 µM CuO (Copper oxide nanoparticle) for 7 days. The doses were then chosen based on screening experiments. During the development of seedlings in these 7 days, the half-strength Hoagland solution, pH 6.8) of each treatment was changed once in 3 days and to prevent root anoxia, the seedlings were aerated daily. The respective doses were chosen based on screening experiments. During these 7 days of development of seedlings, the nutrient solution (half-strength Hoagland solution, pH 6.8) of each treatment was replaced once and to prevent root anoxia, seedlings were daily aerated. The seedlings were taken after 7 days of treatment and analysed for biochemical and morphological parameters.

2.3. Growth parameters analysis

After harvesting, lengths of roots and shoots of seedlings and their fresh weights were determined for the estimation of growth parameters of wheat seedlings. Roots and shoots were separated and their lengths were measured by a centimetre scale. Analytical weighing balance was utilized to measure the fresh weight of roots and shoots. For dry weight measurement, roots and shoots were wrapped in a butter paper and kept in hot air oven for 48 hours at 65 ℃. (Tripathi, Tripathi, Chauhan, & Kumar, 2017) [27, 28]

2.4. Assessment of Photosynthetic performance

For estimation of photosynthetic pigments, 20 mg of leaf sample was homogenized in 80% v/v acetone solution (5ml). The extracts were centrifuged at 10,000 rpm for 15 mins at 4℃ was combined with 4.5 ml acetone. The absorbance of the final solution was recorded at 663.2, 646.5 and 470 nm by using UV-visible double beam spectrophotometer (Labman, Model-LMSPUV1920). Then the chlorophyll content and total carotenoids content were calculated using equations of Lichtenthaler (1987) [11].

2.5. Histochemical staining of wheat roots

Several staining techniques were used to visualize oxidative damage, lipid peroxidation, and cell viability to understand how

wheat roots respond to stress at the cellular level. Root tips and sections were treated with nitro blue tetrazolium (NBT) to detect superoxide anions. After soaking in the solution under light for several hours, areas with superoxide buildup turned dark blue, clearly marking the stress-affected regions. Similarly, the presence of hydrogen peroxide was detected using 3,3′-diaminobenzidine (DAB). This method stained affected cells a reddish-brown colour, helping to pinpoint where hydrogen peroxide had accumulated. To identify damage caused by lipid peroxidation, which often occurs under stress, Schiff's reagent was used. After preparing the root tissues with periodic acid, Schiff's reagent reacted with the aldehydes produced during membrane breakdown, resulting in a distinctive magenta color in damaged areas. Finally, to check for cell viability, roots were stained with Evans Blue, a dye that only enters and stains dead or damaged cells. Blue-stained regions revealed where cells had lost membrane integrity. All stained samples were subsequently analyzed utilizing a light microscope to systematically record and evaluate the degree of damage observed under varying conditions.

2.6 Total protein measurement

The total protein in each sample was measured by using the method of (Lowry *et al*. 1951) [13], modified by (Herbert *et al*. 1971) [7] by adapting bovine serum albumin as the standard. The control and treated wheat seedlings samples were mixed with 10 ml of 50 mM potassium phosphate buffer at pH 6.8 and then centrifuged for 15 mins at 8000 g. For ten minutes a standard volume (0.5ml) of this extract was mixed with 0.5 ml of 1N NaOH and was put in a boiling water bath. Reagent C was prepared by mixing 0.2% Na_2CO_3 in 0.1N NaOH with 0.5% $CuSO_4.5H_2O$ IN 1.0% potassium sodium tartarate in the ratio 100:2, then this was added to the reaction mixture after cooling. The reaction mixture was left to sit at room temperature for 15 mins before adding Folin-Ciocalteu's reagent. After 15 mins of incubation, the resulting blue color was read spectrophotometrically at 650 nm for its OD. The bovine serum albumin (BSA) standard curve was used to estimate the protein content.

2.7 Anatomical analysis

Transverse hand sections (TS) of roots of control and treated seedlings were made, stained with 50% safranin and mounted on glycerin and observed by the Olympus microscope (CH20i) equipped with a digital camera (DSCW-7). Epidermal cells, cortex cells, endodermal cells, vascular bundles, xylem and phloem were observed on 10× and 40× magnifications.

2.8 Stomatal frequency

For the measurement of stomatal frequency in control and treated wheat leaves, epidermal peels were prepared and mounted in glycerine jelly and then the number of stomata was determined in 1 mm^2 area using digital camera attached with an optical microscopy on 10 x.

2.9 Statistical analysis

The data was subjected to one-way analysis of variance using SPSS 16.0 software, following confirmation of normal distribution. Differences between treatments were assessed using Duncan's multiple range test (DMRT) at a significance level of $p<0.05$. The reported values represent the means obtained from three distinct biological replicates (n=3)

3. Results and Discussion

3.1. Effect of FeO and CuO on seedling growth

The effect of FeO NPs and CuO NPs on the growth of wheat seedlings was measured in terms of fresh and dry weight as well as root and shoot length. At lower concentrations, the FeO-NPs (25 µM) resulted in a significant increase in shoot fresh weight by 41.1% and root fresh weight by 18.1% compared to the control. Similarly, CuO NPs at 15 µM enhanced shoot fresh weight by 29.4% and root fresh weight by 30.3%. In contrast, at higher concentrations, both FeO NPs (500 µM) and CuO-NPs (100 µM) led to a decline in shoot fresh weight by 38.23% and 35.29%, respectively, and root fresh weight decreased by 21.2% and 27.2% compared to the control. (As shown in Fig. 3 & Fig. 4)

The dry weight measurements reflected the fresh weight results. At lower concentrations, FeO NPs at 25 µM increased shoot dry weight by 22.2% and root dry weight by 33.3%. CuO NPs at 15 µM showed an even more pronounced effect, with shoot dry weight increasing by 33.3% and root dry weight by 66.6% compared to control. Conversely, at higher concentrations, FeO NPs at 500 µM reduced shoot dry weight by 22.2% and root dry weight by 33.3%, while CuO-NPs at 100 µM resulted in a more severe reduction, with shoot dry weight decreasing by 44.4% and root dry weight by 66.6%. (As shown in Fig. 5 & 6)

In case of seedling length, lower concentrations of FeO NPs at 25 µM and CuO NPs at 15 µM resulted in increased shoot lengths by 12.37% and 19.79%, respectively. Root lengths also showed significant increases of 57.2% with FeO NPs and 77.05% with CuO NPs. However, at elevated concentrations, FeO NPs (500 µM) caused a decrease in shoot length by 25.2% and CuO NPs at 100 µM reduced shoot length by 3.9%. Root lengths were similarly affected, with reductions of 16% and 37.2% for FeO NPs and CuO NPs, respectively, compared to the control (As shown in Fig. 1 & Fig. 2).

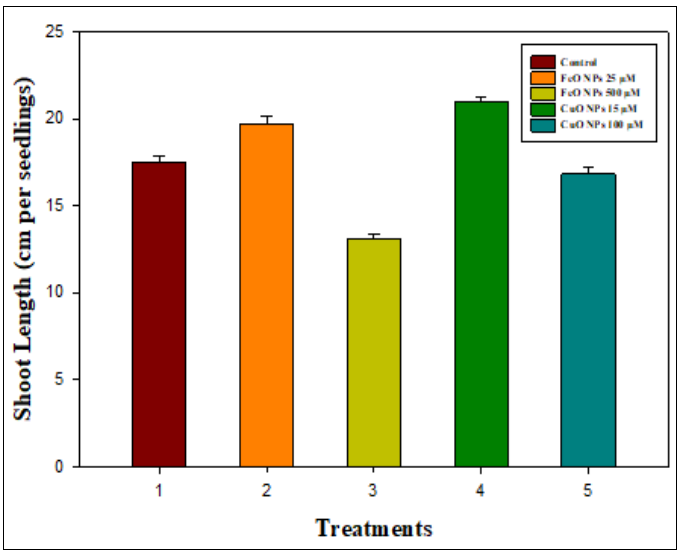

Fig 1: Graphical representation of impact in shoot length of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

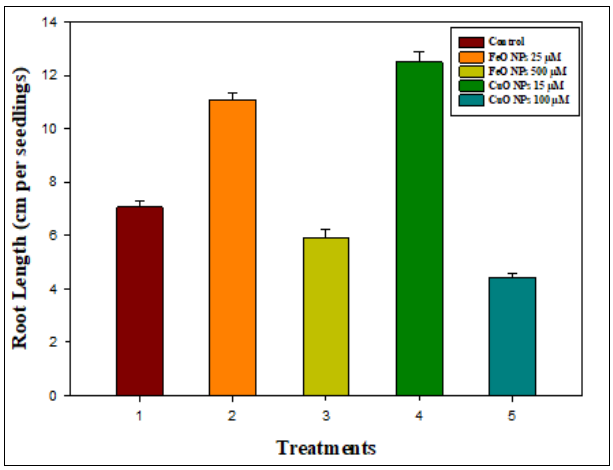

Fig 2: Graphical representation of impact in root length of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

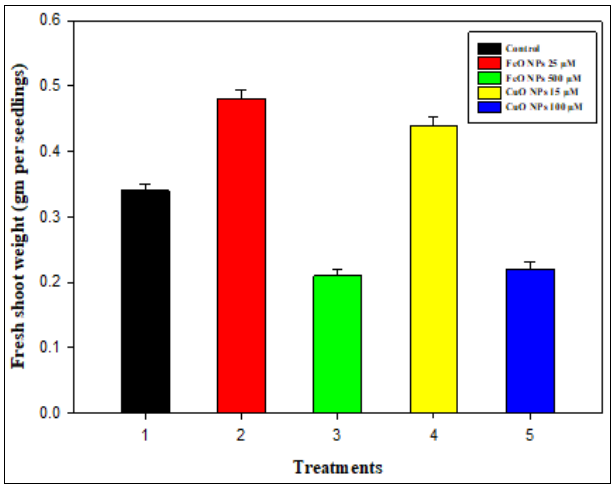

Fig 3: Graphical representation of impact in fresh shoot weight of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

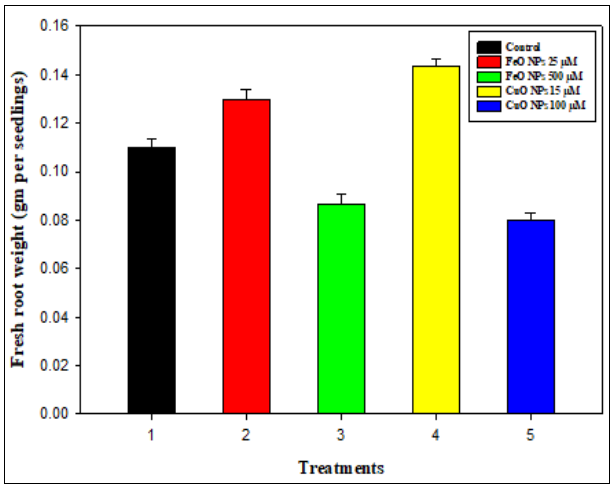

Fig 4: Graphical representation of impact in fresh root weight of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test

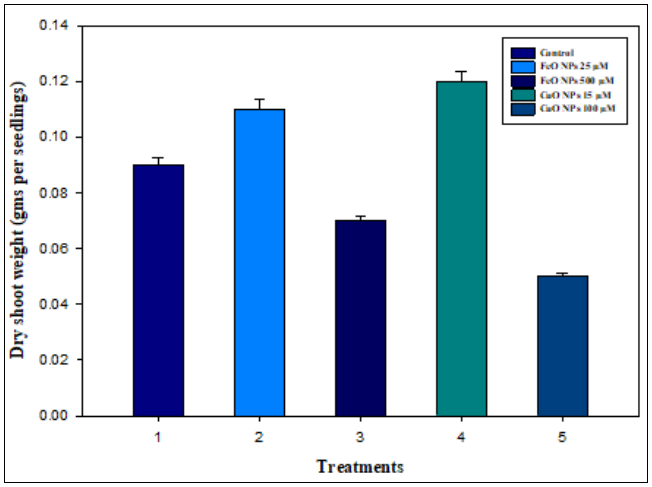

Fig 5: Graphical representation of impact in dry shoot weight of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

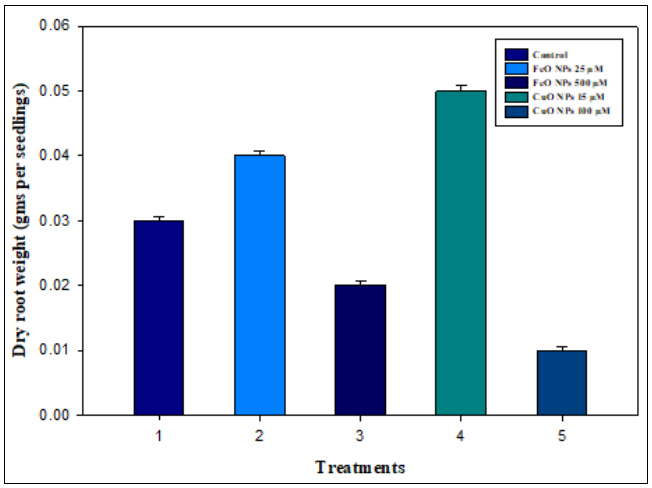

Fig 6: Graphical representation of impact in dry root weight of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

3.2. Effect on Stomatal Frequency

The result suggests that FeO NPs at 25 µM resulted in a notable increase in stomatal frequency by 48.48%, while CuO NPs at 15 µM led to an increase of 31.81% compared to control. This data suggests that at lower concentrations, these nanoparticles can facilitate improved gas exchange efficiency, potentially enhancing photosynthetic performance and overall plant health. However, at higher concentrations, both FeO NPs at 500 µM and CuO NPs at 100 µM caused significant reductions in stomatal frequency, with 21.21% and 25.75% reduction compared to control (As shown in Fig. 7). This data indicate that there is a threshold beyond which the beneficial effects of nanoparticles are negated, likely due to toxic effects or physiological stress induced by the nanoparticles. This duality highlights the importance of nanoparticle concentration in agricultural applications, as excessive levels may adversely affect plant physiology. The increase in stomatal frequency at lower concentrations suggests that these nanoparticles may enhance the physiological processes involved in stomatal development

and function, possibly through improved nutrient availability or stimulation of growth pathways. This enhancement can be beneficial for the plant's photosynthetic efficiency and overall health, as a higher stomatal frequency typically facilitates greater gas exchange. However, the observed decline in stomatal frequency at higher concentrations of nanoparticles indicates a threshold beyond which these substances may become detrimental to plant health. The reduction in stomatal frequency at elevated concentrations could be attributed to potential phytotoxic effects of the nanoparticles, which may disrupt normal cellular processes or lead to oxidative stress (Feng *et al*., 2022 [4]

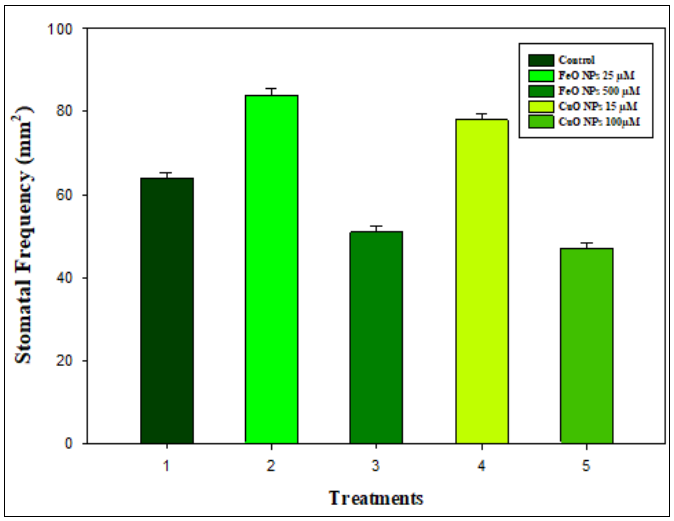

Fig 7: Graphical representation of impact in stomatal frequency of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 3 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

3.3 Photosynthetic pigments: Total chlorophyll (Chl a and Chl b) and Total carotenoids

The shoot chlorophyll pigments and carotenoids exhibited variable responses when subjected to different treatments of FeO and CuO nanoparticles in comparison to control. Specifically, the concentration of chlorophyll a was significantly increased by 48.3% and 43.8% under the treatments of FeO NPs at 25 µM and CuO NPs at 15 µM, respectively. On the other hand, a decrease of 7.2% and 19.9% was recorded with FeO NP at 500 µM and CuO NP at 100 µM, respectively, when compared to the control seedlings (As shown in Fig.8). Correspondingly, chlorophyll b concentrations were elevated by 41.4% and 17.5% under FeO NPs at 25 µM and CuO NPs at 15 µM, while reductions of 33.6% and 18.1% were observed under FeO NP at 500 µM and CuO NPs at 100 µM, respectively (As shown in Fig.9). The total chlorophyll content exhibited a parallel trend, demonstrating a significant increase of 45.7% and 34.2% with the application of FeO NPs at 25 µM and CuO NP at 15 µM, whereas it experienced a decline of 16.8% and 19.3% under the treatments of FeO NPs at 500 µM and CuO NPs at 100 µM, respectively (As shown in Fig.10). Regarding total carotenoid levels, the content was enhanced by 41.6% and 12.0% under the FeO NP at 25 µM and CuO NP at 15 µM treatments, but exhibited a notable decrease of 18.5% and 8.0% under FeO NP at 500 µM and CuO NPs at 100 µM, respectively, in relation to the control plants (As shown in Fig.11).

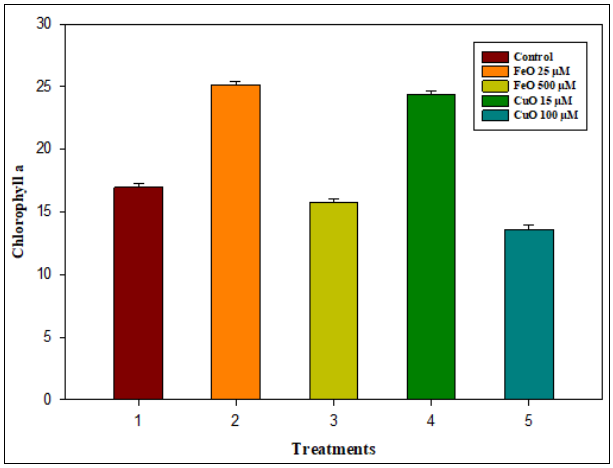

Fig 8: Graphical representation of impact in Chlorophyll a of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 6 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

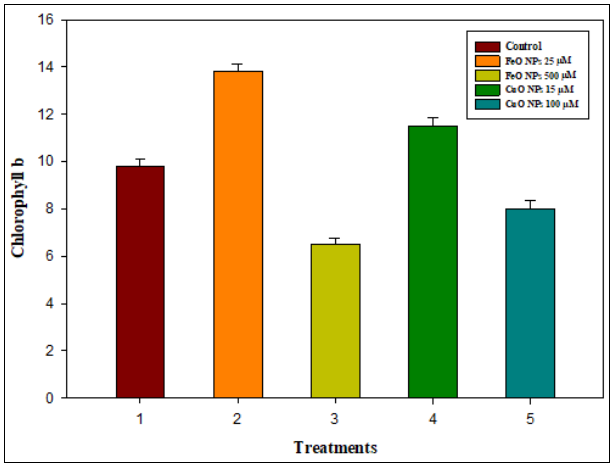

Fig 9: Graphical representation of impact in Chlorophyll b of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 6 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test.

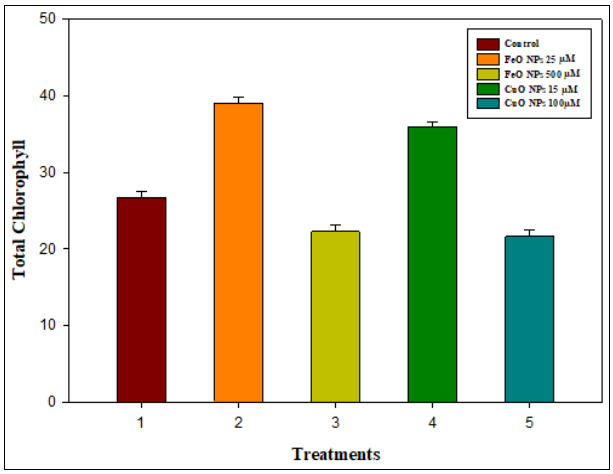

Fig 10: Graphical representation of impact in Total Chlorophyll of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 6 biological replicates. Bars with different letters show significant differences at $P< 0.05$ between treatments according to the Duncans multiple range test

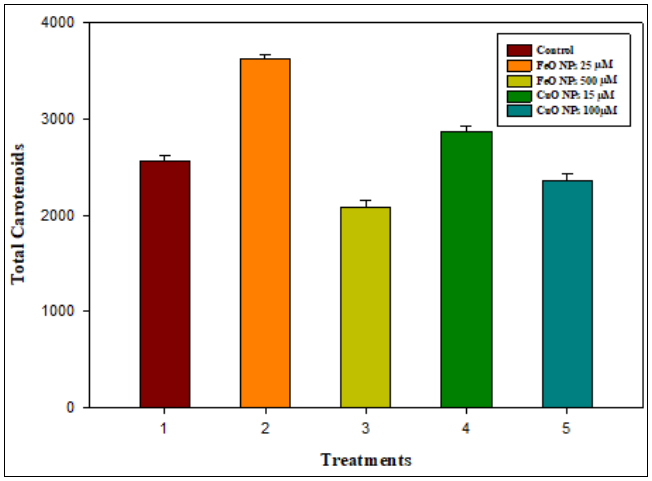

Fig 11: Graphical representation of impact in Total Carotenoids of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 6 biological replicates. Bars with different letters show significant differences at $P < 0.05$ between treatments according to the Duncans multiple range test.

3.4 Effect of FeO and CuO on protein content

It has been reported in previous studies that the protein content in the roots decreased sharply with higher concentrations of CuO-NPs.This reduction in protein content could be a result of oxidative damage or stress responses triggered by the nanoparticles (Zhou, 2011) [32]. The result suggested an increase in protein content by 59.68% and 54.44% in lower concentrations of FeO-NPs (25 µM) and CuO-NPs (15 µM) in comparison to control seedlings. Similarly, the protein content was reduced by 22.77% and 15.26% in higher concentrations FeO-NPs (500 µM) and CuO-NPs (100 µM) in comparison to control seedlings (As shown in Fig. 12), which suggests that these nanoparticles may have a stimulating effect on protein synthesis or an enhancement of physiological processes that support growth and development in wheat seedlings.

However, the reduction in protein content observed at higher concentrations of FeO-NPs (500 µM) and, CuO-NPs (100 µM) indicates a detrimental effect that may be due to oxidative stress (Singh *et al*., 2015) [24]. Elevated levels of these nanoparticles could lead to the generation of reactive oxygen species (ROS), which may cause oxidative damage to cellular structures, including proteins, lipids, and nucleic acids. This oxidative stress can trigger stress responses in plants, decreasing protein synthesis as resources are diverted to mitigate damage. (Tiwari *et al*., 2019; Sharma & Uttam, 2017) [26, 21]

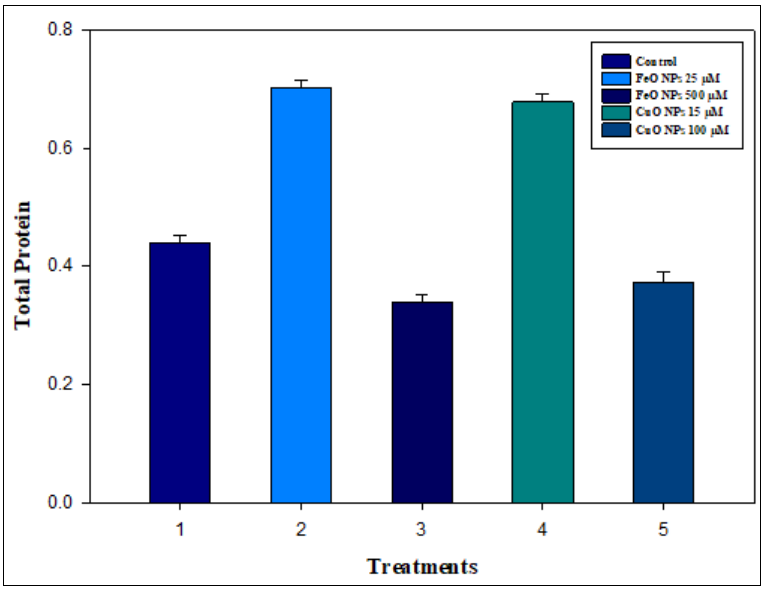

Fig 12: Graphical representation of impact in Total Protein of wheat plants under different treatments; (Control, FeO NP 25 µM, FeO NP 500 µM, CuO NP 15 µM, CuO NP 100 µM). Data are means standard error of 6 biological replicates. Bars with different letters show significant differences at $P < 0.05$ between treatments according to the Duncans multiple range test.

3.5 Histochemical staining of wheat roots

The histochemical staining of wheat seedlings roots exposed to various treatments showed significant differences in reactive oxygen species (ROS) damage. Staining of root tips and root sections with Evans's blue showed that the lower concentrations of FeO NPs at 25 µM and CuO NPs at 15 µM resulted in minimal membrane damage in the root sections of wheat plant, as compared to the elevated concentrations of FeO NPs at 500 µM and CuO NPs at 100 µM, which exhibited more membrane damage (As shown in Fig. 13 and Fig. 14). Similarly, Schiff's staining, showed lipid peroxidation in the root sections and root tips of wheat plant. In case of the lower concentration of nanoparticles the was a faint pink stain which indicates less lipid peroxidation. In contrast, seedlings treated with high concentrations of nanoparticles displayed darker pink staining, indicating lipid peroxidation in the root tips and root sections. Furthermore, DAB staining, which detects hydrogen peroxide (H_2O_2), showed reduced accumulation of H_2O_2 in seedlings treated with lower nanoparticle concentrations, whereas higher concentrations increased oxidative stress (As shown in Fig. 13 and Fig. 14). Similarly, NBT staining for superoxide (O_2^-) production confirmed that the seedlings exposed to lower concentrations of nanoparticles had significantly lower superoxide levels, in contrast to the higher levels observed in seedlings treated with elevated nanoparticle concentrations (As shown in Fig. 13 and Fig. 14). The observed results suggests that the concentration of FeO NPs and CuO NPs plays an important role in determining the extent oxidative stress. At lower concentrations, the nanoparticles may enhance plant growth and physiological responses without inducing significant ROS

production, thereby maintaining cellular integrity. However, at elevated concentrations, the overwhelming generation of ROS can lead to severe oxidative damage, compromising membrane integrity and overall plant health.

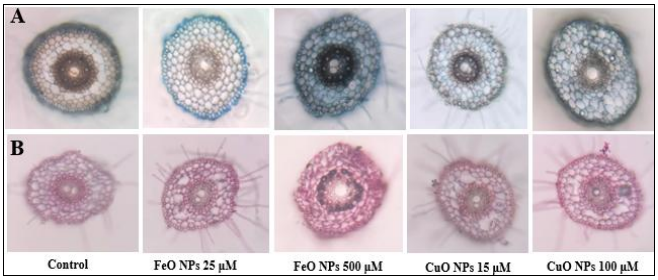

Fig 13: Representation of impact of different treatments on [A: membrane damage] and [B: Lipid peroxidation] in 12 days old wheat plants' root section.

Fig 14: Representation images illustrating the *in vivo* visualization of (A). membrane damage (B). lipid peroxidation (C). hydrogen peroxide (H_2O_2) (D). Superoxide radicals (O_2^-) in the root tips of 12 days old wheat roots grown in under various treatments in wheat. Root images Bar = 100 µM and visualized under 10X objective.

Conclusion

The result of this study indicates that FeO-NPs and CuO-NPs have a concentration-dependent effect on the growth and development of wheat. At low concentrations, FeO-NPs at 25 µM and CuO-NPs at 15 µM, both the nanoparticles promoted plant growth parameters such as fresh and dry weight, shoot and root length, photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), and stomatal frequency. These enhancements indicate a potential function of FeO NPs and CuO NPs as micronutrient supplements, enhancing the efficiency of gas exchange and increasing photosynthetic efficiency which further helps in improved growth and productivity. Additionally, protein content was also enhanced at these reduced concentrations of (FeO-NPs at 25 µM and CuO-NPs at 15 µM), indicating a stimulating influence on metabolic and physiological processes, potentially via enhanced uptake of nutrients or greater enzymatic activity. Histochemical staining indicated very less oxidative stress at these concentrations, indicating maintained cellular integrity and lower reactive oxygen species (ROS) formation. On contrast, toxic effects were produced with greater concentrations of FeO-NPs (500 µM) and CuO-NPs (100 µM), resulting in inhibitions of all growth parameters, chlorophyll and carotenoid content, stomatal frequency, and protein synthesis. This was also verified by intense histochemical staining as well, which reflected the augmented ROS generation, lipid peroxidation, and membrane damage showing characteristics of oxidative stress. These results underscore the bivalent character of metal oxide nanoparticles: beneficial at optimal, low doses but toxic at elevated doses. The outcomes underpin the need for dosage accuracy in the use of nanoparticles in agriculture. While FeO NPs and CuO NPs are potentially useful as growth stimulants in wheat, their use must be carefully titrated to prevent physiologically deleterious effects. Long-term effects, uptake mechanisms, and nanoparticle fate should be the focus of future research into soil-plant systems to allow for the sustainable and safe application in agricultural crop production.

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