

# GRAPHENE OXIDE EFFECT ON THE WHEAT PLANT (*TRITICUM AESTIVUM*) UNDER DIFFERENT APPLICATION AND CONCENTRATION TECHNIQUES

## Abstract

Graphene-based nanomaterial's, with unique chemical and physical properties, are increasingly used in agriculture, raising questions about their impact on soil, plant growth, and nutritional conditions. A pot experiment was conducted to examine how GO affects macronutrient and micronutrient uptake by wheat plants at the booting growth stage and the nutritional status of clay soil. Soil samples were collected at the farm of the Agricultural Research Centre in Egypt's. The experiment applied the treatment in three ways (soaking, soil application, and foliar application) and tested five different amounts of nanocarbon (0, 100, 200, 400, and 600 mg L<sup>-1</sup>) for soaking and soil application. For the foliar treatment, we used different doses (0, 10, 20, 40, and 60 mg L<sup>-1</sup>). Each treatment was repeated three times in a randomized block design. The results indicated that the amount of chlorophyll pigments (A, B, and carotene) in wheat leaves changed a lot depending on how GO was applied and how much was used. Furthermore, GO influences wheat plant growth (fresh and dry weight) relative to controls. The wheat crop responded to a high soaking dosage of 600 mg L<sup>-1</sup>, with analogous effects noted when 400 mg L<sup>-1</sup> of GO was applied to the soil. Moreover, foliar application of GO at 10 mg L<sup>-1</sup> improved shoot and root development in wheat plants under both wet and dry circumstances. When wheat shoots and roots were given different ways and amounts of GO nanoparticles, they consistently absorbed important nutrients like nitrogen, phosphorus, potassium, iron, manganese, zinc, and copper. Finally, soaking wheat grains at high concentrations is safe. While soil treatment is safe at concentrations up to 400 mg L<sup>-1</sup>, GO foliar spray for plants is only effective at a concentration of 10 mg L<sup>-1</sup>, and other tested doses harm wheat development.

Keywords: Graphene oxide; photosynthesis pigments; plant growth; macro and micro nutrients uptake; soil nutrients

## 1-Introduction

The agricultural sector fundamentally depends on producing food, feed, fiber, and fuel. However, it faces significant challenges from abiotic stress, pathogen infestation, and declining soil fertility (Badger *et al.*, 2021). These factors are crucial for sustaining agricultural productivity and ensuring environmental health (El Banna *et al.*, 2025). Furthermore, soils serve as the reservoir of essential nutrients necessary for the growth of plants. These nutrients improve crop yields and are resilient to biotic and abiotic stresses. These nutrient elements enhance human food with these components (El-Ramady *et al.*, 2021). Also, carbon is a fundamental element for all living organisms. This element possesses distinctive characteristics that enhance its potential with each passing day. The nano-forms of these elements exhibit various patterns. Nanocarbon possesses unique properties that offer significant potential in various agricultural applications, including nanocarriers, sensors, light converters, seed treatments, nanofertilizers, and a possible agent for controlling plant pathogens (Prokisch *et al.*, 2025).

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In addition to addressing common challenges, nanotechnology can improve agricultural output quality and efficiency (El-Shahawy *et al.*, 2022). Several studies (Jiao *et al.*, 2016; Zhang *et al.*, 2016) have found that graphene can enhance plant growth. These findings highlight the intricacy of graphene-based materials' phytotoxicity, which varies depending on the kind of plant and graphene concentration. As a result, we assessed wheat plant growth using different graphene oxide application procedures and concentrations.

An essential measure of wheat plant development, well-being, and environmental change, chlorophyll influences plant photosynthesis directly. After 30 days of exposure to graphene, the levels of chlorophyll and photosystem II (PSII) activity in wheat dropped, according to the research (Zhang *et al.*, 2016). As GO concentration increased, its effects shifted. Vochita *et al.* (2019) found that the dosage of GO affects the activity of both the enzymatic and non-enzymatic antioxidant systems. Consequently, at 500 mg L<sup>-1</sup> of GO, the concentration of carotenoid pigments was lower than that of chlorophyll pigment, significantly dropping in concentrations exceeding 2000 mg L<sup>-1</sup>. The pigment chlorophyll b did not alter in a statistically significant way. After one hundred days of exposure, at a maximum concentration of 0.6%, Zhao *et al.* (2022) discovered that GO reduced the total pigment content and the net photosynthetic activity. Zhang *et al.* (2021b) found that GO enhances photosynthesis, promoting *Aloe Vera* development. They showed that the most effective dosage of GO was 50 mg L<sup>-1</sup>, with 10-100 mg L<sup>-1</sup> could enhance the growth of *Aloe Vera* L. by boosting the nutritional value of the plant's leaves as well as the photosynthetic capacity, yield, and morphological characteristics.

Oxidative stress and lipid peroxidation can be caused by reactive oxygen species (ROS), which may increase production (Xiao *et al.*, 2022). The effects of GO on photosynthesis, including increased ROS production, oxidative stress, lipid peroxidation, and cell death, have been reported by Kazlauskas *et al.* (2023). Yuan *et al.* (2011) previously stated that high concentrations of GO in plant cell culture can cause the following issues: (1) disruption of water and nutrient adsorption via aggregation on the root surface; (2) formation of highly affinity pores in the cell membrane; (3) mitochondrial dysfunction in plant cell necrosis; and (4) the generation of reactive oxygen species, which may expedite cellular apoptosis. A critical step in improving the use of nanomaterials in plant development is determining the proper nanoparticle concentrations to minimize cell death and guarantee cell viability. Through transcriptomic and physiological investigations, Liu *et al.* (2022) examined buckwheat's response to GO. High doses of GO (>50 mg L<sup>-1</sup>) inhibited seedling development in buckwheat by triggering various transcriptional responses, and they found that GO infiltrated both the shoot and the root.

Furthermore, the effects of GO on the shoot and root of different plant growths vary depending on the quantities used and the application strategy. Zhu *et al.* (2020) found that a small amount of GO boosted plant height and shoot and leaf biomass in *Medicago sativa* (alfalfa), just one of many studies demonstrating that GO enhances shoot growth. *Festuca arundinacea* plants may grow taller and produce more biomass at a GO concentration of 0.2 mg L<sup>-1</sup>. Wang *et al.* (2018) and Vochita *et al.* (2019) found that graphene oxide may reach plant tissues through roots, affecting germination, growth, ROS production, membrane modifications, and more (Chichiricò and Poma, 2015). Furthermore, Guo *et al.* (2021) discovered that augmenting the number of cortical cells, cross-sectional area, diameter, and vascular column area resulted in a considerable, dose-dependent enhancement of tomato shoots and stem growth with GO therapy. This data proves that GO may be a helpful tomato plant growth regulator, stimulating cell proliferation in stems and shoots in a concentration-dependent fashion. Liu *et al.* (2015) evaluated the negative impacts of graphene on rice germination and seedling morphology

in hydroponic cultures. High amounts of GO may impair plant growth and development, causing undesired morphological changes. The primary process by which plants experience growth inhibition when exposed to high concentrations of GO is the production of reactive oxygen species (ROS) due to oxidative stress, as stated by **Yang et al. (2022)**. Researchers **Zhang et al. (2020)** found that compared to a control group, rice shoots treated with GO at concentrations of 100 and 250 mg L<sup>-1</sup> had lower biomass and elongation. **Wang et al. (2020)** found that Lucerne seedling development was significantly reduced at GO concentrations of 0.5-1.5%, which is in line with the results of the previous study. The effects were shown to be increasingly noticeable with increasing concentration and duration of exposure (**Zhao et al., 2023**). At its highest concentration of 0.6%, GO lowered the control group's plant height, as well as the dry weights of the leaves and shoots. In contrast, soil pollutants are first detected and responded to by the roots. The capacity to transport water and nutrients is shown, for instance, by the quantity and size of xylem arteries, vascular cylinders, and sieve tubes (**Da Cunha Cruz et al., 2020**). Nutrition absorption and physiological function are directly affected by changes to root structure. Modifying the form of a plant's roots can enhance its ability to uptake nutrients (**Lima et al., 2021**). The root development of *Aloe Vera* was seen to be impacted by the administration of 10-100 mg L<sup>-1</sup> of GO, according to **Zhang et al. (2021b)**. At various GO concentrations, root volume, length, fresh weight, and surface area grew dramatically. **Guo et al. (2021)** discovered that 50 -100 mg L<sup>-1</sup> GO increased tomato root tip and hair surface area compared to untreated controls.

For example, GO increased the total surface, length, and surface area of seedling roots in a dose-dependent way, indicating that GO may improve nutrient absorption. In accordance with this, biomass buildup was demonstrated by the much higher dry weight of the roots in graphene-treated seedlings compared to the control group. Higher GO levels cause oxidative stress in plants, which damages them, according to **Yang et al. (2022)**. The results demonstrate that the adverse impacts of GO on plant growth and development are intricate and contingent upon the plant genotype. Consequently, we endorse the application of appropriate GO doses in agricultural techniques for certain plant species. As a consequence of this, we are fully in favor of the utilization of appropriate GO dosages in agricultural methods for certain plant species. Researchers **Ren et al. (2020)** found that the presence of GO at concentrations ranging from 400 to 1000 mg L<sup>-1</sup> hindered the growth of roots in wheat seedlings. Wheat plants had fewer lateral roots and stunted root development when exposed to GO concentrations ranging from 200 to 800 mg L<sup>-1</sup>, according to **Weng et al. (2020)**. Experimental evidence suggests that GO can be contained within root vacuoles, leading to a significant decrease in both the maximum root length and the quantity of lateral roots. Wrinkles, oxidative stress, reduced respiration, and whitened root tips were also found. According to these results, GO is very detrimental to robust root development and prevents the growth of the root absorption region. Furthermore, only a small number of this research examined how nanomaterials in particular, graphene oxide affects the absorption of macro- and micronutrients, the degree to which developing plants react to high concentrations, and the application technique. In a study carried out by **Zhang et al. (2016)**, it was found that the levels of nitrogen (N) in wheat seedling shoots are substantially reduced when exposed to 500 mg L<sup>-1</sup> of graphene. **Weng et al. (2020)** indicated that wheat plants subjected to (GO) at concentrations of 200–800 mg L<sup>-1</sup> exhibited significantly reduced nitrate levels in their roots and that GO could markedly diminish net NO<sub>3</sub><sup>-</sup> influx in the meristematic, elongation, and maturity regions of wheat roots. Lowered levels of various minerals in wheat leaves indicate that GO may affect nutritional homeostasis; GO at a dosage of 5 mg L<sup>-1</sup> dramatically lowered

Micronutrient levels (Hu *et al.*, 2018). Furthermore, Zhang *et al.* (2016) discovered that wheat exposed to graphene for 30 days exhibited lower Concentrations of nitrogen, potassium, calcium, magnesium, iron, zinc, and copper are associated with stunted development, and a nutritional imbalance. Graphene concentrations and treatment periods have differing effects on plant development. The study discovered that graphene impeded plant growth and photosynthesis, causing a decline in chlorophyll content shoot biomass production, photosystem activity, and nutrient levels, disturbing nutritional homeostasis. GO reduced the quantities of N, K and micronutrient to a maximum of 0.6%, according to Zhao *et al.* (2022) and Zhao *et al.* (2023). Graphene is seldom studied in relation to soil characteristics such as chemical characteristics, enzymatic activity and the cycling of nutrients. Nonetheless, the impacts of multi-walled carbon nanotubes containing GO characteristics, on the activity of soil enzymes have been reported. Graphene in soils has been shown to interact with organic materials, reducing its likelihood of entering organisms (Lammel *et al.*, 2013). Additionally, it has limited interaction with enzymes like catalase, which means that it may not have immediate deleterious effects on plants (Shrestha *et al.*, 2013).

This research aims to look at wheat plant development in response to diverse techniques and amounts of GO application while also tracking chemical changes in soil nutrients.

## 2. Materials and Methods

In a pot experiment, we examined the influence of GO on wheat plant development, macronutrient and micronutrient absorption, and nutrient availability in clay soil. Samples of soil (0–30 cm) were collected at the Agricultural Research Center's farm in the Egyptian governorate of El-Giza. The research farm is located at 29° 58/55.046 N and 31° 12/49.272 E. Following air drying, crushing, and fine grinding, the soil samples were sieved through a 2 mm sieve and placed in 30 cm-diameter plastic pots. The physical and chemical properties of the soil samples evaluated following the procedure described by Page *et al.* (1982) are shown in Table 1. For this experiment, the seeds of wheat (*Triticum aestivum* L., Giza 171) were sown in open fields. Following preparation, 15 grains were planted in each pot, which had four holes and measured 25 cm in diameter by 21 cm in height. Six kilograms of soil samples were placed within each pot. Three replicates for each treatment were employed in the statistical design, which was entirely blocked and adequately randomized. From an initial stock solution of 2000 mg L<sup>-1</sup> made by dissolving GO powder in ultrapure distilled water using ultrasonication at 20 kHz for 4 hours, nanocarbon treatments were made in five concentrations (0, 100, 200, 400, and 600 mg L<sup>-1</sup>) and then added in three application forms (soaking, soil, and foliar). The same concentrations were used for soaking and soil treatment, whereas foliar was applied at varied doses (0, 10, 20, 40, and 60 mg L<sup>-1</sup>). The grains from the soaking treatment were soaked in each group for 24 hours at their respective concentrations before planting in pots on January 3, 2022.

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### Recommended fertilizers

The required N, P, K, and micronutrients were added to all pots. The soil's moisture content was maintained at field capacity with tap water throughout the experiment.

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### Plant analysis

At 60 days after seeding, plants were split into shoots and roots and weighed for fresh and dried weights. Samples from each treatment were subjected to oven drying at 70 degrees

Celsius for 72 hours, subsequently ground in a stainless-steel mill, and wet-digested using the methodology outlined by **Cottenie et al. (1982)**. The concentrations of macronutrients and micronutrients in the roots and shoots were assessed.

#### Soil analysis

A soil sample for each pot at harvest was collected, air-dried, crushed, and sieved to pass through a 2 mm screen, which was finally used by **Page et al. (1982)** to determine the availability of macro- and micronutrients.

**Table (1): Some physical and chemical properties of the investigated soil.**

Soil characteristics	Value	Soil characteristics	Value
<b>Soil particles distribution (%)</b>		SP	52.84
Sand	29.30	EC( dS m <sup>-1</sup> ) **	3.93
Silt	36.13	<b>Soluble cations and anions, (mmolc l<sup>-1</sup>)**</b>	
Clay	34.57	Ca <sup>++</sup>	20
Textural class	Clay Loam	Mg <sup>++</sup>	10
Bulk Density (Mg.m <sup>-3</sup> )	1.26	Na <sup>+</sup>	8.51
pH *	8.72	K <sup>+</sup>	1.74
CEC (cmolc/Kg)	21.33	CO <sub>3</sub> <sup>=</sup>	Nil
OC (g kg <sup>-1</sup> )	11.80	HCO <sub>3</sub> <sup>-</sup>	11.50
OM (g kg <sup>-1</sup> )	20.30	Cl <sup>-</sup>	27.5
CaCO <sub>3</sub> (g kg <sup>-1</sup> )	54.00	SO <sub>4</sub> <sup>=</sup>	1.25
<b>Available macro nutrients (mg kg<sup>-1</sup>)</b>		<b>Available micro nutrients (mg Kg<sup>-1</sup>)</b>	
N	140.3	Fe	2.14
P	60.0	Mn	27.1
K	276.0	Zn	4.22
		Cu	7.64

• pH (1:2.5 soil: water suspension)

\*\* soil paste extract

#### Statistical analysis:

All the experiments were carried out, at least in triplicate. All statistical analyses were performed employing MSTAT computer software package. The averages were compared utilizing Duncan's multiple-range test (**Duncan, 1955**)

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### 3. Results

As previously noted in the section on materials and techniques, a pot methodology was used to evaluate the behavior of wheat plants in the presence of GO nanocarbons at various concentrations and different ways of applying GO to wheat plants. This was accomplished by assessing the photosynthesis pigments, development, and status of the specified macronutrients (N, P, and K) and micronutrients (Fe, Mn, Zn, and Cu) in the plants and soil.

#### Responses of the wheat plant to graphene oxide treatments

##### a- Effect of GO on photosynthesis pigments

Photosynthesis is plants' primary activity, converting CO<sub>2</sub> into organic matter required for plant development and the carbon cycle. The influence of graphene oxide nanoparticles, application techniques, and concentrations on chlorophyll content was examined, and the results are presented in Table 2. In terms of photosynthetic machinery, the chlorophyll pigment concentration of wheat plants has changed significantly in response to varied GO dosages and application techniques.

**Table 2:- Effect of GO method of application and different concentrations on chlorophyll a, b, and carotene**

Treatments		Chlorophyll content (mg g <sup>-1</sup> )		
Method of application(A)	Concentration of GO (mgL <sup>-1</sup> ) (B)	Chloro (a)	Chloro (b)	Carotene
<b>Control</b>		<b>0.87<sub>E</sub></b>	<b>0.30<sub>F</sub></b>	<b>0.25<sub>G</sub></b>
<b>Soaking</b>	<b>100</b>	1.09 <sub>D</sub>	0.34 <sub>EF</sub>	0.26 <sub>G</sub>
	<b>200</b>	1.11 <sub>CD</sub>	0.35 <sub>DEF</sub>	0.27 <sub>FG</sub>
	<b>400</b>	1.19 <sub>BCD</sub>	0.36 <sub>CDF</sub>	0.29 <sub>DE</sub>
	<b>600</b>	1.29 <sub>ABC</sub>	0.37 <sub>B-E</sub>	0.33 <sub>B</sub>
<b>Mean</b>		<b>1.11<sub>B</sub></b>	<b>0.35<sub>B</sub></b>	<b>0.2<sub>A</sub></b>
<b>Soil application</b>	<b>100</b>	1.34 <sub>AB</sub>	0.37 <sub>B-E</sub>	0.30 <sub>CD</sub>
	<b>200</b>	1.39 <sub>A</sub>	0.40 <sub>A-D</sub>	0.31 <sub>CD</sub>
	<b>400</b>	1.43 <sub>A</sub>	0.41 <sub>ABC</sub>	0.31 <sub>BC</sub>
	<b>600</b>	1.42 <sub>A</sub>	0.38 <sub>A-E</sub>	0.31 <sub>BC</sub>
<b>Mean</b>		<b>1.29<sub>A</sub></b>	<b>0.37<sub>A</sub></b>	<b>0.30<sub>A</sub></b>
<b>Foliar application</b>	<b>10</b>	1.45 <sub>A</sub>	0.42 <sub>A</sub>	0.35 <sub>A</sub>
	<b>20</b>	1.39 <sub>A</sub>	0.41 <sub>AB</sub>	0.32 <sub>BC</sub>
	<b>40</b>	1.37 <sub>AB</sub>	0.39 <sub>A-E</sub>	0.30 <sub>CD</sub>
	<b>60</b>	1.34 <sub>AB</sub>	0.38 <sub>A-E</sub>	0.28 <sub>EF</sub>
<b>Mean</b>		<b>1.29<sub>A</sub></b>	<b>0.38<sub>A</sub></b>	<b>0.30<sub>A</sub></b>
<b>Mean of concentration</b>	<b>C1</b>	1.30 <sub>A</sub>	0.38 <sub>A</sub>	0.31 <sub>A</sub>
	<b>C2</b>	1.30 <sub>A</sub>	0.39 <sub>A</sub>	0.30 <sub>A</sub>
	<b>C3</b>	1.33 <sub>A</sub>	0.38 <sub>A</sub>	0.30 <sub>A</sub>
	<b>C4</b>	1.35 <sub>A</sub>	0.38 <sub>A</sub>	0.31 <sub>A</sub>

Overall, the data in Table (2) reveal that chlorophyll a, b, and carotene contents rose across all concentrations and application methods compared to the control treatment. The way graphene oxide was applied, and the concentration worked together to show that soaking wheat grains had less effect on the amounts of chlorophyll a and b than other ways of applying it at different concentrations. Furthermore, the results in Table (2) demonstrated that wheat grains responded to GO soaking at various concentrations; higher soaking concentrations of up to 600 mg L<sup>-1</sup> produced significant chlorophyll a, b, and carotene levels. This enhanced reach was 48.3, 23.3, and 0.32.0% for chlorophyll a, b, and carotene, respectively, compared to the control treatment. In addition, when GO was applied to soil, the study discovered that 400 mg L<sup>-1</sup> was the ideal dosage for chlorophylls a, b, and carotene compared to untreated treatment. Wheat leaves received 400 mg L<sup>-1</sup> of GO exhibited elevated chlorophyll levels compared to the control treatment, the percentages climbed to 64.4, 36.7, and 24.0% for chlorophyll a, b, and carotene, respectively.

Furthermore, increasing the concentration to 600 mg L<sup>-1</sup> hurt chlorophylls a and b, while increasing the applied concentrations from 100 to 600 mg L<sup>-1</sup> of soil application did not affect

the amount of carotene. In contrast, Table (2) shows that foliar application of GO at a concentration of 10 mg L<sup>-1</sup> increased wheat chlorophyll a, b, and carotene content. They boosted the content of chlorophyll a, b, and carotene to 66.7, 40.0, and 40.0%, respectively. Furthermore, the total chlorophyll content decreased with each increment when different concentrations were used. According to the findings, applying GO as a foliar treatment at a high concentration was more detrimental to chlorophyll a, b, and carotenes in wheat leaves. The suppression of photosynthesis caused by GO treatment might be related to damage to the leaf chloroplast structure.

However, regardless of the doses utilized, mean values of the individual treatment technique of application (Table 2) demonstrate that all ways of graphene oxide administration result in a considerable increase in chlorophyll a, b, and carotene content in wheat leaves compared to the control treatment. In addition, there was no significant change in chlorophyll a, b, or carotene content across the three application techniques. The soaking application method produced lower chlorophyll a, b, and carotene levels than soil or foliar application.

In addition, regardless of the application technique, Table 2 shows that the mean values of the concentrations used revealed that no significant changes were observed in chlorophyll a, b, and carotene when all concentrations of GO nanoparticles were used on wheat leaves.

#### **b- Biomass production and moisture content**

Plant development is known to be influenced by nanocarbons; the consequences of this influence vary depending on the application technique and concentrations of the relevant element. Fresh weight, dry weight, and the moisture content of the shoot and root of the wheat plant are the three measurements used to describe plant growth. The results in Table (3) demonstrate how, depending on the application technique, applied graphene oxide nanoparticles at varying concentrations affect the development of wheat plants during the booting growth stage. In general, applying GO in various methods and concentrations positively affected wheat plant organs, whether fresh or dry weight, for both shoots and roots, compared to control treatments.

Moreover, the soaking technique benefitted all of the examined features, with a steady rise in the fresh and dry weights of the wheat shoot and root. When the wheat grains were soaked in 600 mg L<sup>-1</sup> of GO nanocarbon, the fresh weight of the shoot and root increased by 37.6% and 50%, respectively, compared to the control treatment. Similar increases were seen, reaching 14.7% and 23.8% in dry weight for both shoot and root, respectively, compared to the control.

Concerning applied GO as soil application, the results in Table (3) showed that applied GO at a concentration of 400 mg L<sup>-1</sup> increased wheat growth parameters by 24.1, 41.7% for shoot and root fresh weight, and 16.6, 19.0% for shoot and root dry weight, respectively, when compared to the untreated treatment. Raising the additive content to 600 mg L<sup>-1</sup> reduced all growth markers, including fresh weight, dry weight, and plant organs (shoot and root).

<b>Table 3: The application technique and concentration of GO have an impact on the fresh and dry weight along with the moisture content of shoot and root wheat plants during the booting growth stage.</b>							
<b>Treatments</b>		<b>Fresh weight (g plant<sup>-1</sup>)</b>		<b>Dry weight (g plant<sup>-1</sup>)</b>		<b>Moisture (%)</b>	
<b>Method of application (A)</b>	<b>Concentration of GO (mgL<sup>-1</sup>) (B)</b>	<b>Shoot</b>	<b>Root</b>	<b>Shoot</b>	<b>Root</b>	<b>Shoot</b>	<b>Root</b>
<b>Control</b>		<b>5.03<sub>DE</sub></b>	<b>0.48<sub>CDE</sub></b>	<b>1.63<sub>DEF</sub></b>	<b>0.21<sub>ABC</sub></b>	<b>67.5<sub>C</sub></b>	<b>55.9<sub>A</sub></b>
<b>Soaking</b>	<b>100</b>	5.30 <sub>CDE</sub>	0.53 <sub>BC</sub>	1.70 <sub>CDE</sub>	0.22 <sub>AB</sub>	68.1 <sub>BC</sub>	58.0 <sub>A</sub>
	<b>200</b>	5.93 <sub>BCD</sub>	0.54 <sub>BC</sub>	1.79 <sub>ABC</sub>	0.23 <sub>AB</sub>	69.8 <sub>ABC</sub>	57.8 <sub>A</sub>
	<b>400</b>	5.77 <sub>ABC</sub>	0.55 <sub>BC</sub>	1.80 <sub>ABC</sub>	0.24 <sub>A</sub>	70.6 <sub>ABC</sub>	54.7 <sub>A</sub>
	<b>600</b>	6.92 <sub>A</sub>	0.72 <sub>A</sub>	1.87 <sub>AB</sub>	0.26 <sub>A</sub>	72.1 <sub>AB</sub>	63.8 <sub>A</sub>
<b>Mean</b>		<b>5.80<sub>A</sub></b>	<b>0.56<sub>A</sub></b>	<b>1.76<sub>A</sub></b>	<b>0.23<sub>A</sub></b>	<b>69.6<sub>A</sub></b>	<b>58.0<sub>A</sub></b>
<b>Soil application</b>	<b>100</b>	5.81 <sub>B-E</sub>	0.53 <sub>BC</sub>	1.72 <sub>BCD</sub>	0.22 <sub>ABC</sub>	70.2 <sub>ABC</sub>	58.3 <sub>A</sub>
	<b>200</b>	6.08 <sub>ABC</sub>	0.57 <sub>BC</sub>	1.76 <sub>A-D</sub>	0.23 <sub>AB</sub>	70.9 <sub>ABC</sub>	59.2 <sub>A</sub>
	<b>400</b>	6.24 <sub>AB</sub>	0.68 <sub>A</sub>	1.90 <sub>A</sub>	0.25 <sub>A</sub>	69.3 <sub>ABC</sub>	63.0 <sub>A</sub>
	<b>600</b>	6.12 <sub>ABC</sub>	0.52 <sub>BCD</sub>	1.65 <sub>CDE</sub>	0.18 <sub>BCD</sub>	73.0 <sub>A</sub>	64.7 <sub>A</sub>
<b>Mean</b>		<b>5.86<sub>A</sub></b>	<b>0.56<sub>A</sub></b>	<b>1.73<sub>A</sub></b>	<b>0.22<sub>A</sub></b>	<b>70.2<sub>A</sub></b>	<b>60.2<sub>A</sub></b>
<b>Foliar application</b>	<b>10</b>	5.87 <sub>BCD</sub>	0.58 <sub>B</sub>	1.70 <sub>CDE</sub>	0.23 <sub>AB</sub>	71.0 <sub>ABC</sub>	58.4 <sub>A</sub>
	<b>20</b>	5.76 <sub>B-E</sub>	0.44 <sub>DEF</sub>	1.65 <sub>CDE</sub>	0.18 <sub>BCD</sub>	71.4 <sub>ABC</sub>	58.5 <sub>A</sub>
	<b>40</b>	5.29 <sub>CDE</sub>	0.40 <sub>EF</sub>	1.56 <sub>EF</sub>	0.17 <sub>CD</sub>	70.4 <sub>ABC</sub>	59.2 <sub>A</sub>
	<b>60</b>	4.95 <sub>E</sub>	0.38 <sub>F</sub>	1.50 <sub>F</sub>	0.14 <sub>D</sub>	69.7 <sub>ABC</sub>	62.2 <sub>A</sub>
<b>Mean</b>		<b>5.39<sub>A</sub></b>	<b>0.46<sub>B</sub></b>	<b>1.61<sub>A</sub></b>	<b>0.19<sub>B</sub></b>	<b>70.1<sub>A</sub></b>	<b>58.8<sub>A</sub></b>
<b>Mean of concentration</b>	<b>C1</b>	5.67 <sub>A</sub>	0.55 <sub>A</sub>	1.70 <sub>AB</sub>	0.22 <sub>A</sub>	69.8 <sub>AB</sub>	58.2 <sub>A</sub>
	<b>C2</b>	5.92 <sub>A</sub>	0.51 <sub>AB</sub>	1.73 <sub>A</sub>	0.21 <sub>A</sub>	70.7 <sub>A</sub>	58.5 <sub>A</sub>
	<b>C3</b>	5.97 <sub>A</sub>	0.55 <sub>A</sub>	1.75 <sub>A</sub>	0.22 <sub>A</sub>	70.1 <sub>A</sub>	59.0 <sub>A</sub>
	<b>C4</b>	5.99 <sub>A</sub>	0.54 <sub>A</sub>	1.67 <sub>AB</sub>	0.19 <sub>A</sub>	71.6 <sub>A</sub>	63.5 <sub>A</sub>

Furthermore, compared to other treatment techniques, growth parameters significantly impacted the growth of wheat plants treated with foliar applications of GO at different doses. Compared to the control treatment, Table (3) results demonstrate that applying 10 mg L<sup>-1</sup> had a more favorable impact on wheat plants. This treatment increased 16.7 and 20.8% for shoot and root fresh weight and 4.30 and 9.50% for shoot and root dry weight, respectively. Notably, while GO concentrations increased, the plant developmental indicators examined decreased, indicating that increasing GO concentrations hurt wheat plant growth.

On the other hand, GO addition impacts moisture content in both the shoot and the root of wheat plants; this effect varies depending on the mode of administration and the concentration used. In general, applied treatments generated an increase in moisture content when compared to the control treatment. Shoots have greater moisture content than roots. Furthermore, moisture content increased, particularly during the soaking and soil application treatments.

Finally, regardless of the different concentrations, the results in Table (3) illustrate the influence of the GO application methods on wheat plant development at the booting growth stage. In general, results show that mean values of the nanocarbon method of application were increased

compared to untreated treatment of all study growth parameters, along with moisture concentration. Also, Table (3) shows that no significant difference was recorded between all application methods for all study growth parameters. This is true even though applying GO by foliar applications appears to decrease the mean values of both fresh and dry weight of both shoots and roots, along with moisture content. Similar findings were observed using the mean values of the tested concentrations; the results demonstrate that using GO at various doses boosted all evaluated growth parameters compared to the control treatment. In general, Table (3) shows that wheat plant growth parameters, except for shoot fresh wheat, increased with different concentrations up to C3 (400 mg L<sup>-1</sup> for soaking and soil application as well as 10 mg L<sup>-1</sup> for foliar application) and then decreased with C4 using the same previously used method of application. A relatively different trend was observed with moisture content, which increased with GO concentrations increased.

### **c- Impact of GO on wheat plants' absorption of macro and micronutrients.**

Mineral nutrients are vital for the growth, development, productivity, and quality of plants. Table 4 presents the data demonstrating the impact of various techniques and concentrations of GO nanoparticles on the absorption of macro and micro-nutrients, by wheat shoots and roots during the booting growth stage. Overall, data indicate that the application of GO nanoparticles, with the exception of elevated concentrations in foliar treatments, enhanced the uptake of nitrogen, phosphorus, and potassium in both the shoots and roots of wheat plants during the booting developmental phase (Table 4). The wheat crop showed a similar trend in the uptake of micronutrients for both shoots and roots.

Moreover, Table 4 indicates that immersing seeds in nanocarbons at varying concentrations enhanced micronutrient absorption, which escalated with increasing concentrations. Soaking wheat grains at 600 mg L<sup>-1</sup> augmented nitrogen uptake by 43.4% and 48.1% in shoots and roots, respectively, relative to the untreated group. Furthermore, phosphorus and potassium exhibited identical trends under the same conditions. Conversely, phosphorus rose by 40.2% and 70.3% for the shoot and root, respectively, while potassium exhibited comparable increases of 27.6% and 22.0% for both shoot and root. Conversely, immersing wheat grains in evaluated GO nanoparticles affected the absorption of micronutrients; this enhancement was shown incrementally in both shoots and roots as concentrations increased (Table 4). The findings indicated that the administered doses positively influenced wheat plant development. Compared to the untreated treatment, the application of GO to the soil at a dosage of 400 mg L<sup>-1</sup> led to a 44.5% enhancement in nitrogen absorption in shoots and a 63.8% enhancement in roots. Increasing soil GO concentration to 600 mg L<sup>-1</sup> negatively impacts the shoot and root development of wheat plants. Table 4 demonstrates that the incorporation of 400 mg L<sup>-1</sup> of GO improves phosphorus absorption, leading to increases of 46.6% in shoots and 83.8% in roots. Furthermore, the shoot and root of the wheat plant exhibited similar enhancements in potassium absorption, attaining 36.5% and 42.8%, respectively, in comparison to untreated treatment. Elevating the dosage of GO to 600 mg L<sup>-1</sup> adversely impacted macronutrient absorption by both the shoot and root, diminishing the nutritional intake of the wheat plant.

A comparable pattern was noted in the uptake of micronutrients (Fe, Mn, Zn, and Cu) in both the shoot and root of the wheat plant (Table 4). Furthermore, with the exception of 10 mg L<sup>-1</sup>, the foliar application of GO nanoparticles adversely affected nitrogen uptake in the shoots and roots of wheat plants. The results demonstrate that the examined amounts adversely affected

nitrogen absorption in wheat plants. Furthermore, the foliar application of wheat plants at 10 mg L<sup>-1</sup> enhanced nitrogen absorption in shoots and roots by 17.9% and 30.5%, respectively, in comparison to the control treatment. Phosphorus and potassium exhibit a comparable tendency to nitrogen. Incorporating 10 mg L<sup>-1</sup> of GO into wheat plants during the booting growth stage positively influenced phosphate and potassium levels (Table 4). Relative to the control, there was a 22.8% and 56.8% augmentation in phosphorus uptake and a 14.1% and 23.8% augmentation in potassium uptake in both shoots and roots. The elevated concentration of GO, administered via foliar application, resulted in a reduction of macronutrient (N, P, and K) absorption. Furthermore, the use of graphene oxide as a foliar treatment modified the absorption dynamics of micronutrients (Fe, Mn, Zn, and Cu) in wheat plants. Table 4 reveals a comparable trend to macronutrients, showing that concentrations over 10 mg L<sup>-1</sup> adversely affect micronutrient absorption by wheat plant parts.

**Table 4: - Effects of graphene oxide application technique and concentration on macronutrient and micronutrient uptake by wheat plant at booting growth stage**

Treatments		Macronutrients uptake (mg/plant)						Micronutrients uptake (µg/plant)							
		N		P		K		Fe		Mn		Zn		Cu	
Method of appl. (A)	GO Conc. mgL <sup>-1</sup> (B)	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
<b>Control</b>		<b>36.2E</b>	<b>3.18EFG</b>	<b>4.38F</b>	<b>0.37EF</b>	<b>75.0G</b>	<b>6.68C-F</b>	<b>2265C</b>	<b>1406DE</b>	<b>117E</b>	<b>28.7CDE</b>	<b>42.8C</b>	<b>10.6EF</b>	<b>110D</b>	<b>2.60D</b>
<b>Soaking</b>	<b>100</b>	40.4DE	3.66DEF	4.66EF	0.40DEF	81.4EFG	6.74C-F	2246C	1371DE	136E	30.2B-E	46.3C	11.1EF	109D	2.67D
	<b>200</b>	42.0CD	3.92CDE	5.18C-F	0.45B-F	85.4C-F	7.28B-E	2268C	1557CDE	143DE	31.6B-E	45.9C	11.7DEF	116D	3.60BCD
	<b>400</b>	48.9AB	4.15BCD	6.07ABC	0.55A-E	91.1BCD	7.77A-D	2504ABC	1833CD	171CD	37.6ABC	63.1BC	13.1C-F	141BCD	3.47CD
	<b>600</b>	51.9A	4.71ABC	6.14AB	0.63AB	95.7AB	8.15ABC	2984A	2939A	216AB	41.1A	81.2AB	14.9BCD	183A	6.30A-D
<b>Mean</b>		<b>43.9A</b>	<b>3.92A</b>	<b>5.29A</b>	<b>0.48A</b>	<b>85.7AB</b>	<b>7.32AB</b>	<b>2453AB</b>	<b>1821A</b>	<b>157B</b>	<b>33.9A</b>	<b>55.9AB</b>	<b>12.3B</b>	<b>132B</b>	<b>3.77A</b>
<b>Soil appl.</b>	<b>100</b>	46.6BC	4.51A-D	5.38B-E	0.56A-E	88.6B-E	8.23ABC	2573ABC	1379DE	199BC	25.1DEF	93.5A	12.8C-F	164ABC	3.93A-D
	<b>200</b>	50.7AB	4.85AB	5.98A-D	0.62ABC	94.4ABC	9.04AB	2602ABC	1849CD	211AB	33.9A-D	99.6A	15.9ABC	179AB	6.87A-D
	<b>400</b>	52.3A	5.21A	6.42A	0.68A	102.4A	9.54A	2915AB	2170BC	238A	38.0AB	77.1AB	18.5AB	197A	8.10AB
	<b>600</b>	40.4DE	3.00EFG	5.78A-D	0.41C-F	89.1B-E	5.81DEF	2335BC	1157DE	171CD	26.5DE	58.5BC	11.1EF	119D	3.77BCD
<b>Mean</b>		<b>45.2A</b>	<b>4.15A</b>	<b>5.59A</b>	<b>0.53A</b>	<b>89.9A</b>	<b>7.86A</b>	<b>2538A</b>	<b>1592A</b>	<b>187AB</b>	<b>30.4A</b>	<b>74.3A</b>	<b>13.8A</b>	<b>154A</b>	<b>5.05A</b>
<b>Foliar appl.</b>	<b>10</b>	42.7CD	4.15BCD	5.38B-E	0.58A-D	85.6C-F	8.27ABC	2533ABC	2599AB	232AB	31.6B-E	63.2BC	19.2A	131CD	8.37A
	<b>20</b>	41.5D	3.07EFG	5.10DEF	0.33F	83.3D-G	6.35C-F	2316C	1589CDE	228AB	24.4EFG	56.8BC	14.1CDE	123D	7.50ABC
	<b>40</b>	39.6DE	2.82FG	5.11DEF	0.33F	78.4FG	5.70EF	2064C	1276DE	217AB	16.5FG	50.1C	11.8DEF	110D	8.10AB
	<b>60</b>	38.5DE	2.61G	4.76EF	0.31F	75.0G	5.02F	2037C	992E	240A	15.6BG	46.3C	10.1F	102D	5.33A-D
<b>Mean</b>		<b>39.7B</b>	<b>3.17B</b>	<b>4.94A</b>	<b>0.38A</b>	<b>79.5B</b>	<b>6.41B</b>	<b>2243B</b>	<b>1566A</b>	<b>207A</b>	<b>23.5B</b>	<b>51.8B</b>	<b>13.2AB</b>	<b>115C</b>	<b>6.39A</b>
<b>Mean of conc.</b>	<b>C1</b>	43.2B	4.11A	5.14B	0.51A	85.2A	7.75A	2450A	1.78A	189B	29.0A	67.6A	14.4A	134A	4.99AB
	<b>C2</b>	44.7AB	3.95AB	5.42AB	0.47AB	87.7A	7.56A	2395A	1.67A	194AB	30.0A	67.4A	13.9AB	139A	5.99A
	<b>C3</b>	46.9A	4.06A	5.87A	0.52A	90.7A	7.67A	2494A	1.76A	209A	30.7A	63.4A	14.5A	149A	6.56A
	<b>C4</b>	43.6B	3.44BC	5.56AB	0.45AB	86.6A	6.33B	2452A	1.70A	209A	27.7A	62.0A	12.1BC	135A	5.13AB

#### d- Impact of Graphene Oxide on macro and micronutrient availability in soil

The availability of nutrients can influence plant development and changes in root architecture. After 60 days of wheat plant growth, the data in Table 5 demonstrate the impact of GO nanoparticles with varying application techniques and concentrations on the availability of macro and micronutrients in soil. Applying graphene oxide using various procedures and concentrations substantially impacted macronutrient availability (nitrogen, phosphorus, and potassium) compared to the control treatment. Using GO as a soaking treatment at varying concentrations increased macronutrient and micronutrient availability in the soil. This increase in nutrient availability was linked to the increasing application of various concentrations. Table 5 shows that micronutrient availability during soaking treatment follows a similar trend to macronutrients. Results suggested that soaking at a high concentration of 600 mg L<sup>-1</sup> was beneficial for micronutrient availability in soil. Furthermore, soil application of GO nanoparticles significantly boosted (N, P, and K) availability in soil, as shown in Table 5. This increase happens gradually as the concentration rises to 400 mg L<sup>-1</sup>.

**Table 5:- Effects of graphene oxide application technique and concentration on soil macronutrient and micronutrient availability following wheat plant at booting growth stage**

Treatments		Macronutrients availability mg kg <sup>-1</sup>			Micronutrients availability mg kg <sup>-1</sup>			
Method of Application (A)	Concentration of GO (mg L <sup>-1</sup> ) (B)	N	P	K	Fe	Mn	Zn	Cu
<b>Control</b>		<b>107.3<sub>C</sub></b>	<b>48.13<sub>E</sub></b>	<b>1014<sub>G</sub></b>	<b>4.26<sub>BC</sub></b>	<b>15.30<sub>E</sub></b>	<b>4.04<sub>B</sub></b>	<b>8.07<sub>D</sub></b>
<b>Soaking</b>	<b>100</b>	112.0 <sub>BC</sub>	50.67 <sub>E</sub>	1014 <sub>G</sub>	4.53 <sub>BC</sub>	16.55 <sub>DE</sub>	4.07 <sub>B</sub>	8.07 <sub>D</sub>
	<b>200</b>	116.7 <sub>BC</sub>	51.07 <sub>E</sub>	1079 <sub>FG</sub>	4.63 <sub>ABC</sub>	17.82 <sub>CDE</sub>	4.09 <sub>B</sub>	8.08 <sub>D</sub>
	<b>400</b>	119.0 <sub>ABC</sub>	53.10 <sub>DE</sub>	1092 <sub>EFG</sub>	5.37 <sub>AB</sub>	18.43 <sub>CDE</sub>	4.17 <sub>B</sub>	8.32 <sub>BCD</sub>
	<b>600</b>	122.7 <sub>ABC</sub>	70.07 <sub>BC</sub>	1131 <sub>DEF</sub>	6.25 <sub>A</sub>	19.70 <sub>BCD</sub>	4.72 <sub>A</sub>	8.67 <sub>A-D</sub>
<b>Mean</b>		<b>115.5<sub>B</sub></b>	<b>54.61<sub>B</sub></b>	<b>1066<sub>B</sub></b>	<b>5.01<sub>A</sub></b>	<b>17.56<sub>C</sub></b>	<b>4.22<sub>A</sub></b>	<b>8.24<sub>B</sub></b>
<b>Soil application</b>	<b>100</b>	123.7 <sub>ABC</sub>	64.50 <sub>CD</sub>	1157 <sub>C-F</sub>	4.14 <sub>BC</sub>	17.52 <sub>CDE</sub>	4.13 <sub>B</sub>	8.20 <sub>BCD</sub>
	<b>200</b>	130.7 <sub>AB</sub>	64.87 <sub>CD</sub>	1183 <sub>B-E</sub>	4.64 <sub>ABC</sub>	18.84 <sub>CDE</sub>	4.17 <sub>B</sub>	8.31 <sub>BCD</sub>
	<b>400</b>	137.7 <sub>A</sub>	67.87 <sub>BC</sub>	1222 <sub>A-D</sub>	5.18 <sub>ABC</sub>	21.18 <sub>ABC</sub>	4.23 <sub>B</sub>	8.48 <sub>A-D</sub>
	<b>600</b>	123.7 <sub>ABC</sub>	65.97 <sub>BC</sub>	1222 <sub>A-D</sub>	3.50 <sub>C</sub>	20.88 <sub>ABC</sub>	4.03 <sub>B</sub>	8.11 <sub>CD</sub>
<b>Mean</b>		<b>124.6<sub>A</sub></b>	<b>62.27<sub>B</sub></b>	<b>1160<sub>A</sub></b>	<b>4.35<sub>AB</sub></b>	<b>18.74<sub>B</sub></b>	<b>4.12<sub>A</sub></b>	<b>8.24<sub>B</sub></b>
<b>Foliar application</b>	<b>10</b>	123.7 <sub>ABC</sub>	83.50 <sub>A</sub>	1313 <sub>A</sub>	4.47 <sub>BC</sub>	23.89 <sub>A</sub>	4.32 <sub>B</sub>	9.16 <sub>A</sub>
	<b>20</b>	121.3 <sub>ABC</sub>	78.20 <sub>AB</sub>	1261 <sub>AB</sub>	3.69 <sub>BC</sub>	23.32 <sub>AB</sub>	4.20 <sub>B</sub>	8.84 <sub>ABC</sub>
	<b>40</b>	115.5 <sub>BC</sub>	76.07 <sub>ABC</sub>	1248 <sub>ABC</sub>	3.58 <sub>C</sub>	23.32 <sub>AB</sub>	4.17 <sub>B</sub>	8.90 <sub>AB</sub>
	<b>60</b>	102.5 <sub>ABC</sub>	75.50 <sub>ABC</sub>	1235 <sub>ABC</sub>	3.51 <sub>C</sub>	23.08 <sub>AB</sub>	4.09 <sub>B</sub>	8.66 <sub>A-D</sub>
<b>Mean</b>		<b>118.1<sub>B</sub></b>	<b>72.28<sub>A</sub></b>	<b>1214<sub>A</sub></b>	<b>3.90<sub>B</sub></b>	<b>21.78<sub>A</sub></b>	<b>4.16<sub>A</sub></b>	<b>8.73<sub>A</sub></b>
<b>Mean of concentration</b>	<b>C1</b>	119.8 <sub>A</sub>	66.22 <sub>A</sub>	1161 <sub>A</sub>	4.38 <sub>A</sub>	19.32 <sub>A</sub>	4.17 <sub>AB</sub>	8.48 <sub>AB</sub>
	<b>C2</b>	122.9 <sub>A</sub>	64.71 <sub>A</sub>	1174 <sub>A</sub>	4.32 <sub>A</sub>	20.00 <sub>A</sub>	4.15 <sub>AB</sub>	8.41 <sub>AB</sub>
	<b>C3</b>	124.1 <sub>A</sub>	65.68 <sub>A</sub>	1187 <sub>A</sub>	4.71 <sub>A</sub>	20.97 <sub>A</sub>	4.19 <sub>AB</sub>	8.57 <sub>A</sub>
	<b>C4</b>	123.0 <sub>A</sub>	70.51 <sub>A</sub>	1196 <sub>A</sub>	4.42 <sub>A</sub>	21.22 <sub>A</sub>	4.28 <sub>A</sub>	8.48 <sub>AB</sub>

As a consequence, increasing the concentration to 600 mg L<sup>-1</sup> reduces these nutrients in the soil. Except for Zn and Cu, micronutrient availability (Fe and Mn) grew gradually when the

soil was treated with GO up to 400 mg L<sup>-1</sup>. Micronutrient availability followed similar patterns to macronutrient availability. Increasing the GO concentration to 600 mg L<sup>-1</sup> resulted in a decrease in the nutrients provided below. On the other hand, foliar techniques of GO with different concentrations, as shown in Table 5, reveal that macronutrient availability (N, P, and K) decreased gradually as concentrations increased. These findings demonstrated that the large amounts of GO added were unsuitable for macronutrient availability. However, foliar application with 10 mgL<sup>-1</sup> was suitable for applying GO nanoparticles for macronutrient availability in soil after wheat plant cultivation.

Finally, when comparing the application of different GO application methods and their effect on the availability of nutrients in the soil, mean values in Table 5 reported that, regardless of different concentrations, the soil application method significantly increased nitrogen availability in the soil after wheat plant cultivation as compared to either the soaking method or the foliar method.

#### 4. Discussion

Relative to the control group, wheat leaves rose across all concentrations and treatment approaches except from elevated foliar application doses, chlorophyll a, b, and carotene levels. The results are ascribed by **Siddiqui et al. (2019)** to direct impact of chlorophyll on plant photosynthesis and its importance as a vital indication of plant development, vitality, and environmental changes. Using GO nanoparticles greatly improved carrot growth, chlorophyll levels, and carotenoid concentrations. Using 500 mg L<sup>-1</sup> of graphene led to a notable decrease in chlorophyll levels in wheat leaves. Research conducted by **Zhang et al. (2016)** and **Song et al. (2020)** revealed that after 30 days of exposure to graphene, the chlorophyll content in wheat was significantly reduced. After 100 days of exposure, graphene oxide at the highest concentration of 0.6% lowered the total chlorophyll concentration and net photosynthetic rate, **Zhao et al. (2022)** found This corresponds with the results of **Hu et al. (2014c)** and **Du et al. (2015)**, who found that graphene reduces plant synthesis of chlorophyll a and b. According to **Hu et al. (2014b)**, exposure to GO changed the chloroplasts' ultrastructure in wheat leaves. The application of high-concentration GO markedly disrupted the cell wall structure of wheat roots (**Chen et al., 2018**) due to elevated ROS generation, corroborating our results. Further research has shown that GO inhibits photosynthesis (**Zhao et al., 2022**). The reduction in chlorophyll content may stem from the inhibition of chlorophyll synthesis or the degradation of chloroplasts. The application of GO by various techniques and concentrations significantly enhanced the fresh and dry weight of wheat plant organs, including shoots and roots, relative to control treatments. **Guo et al. (2021)** discovered that moderate doses of graphene oxide (50 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>) enhanced root biomass retention and morphological development in tomato seedlings.

This was achieved by upregulating genes associated with root growth and increasing the levels of indole-3-acetic acid (IAA). A notable quantity of graphene oxide was observed to enhance plant growth by promoting root length, leaf area and count, and flower bud development (**Park et al., 2020**). Additionally, graphene oxide influenced the maturation of watermelon, resulting in an increase in both the fruit's diameter and sugar content. Our findings suggest that graphene oxide has the potential to enhance plant growth and accelerate the ripening process of fruits. **Chakravarty et al. (2015)** utilized 0.2 g L<sup>-1</sup> graphene in a seed soak pretreatment to enhance the growth of coriander and garlic plants. **Zhao et al. (2023)** found that graphene oxide significantly affected the growth and shape of Lucerne roots, with the impact dependent on the amount of graphene oxide utilized. The root length, diameter, volume, dry weight, lateral root

count, and root activity were all reduced by the highest concentration of GO (0.6%). Conversely, the periderm and phellem's thicknesses, as well as the diameters of the vascular cylinder and arteries, all decreased. The results could be linked to the oxidative damage caused by GO. **Zhang et al. (2016)** observed that a concentration of 500 mg L<sup>-1</sup> of graphene had a negative impact on the growth of wheat plants. The phytotoxic effects of graphene appear to be linked to compromised root hair development, heightened oxidative stress, nutritional deficiencies, and the inhibition of photosynthesis. **Hu et al. (2014a)** determined that decreasing graphene content to 200 mg L<sup>-1</sup> for 5 days did not substantially affect wheat grain germination, root length, or fresh weight. **Yu et al. (2016)** demonstrated that the incorporation of GO into the soil enhanced root development. The impact of GO is essential for plant development and resilience to stress in agricultural and forestry contexts. Initially, an increase in total root length and surface area improved water and mineral nutrient availability for plant growth, thereby enhancing biomass when associated with higher pigment content and photosynthetic efficiency. Secondly, enhanced root systems and elevated total amino acid and protein levels confer onto plants stronger osmotic management abilities, allowing them to endure various adverse conditions. **Zhang et al. (2021a)** validated the enhancement of leaf and root development subsequent to the application of GO to the soil. This led to a substantial improvement in fresh weight and morphological characteristics, such as average maximum leaf length, width, total root length, surface area, and volume. The GO treatment elevated overall amino acid and protein concentrations while preserving the leaf's water content. This signifies that GO promotes plant growth by increasing water absorption and total physical activity. Oxidative stress and damage to cell membranes may be caused by high doses of GO therapy, according to research conducted by **Chen et al. (2018)**. **Weng et al. (2020)** noted that increased quantities of GO can be retained in root vacuoles, resulting in a marked decrease in both the maximum root length and the number of lateral roots. Furthermore, observations indicated a whitening of the root tips, the presence of wrinkles, signs of oxidative stress, and a decrease in respiration rates. The findings indicate that GO plays a role in inhibiting significant root growth and limiting the area available for root absorption. Additionally, **Ren et al. (2020)** observed that plants react to xenobiotic stress by engaging various physiological processes, such as antioxidant systems, ion pumps, and membrane potential, to safeguard themselves from stressors and sustain normal metabolic functions. This study demonstrated that oxidative stress serves as the primary mechanism underlying the reduced growth of wheat seedlings when exposed to elevated GO concentrations. According to **He et al. (2018)**, who found significantly increased moisture content in the GO-treated zone compared to the GO-free area, the obtained data were in agreement with their findings. As a result, GO's hydrophilic oxygen-containing functional groups probably explain its extraordinary capacity to absorb and hold soil moisture. In addition to increased concentrations of foliar applications, the study revealed that varying concentrations and techniques of GO markedly enhanced the absorption of macronutrients and micronutrients by wheat plants (shoots and roots) throughout the booting growth stage. Additional factors influencing diminished nutrient absorption encompass the effect of GO on root surface chemistry (**Zhang et al., 2016**), the decrease in NO<sub>3</sub><sup>-</sup> absorption, and the inhibition of transporter gene expression (**Weng et al., 2020; Zhu et al., 2022**). **Hu et al. (2014a)** and **Zhang et al. (2016)** contend that GO can adhere to root surfaces, obstruct nutrient uptake, harm root hairs, and provoke oxidative stress, hence limiting nutrient absorption. Moreover, the findings corresponded with those of **Zhang et al. (2016)**, who demonstrated that 500 mg L<sup>-1</sup> graphene significantly diminished the contents of two macronutrients, nitrogen and potassium. Furthermore, graphene impeded the accumulation of Ca, Mg, Fe, Zn, and Cu compared to the

control group. However, the supply of graphene did not affect the concentrations of phosphorus and sodium. The studies demonstrate that graphene modified the concentrations of key nutritional constituents in shoots. The reduction in amounts of nitrogen, potassium, calcium, magnesium, iron, zinc, and copper in shoots may be linked to the process by which graphene modifies the surface chemistry of roots, subsequently influencing their environmental interactions; root injury was observed in plants subjected to graphene exposure. The findings correspond with those of **Weng et al. (2020)**, who noted a substantial decrease in nitrate levels in the roots of wheat plants treated with 200-800 mg L<sup>-1</sup> graphene oxide. They also demonstrated that GO may substantially reduce the net influx of NO<sub>3</sub><sup>-</sup> into the meristematic, elongation, and maturation zones of wheat roots. **Zhao et al. (2022)** discovered that GO at the B and Si sites enhanced the activities of antioxidant enzymes, lipid peroxidation, reactive oxygen species, and electrolyte leakage, particularly with extended exposure to elevated concentrations. Research indicates that GO can reduce photosynthesis, provoke oxidative stress, and create nutritional imbalances, so impeding plant growth. Our findings indicate that elevated levels of GO impede root growth and development by inducing structural damage, nutritional imbalance and oxidative stress. Prioritizing the precise control of soil GO is crucial. Additionally, **Hu et al. (2014c)** discovered that graphene significantly modified the quantity of metabolites (fatty acids, amino acids, and carbs) in wheat shoots. We assert that graphene-induced nutrient deprivation interferes with these metabolites, hence reducing biomass production, as nutritional elements are essential for plant growth and productivity (**López- Buico et al., 2003**) and act as precursors for certain metabolites (**Rasmussen et al., 2008**). Moreover, following a 30-day exposure to graphene, wheat growth was impeded, and the levels of many important elements (N, K, Ca, Mg, Fe, Zn, and Cu) decreased, leading to a nutritional imbalance (**Zhang et al., 2016**). Thus, the impact of graphene on plant growth varied according to the dosage and length of exposure. Using graphene oxide in different methods and amounts greatly affected the availability of macronutrients (nitrogen, phosphorus, and potassium) compared to the untreated group. **Zhang et al. (2022)** delineate the principal applications of GFN as in vivo injection, integration into solid or liquid media, and soil irrigation. The method of integrating chemicals into aqueous solutions or growth media is extensively employed in research due to its simplicity, high absorption efficacy, and lack of interference complications. Nevertheless, owing to the similarity of soil treatments to the plants' natural growth conditions, a growing number of studies have utilized them in recent years. Furthermore, many direct injection and foliar treatments have been executed. **Feriancikova and Xu (2012)**; **Wu et al. (2013)**. GO is more challenging to maneuver on solid mediums than to liquid surroundings. Owing to its robust soil-binding characteristics, GO poses difficulties for plant roots to penetrate, potentially reducing its toxicity. Fewer GO sheets attached to the roots in soil culture than in hydroponic culture, possibly due to the interaction between graphene and soil. According to **L'opez-Bucio et al. (2003)**, micronutrients and macronutrients were all negatively impacted by high concentrations of GO, indicating that GO disrupted the mineral nutritional composition of roots. Thus, GO has demonstrated significant potential for improving agricultural output and soil system quality (**He et al., 2019**). Multiple studies have thoroughly examined the "indirect" nano toxicity of generally innocuous graphene oxide (GO), which increased phytotoxicity and is extensively distributed in wheat. Five variables have led to the indirect nanotoxicity of graphene oxide (GO). (i) Increasing oxidative stress and impeding growth; (ii) Modulating essential metabolic processes, encompassing secondary, amino, and carbohydrate metabolism; (iii) Amplifying cellular permeability and causing structural damage; (iv) Controlling uptake and transformation via chemical interactions, including cellular entry; and (v)

Governing uptake and transformation through biological mechanisms. **He et al. (2019)** contend that GO acts as a soil conditioner, providing moisture and nutrients to enhance plant growth (**He et al., 2018**). The addition of GO resulted in a darker soil composition. In addition to functioning as an organic component that improves soil fertility and carbon content, GO can markedly diminish soil compaction. Furthermore, foliar spray increased the availability of phosphate and potassium compared to alternative treatment approaches. A significant trend was seen for micronutrient availability in soil; the average values in Table 5 indicated that soaking with GO improved the availability of both Fe and Zn in the soil. Compared to other treatment strategies, foliar spray most effectively enhanced the amount of manganese and copper that were available in the soil. In contrast, **Guo et al. (2021)** demonstrated that graphene modifies the composition, diversity, and activity of soil bacteria essential for nutrient cycling and soil quality, depending on the duration of exposure (**Ren et al., 2015**).

## 5. Conclusion

To summarize, soil nutrient components are critical for plant development and production because their bioavailability influences root growth and functional responses. However, soil factors like chemical composition, enzyme activity, and nutrient cycling have hardly been studied concerning graphene. Graphene oxide is a potential addition to stimulate plant development, particularly wheat, which is regarded as a strategic crop. The addition method and concentrations employed in each technique affect graphene oxide's effect. Soaking wheat seeds is considered safe, even in large numbers. While soil treatment is considered adequate, up to 400 mg L<sup>-1</sup>, increasing the dosage affects plant growth and element content in the soil. Adding GO as a foliar spray to the plant is applicable only at low concentrations of 10 mg L<sup>-1</sup>, while the other evaluated doses are considered damaging and toxic to wheat development. GO may affect nutritional homeostasis, as seen by the reduced levels of some nutrients in wheat leaves. Future studies should look into the mechanism of GO toxicity on root architecture and the consumption of nutrients using metabolomics approaches.

## 6. Conflicts of interest

There are no conflicts to declare.

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