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Investigating the Morpho-physiological and Biochemical Characteristics of Bell Peppers under Drought Stress and Carbon Nanoparticle Foliar Application in Greenhouse Conditions

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ABSTRACT

Dehydration stress is the most significant abiotic factor affecting plant growth and development. In recent years, the use of engineered nanomaterials has emerged as a promising solution to mitigate the destructive effects of drought stress on plants. This study, conducted in 2021, aimed to investigate the impact of carbon nanoparticles on the properties of bell peppers under drought stress in greenhouse conditions. A completely randomized design with three replications was used. The first factor was irrigation levels, based on field capacity (FC), with three treatments: 100% FC, 75% FC, and 50% FC. The second factor involved the application of carbon nanoparticles at ten levels: no nanoparticles, fullerene (100, 200, 1000 mg L^{-1}), graphene (100, 200, 1000 mg L^{-1}), and multi-walled carbon nanotubes (100, 200, 1000 mg L^{-1}). The results indicated that foliar application of carbon nanoparticles alleviated the effects of water-deficit stress on root dry weight but did not significantly reduce the negative impact on fruit yield. However, the application of 200 mg L^{-1} of nanographene and 1000 mg L^{-1} of multi-walled carbon nanotubes under 100% FC irrigation improved fruit production compared to the absence of nanoparticles at the same irrigation level. Additionally, the use of multi-walled carbon nanotubes enhanced the activity of catalase and peroxidase enzymes. However, the effect of nanoparticles on enzyme activity varied across different irrigation levels.

Introduction

The development of greenhouse crops is essential for increasing yield, reducing water consumption, and protecting crops against adverse climatic conditions (Thakur et al., 2018). According to published statistics, an average of 65.51% of Iran's total cultivated greenhouse area is dedicated to growing vegetable crops (Agricultural Statistics, 2017). The bell pepper, scientifically known as *Capsicum annuum* L., is a significant fruit vegetable from the Solanaceae family (Getahun and Habtie, 2017). Bell peppers can be harvested at various stages of maturity (Mardanluo et al., 2018) and are popular among consumers worldwide for their nutritional value, excellent flavor, and taste (Ge et al., 2020). They are rich in natural antioxidants and essential nutrients such as vitamin C, carotenoids, phenolic compounds, and potassium. This high nutritional value has secured their place in household consumption (Bosland and Votova, 2000).

Globally, agriculture is the largest consumer of water, accounting for approximately 70% of water resource withdrawals in developed countries and up to 95% in developing countries (Wada et al., 2011). Dehydration stress is the most significant abiotic factor affecting plant growth and development (Mumivand et al., 2021) and has a profound impact on the growth, physiology, and performance of crop plants worldwide (Jalil and Ansari, 2020). Therefore, increasing the productivity of agricultural products by alleviating drought will be one of the most important challenges in the coming years.

Huitzimengari et al. (2013) reported that ribulose bisphosphate activity decreased during drought stress in pepper plants due to reduced stomatal activity and closure, leading to diminished photosynthesis. Additionally, research investigating the effects of ascorbic acid on drought stress tolerance in bell pepper (*C. annuum* L.) revealed that drought stress not only reduced the number of fruits, plant height, weight, yield, and chlorophyll a and b levels but also increased the activity of catalase, peroxidase, superoxide dismutase, proline, anthocyanins, soluble sugars, malondialdehyde, and H_2O_2 in the leaves (Khazaei et al., 2020). Furthermore, a study examining how drought stress affects the growth, physiological, and biochemical reactions of red pepper (*C. annuum* L.) cultivars found that drought stress resulted in a decrease in growth, physiological, and biochemical parameters compared to the control group (Molla et al., 2023).

The potential impact of carbon nanoparticles on plants is significant, whether from their intentional use in agricultural and environmental programs or their accidental release as pollutants (Zaytseva and Neumann, 2016). Some nanoparticles, possessing unique physicochemical properties, not only act as nanocarriers but also enhance plant growth and stress tolerance. This biological role of nanoparticles is influenced by their physicochemical properties, application method (such as foliar application, hydroponics, or soil), and concentration used (Zhao et al., 2020).

Carbon nanoparticles can increase plant photosynthesis, promote crop growth, and enhance water uptake (Mukherjee et al., 2016). They also boost antioxidant levels (Ghasempour et al., 2019) and improve the efficiency of nitrogen (N), phosphorus (P), and potassium (K) use (Zhao et al., 2021). Ahmadi et al. (2020) confirmed the absorption and distribution of fullerene C60 in the leaf system through scanning electron microscopy (SEM) images after foliar spraying on two Feverfew genotypes. Research investigating the interaction effects of compost, arbuscular mycorrhizal fungi, and carbon nanoparticles on corn under drought stress found that combined treatments significantly increased soil fertility and improved corn plant growth in both control and drought conditions, with increases of 1.20% and 4.39%, respectively (Alsherif et al., 2023).

In a study examining the effect of carbon nanoparticles on various characteristics of chili pepper plants under drought stress, the results indicated that drought stress significantly reduced plant height, fresh weight, and dry weight. However, the application of functionalized carbon nanoparticles increased relative water content, chlorophyll stability index, and chlorophyll fluorescence (Fv/Fm) under drought conditions. These nanoparticles also significantly elevated proline content during drought by reducing abscisic acid levels in leaves and enhancing antioxidant activities, including superoxide dismutase and catalase (Alluqmani and Alabdallah, 2023).

Drought is one of the most significant abiotic stresses that can adversely affect crop performance. Given their potential benefits, carbon nanoparticles have garnered considerable attention for their impact on plants experiencing abiotic stress conditions. This study aims to examine the effects of carbon nanoparticles on the morpho-physiological and biochemical characteristics of bell pepper plants under drought stress in greenhouse conditions. The findings from this research could prove valuable in developing strategies to enhance plant tolerance to drought stress.

Material and Methods

Planting, growth conditions, and applying treatments

The current research was conducted in 2022 using a factorial design in a completely random layout with three replications in the greenhouse of the Faculty of Agriculture, Lorestan University. The first factor involved irrigation levels based on field capacity at three levels (100% FC, 75% FC and 50% FC), while the second factor involved the use of carbon nanoparticles at ten levels (no nanoparticles, fullerene 100 mg L^{-1}, fullerene 200 mg L^{-1}, fullerene 1000 mg L^{-1}, graphene 100 mg L^{-1}, graphene 200 mg L^{-1}, graphene 1000 mg L^{-1}, multi-walled nanotube 100 mg L^{-1}, multi-walled nanotube 200 mg L^{-1} and multi-walled nanotube 1000 mg L^{-1}). Each pot in the study contained five bell pepper seeds, with only one seedling retained after germination. The pots, measuring 20 cm in diameter and 30 cm in height, were filled with 10 kg of a substrate composed of field soil, sand, and manure in a 1:1:1 ratio. To prepare the stock solution of carbon nanoparticles, 0.55 g of each nanoparticle was mixed with 50 mL of distilled

water for 30 min and then ultrasonicated to ensure even distribution. The solution was diluted with distilled water to 500 mL to create different concentrations of carbon nanoparticles. The nanoparticles were sprayed onto the plants at the four-leaf stage, with a second application two weeks later (Ahmadi et al., 2020).
Soil moisture levels were monitored using tensiometers, starting two days after the second foliar spraying and continuing until harvest. To determine the field capacity, the pots were weighed before and after irrigation, with the difference in weight indicating the amount of water available to the plants. The soil characteristics of the pots are provided in Table 1. Seeds were purchased from Keshtzar Company in Tehran, while carbon nanoparticles were purchased from Iranian Nano Materials Pioneers Company in Mashhad for the experiment. The specific characteristics of the carbon nanoparticles can be found in Table 2.

Table 1. Analysis of soil physical and chemical testing.

Texture	EC	pH	O.C	N	P	K
	($ds\ m^{-1}$)			(%)	($mg\ kg^{-1}$)	
Sandy Clay loam	2.45	6.9	1.2	0.17	14.85	367

Table 2. Characteristics of carbon nanoparticles used in this study.

C60 fullerene	Morphology	Color	Decoloration rate	Purity	Sterilization	APS	H_2O	Ash	pH	True density	Bulk density
	Nanospherical	Black	99%	>95%	Cobalt-60 Radiation	20-40 nm	<5%	<2%	7-10	0.44 g mL^{-1}	0.32 g mL^{-1}
Multi walled nanotubes (MWNTs)	Morphology	Color	Outside diameter	Purity	Inside diameter	SSA	Length	Ash	EC	True density	Bulk density
	nanotube	Black	20-30 nm	>95%	5-10 nm	>110 $m^2\ g^{-1}$	10-30 um	<1.5%	>100 s cm^{-1}	~2.1 g cm^{-3}	0.28 g cm^{-3}
Graphene Nanoplatelets	Morphology	Color	Volume Resistivity	Purity	diameter	SSA	Thickness	The Product COA	pH	True density	Bulk density
	Nanoplatelets Powder	Black	$4x10^{-4}$ ohm.cm	99.5%	4 -12 um	500 - 1200 $m^2\ g^{-1}$	2-18 nm, Less Than 32 Layers	C = 99.7%, O<0.3%	7-7.7	-	-

Measurement of morphological traits

At the end of the experiment, plant height was measured using a ruler. The plants were then cut and placed in an oven at 70°C for 48 h to measure their dry weight. The dry weight was determined using a digital scale with an accuracy of 0.001 g. To assess root volume and dry weight, the roots were carefully removed from the soil to prevent damage. Any soil clinging to the roots was washed away with water. Root volume was calculated by measuring the difference in water volume before and after submerging the roots. For dry weight measurement, the root samples were placed in an oven at 70°C for 48 h.

Agronomic traits

At 132 days after seed germination, the fruits from each plant were harvested and counted separately. A digital scale was used to measure both the fresh weight and dry weight of the fruits.

Physiological traits

Membrane stability

To evaluate cell membrane stability in the leaves, an electrolyte leakage test was conducted. Identical circles were cut from fully developed leaves for each treatment, then placed in glass tubes containing distilled water and left at room temperature for 24 h. After this period, the electrical conductivity (EC1) of the solution was measured.
To assess electrolyte leakage from dead cells, the tubes were autoclaved at 120°C for 20 min. Once cooled, the electrical conductivity of the solution (EC2) was measured again. The percentage of electrolyte leakage from the membranes was then calculated using the formula provided by Shi et al. (2006).

$$MS\ (\%) = 1 - \left(\frac{EC1}{EC2}\right) \times 100 \qquad \text{Equation 1}$$

Gas exchanges

Gas exchange factors were measured in the upper leaves using a portable gas exchange measurement device model CI-340 CID, manufactured in the USA. During the gas exchange measurement, the carbon dioxide concentration under the aperture was 350 μmol mol^{-1}, the temperature under the chamber ranged from 29-26 °C, and the relative humidity was 58-62%. This device operates based on the amount of carbon dioxide consumed. Stomatal resistance (s m^{-2}), rate of photosynthesis (µmol H_2O m^{-2} s^{-1}), carbon dioxide concentration substomatal (µmol CO_2 mol^{-1}), transpiration rate (µmol H_2O m^{-2} s^{-1}), vapor pressure difference (kPa), photosynthetic water use efficiency (µmol CO_2 mol^{-1} H_2O), and carboxylation efficiency (mol CO_2 m^{-2} s^{-1}) were all measured.

Biochemical traits

Photosynthetic pigments

Chlorophyll content was determined using the method of Arnon (1949), while carotenoid content was calculated following the method of Lichtenthaler and Wellburn (1983). Thus, 0.5 g of fresh leaf samples were weighed and extracted in a Chinese mortar with 10 mL of 80% acetone. The extract was then centrifuged at 3000 rpm for 10 min. Three ml of the supernatant were transferred to a spectrophotometer (Speco 200 model, manufactured by Analytic Jena, Germany), and the optical absorption of chlorophyll a, chlorophyll b, and carotenoids was measured at wavelengths of 663 nm, 645 nm, and 470 nm, respectively. The concentrations of chlorophyll a, chlorophyll b, and carotenoids were then calculated in mg g^{-1} of fresh leaves using the appropriate formulas.

$$Chlorophyll\ a\ =\ 12.9\ (A_{663})\ -\ 2.9\ (A_{645})$$
Equation 2

$$Chlorophyll\ b\ =\ 22.9\ (A_{645})\ -\ 4.68\ (A_{663})$$
Equation 3

$$Carotenoid\ =\ \frac{(1000\ (A_{470})\ -\ 3.27\times Chl\ a\ -104\ (Chl\ b))}{229}$$
Equation 4

Measurement of catalase enzyme activity

Catalase (CAT) enzyme activity was measured at 25 °C using a spectrophotometer manufactured in Japan, following a method outlined by Aebi (1984). The reaction mixture consisted of 400 µL of 50 mM phosphate buffer (pH = 7), 300 µL of 30% H_2O_2, and 10 µL of enzyme extract. CAT enzyme activity was determined by measuring the decrease in absorbance over one min at a wavelength of 240 nm. The activity of the enzyme was calculated using the quenching coefficient (Ɛ = 39.4 mM^{-1} cm^{-1}) and the provided formula.

$$\text{Activity} = \frac{\Delta A_{240}\times V_t}{\varepsilon\times V_s}$$
Equation 5

Assay of peroxidase enzyme activity

The enzyme activity mentioned above was measured using a method developed by Nakano and Asada (1981), based on the conversion of guaiacol to tetraguaiacol in the presence of hydrogen peroxide and enzyme extract. The reaction mixture contained 200 µL of 50 mM phosphate buffer (pH = 7), 50 µL of 1% guaiacol, 50 µL of 0.3% hydrogen peroxide, and 50 µL of the enzyme extract. After adding the enzyme extract, the increase in absorbance at a wavelength of 470 nm was measured within one min. The amount of tetraguaiacol was determined using an extinction coefficient (Ɛ = 26.6 mM^{-1} cm^{-1}).

$$\text{Activity} = \frac{\Delta A_{470}\times V_t}{\varepsilon\times V_s}$$
Equation 6

Statistical analysis

Data analysis was conducted using SAS software (Ver.9.1). The comparison of mean values was performed using Duncan’s test at a significance level of 5%. Graphs were generated using Microsoft Excel.

Results

The analysis of variance (Table 3) indicated that the effect of dehydration stress on carbon dioxide concentration within the chamber and peroxidase enzyme activity was not statistically significant. However, significant effects were observed for membrane stability, leaf water vapor pressure deficit, and catalase enzyme activity at the 5% probability level. In addition, other traits evaluated in this study were significantly affected at the 1% probability level.

Furthermore, the foliar application of carbon nanoparticles had a significant impact on all traits evaluated, with the exception of plant height, shoot dry weight, root volume, and membrane stability at the 1% probability level. The interaction effects of treatments on plant height, shoot dry weight, carbon dioxide concentration within the chamber, and transpiration rate were not significant. However, significant interaction effects were noted for root volume and total chlorophyll content at the 5% probability level, and for other traits at the 1% probability level.

Plant height and shoot dry weight

The results of the comparison of the average effects of drought stress on plant height (Table 4) indicated that the highest plant height was achieved under irrigation at 100% field capacity (60.19 cm), while the lowest height was observed under irrigation at 50% field capacity (42.88 cm). Similarly, the maximum shoot dry weight was recorded under irrigation at 100% field capacity (15.85 g), whereas the lowest shoot dry weight was observed under irrigation at 50% field capacity (8.59 g).

Table 3. Analysis variance of the effects of foliar spraying of carbon nanoparticles and drought stress on specific morphophysiological and phytochemical characteristics of bell pepper.

S.O. V	D.f	Mean of squares					
		Height	Dry weight of shoot	Root volume	Dry weight of root	Number of fruits	Fresh weight of fruit
Block	2	95.59^{ns}	1.72^{ns}	935.66**	0.55^{ns}	0.31^{ns}	7.24^{ns}
Drought stress (A)	2	2481.74**	407.35**	1268.04**	31.10**	53.15**	67.77**
Carbon nanoparticles(B)	9	76.01^{ns}	6.04^{ns}	269.98^{ns}	30.54**	2.03**	22.37**
A*B	18	55.01^{ns}	5.36^{ns}	384.26*	25.55**	3.55**	48.02**
Error	57	68.61	4.58	186.12	2.57	0.51	7.22
CV.	-	15.58	17.00	20.42	15.10	24.75	33.93

**Significant at 1% level, *Significant at 5%, ^{ns} not statistically significant.

Continuation of Table 3. Analysis variance of the effects of foliar spraying of carbon nanoparticles and drought stress on specific morphophysiological and phytochemical characteristics of bell pepper.

S.O. V	D.f	Mean of squares					
		Membrane stability	Total chlorophyll	The ratio of chlorophyll a to *b*	Carbon dioxide substomatal	transpiration rate	Rate of photosynthesis
Block	2	416.73^{ns}	0.01^{ns}	0.10^{ns}	8516.59^{ns}	0.001^{ns}	22.33^{ns}
Drought stress (A)	2	622.48*	0.35**	1.53**	10130.47^{ns}	3.44**	1216.55**
Carbon nanoparticles(B)	9	127.03^{ns}	0.10**	0.48**	25117.66**	2.76**	111.24**
A*B	18	423.06**	0.04*	0.54**	5739.80^{ns}	0.70^{ns}	113.19**
Error	57	196.73	0.02	0.08	9040.24	0.46	15.76
CV.	-	34.92	12.69	22.42	25.85	25.93	20.45

**Significant at 1% level. *Significant at 5%, ^{ns} not statistically significant.

Continuation of Table 3. Analysis variance of the effects of foliar spraying of carbon nanoparticles and drought stress on specific morphophysiological and phytochemical characteristics of bell pepper.

S.O. V	D.f	Mean of squares					
		Vapor pressure difference	Photosynthetic water use efficiency	Carboxylation Efficiency	stomata resistance	Catalase enzyme	Peroxidase enzyme
Block	2	0.32^{ns}	0.007^{ns}	2.36^{ns}	0.0000003^{ns}	0.003^{ns}	0.03^{ns}
Drought stress (A)	2	0.61*	0.11**	76.92**	0.000004**	0.04*	0.04^{ns}
Carbon nanoparticles(B)	9	2.87**	0.555**	53.61**	0.000006**	0.12**	0.47**
A*B	18	1.25**	0.87**	33.55**	0.000003**	0.18**	0.12**
Error	57	0.15	0.007	11.07	0.0000005	0.02	0.04
CV.	-	26.78	15.14	41	18.46	13.88	35.86

**Significant at 1% level. *Significant at 5%, ^{ns} not statistically significant.

Table 4. Mean comparisons of the morphological traits of bell pepper plants under the influence of drought stress treatments.

Treatments	Treatment levels	Height (cm)	Dry weight of shoot(g)
Drought stress	100% FC	60.19^{a}	15.85^{a}
	75% FC	56.53^{b}	13.34^{b}
	50% FC	42.88^{c}	8.59^{c}

Non-identical letters indicate a significant difference at the 5% level among the means based on Duncan's test.

Volume and dry weight of the root

Comparison of the mean interaction effects of the treatments on the traits of root volume and dry weight (Figs. 1 and 2) showed that the highest root volume was achieved with the application of 100 mg L^{-1} of nano fullerene and irrigation at 75% FC (90.45 cm^3), with no significant difference compared to the control. The lowest root volume was obtained by applying a concentration of 1000 mg L^{-1} of nanographene at 50% FC (47.55 cm^3), which was 37.3% less than the control. Similarly, the highest root dry weight was obtained by applying a concentration of 200 mg L^{-1} of multi-walled nanotubes and irrigation at 100% FC (15.90 g), which was 53% higher than the control. The lowest root dry weight was achieved by applying a concentration of 1000 mg L^{-1} of nano fullerene at 75% FC irrigation (4.34 g), which was 58.2% less than the control.

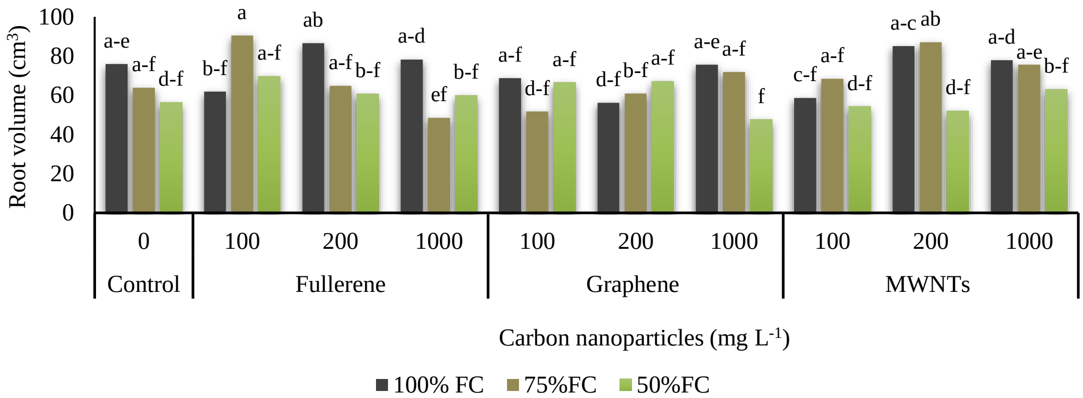

Fig. 1. Changes in root volume in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

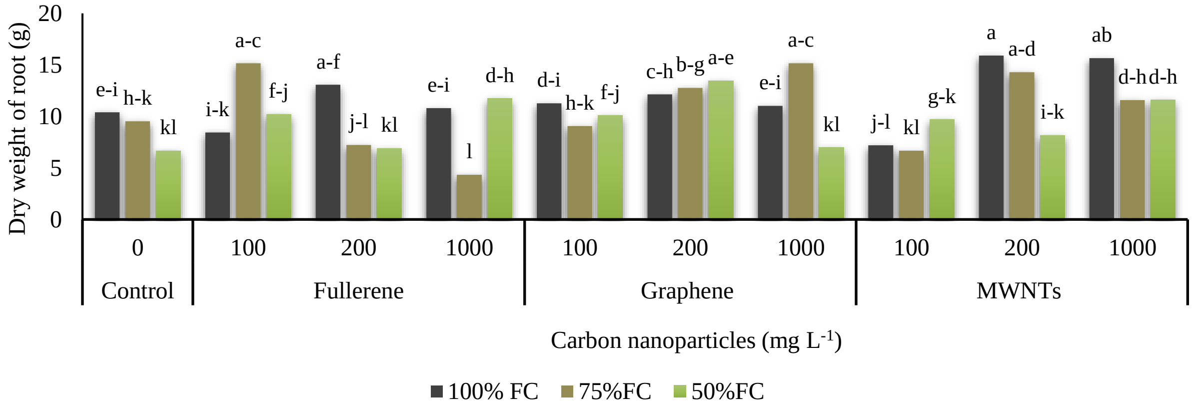

Fig. 2. Changes in the dry weight of roots in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

Number and fresh weight of fruit

The investigation of the interaction effect of carbon nanoparticle application and irrigation levels on fruit count (Fig. 3) showed that the highest fruit count (5.62) was achieved with the application of 1000 mg L^{-1} of multi-walled nanotubes under irrigation at 100% field capacity (FC), representing a 76.17% increase compared to the control. This result was not significantly different from the application of 200 mg L^{-1} nanographene at the same irrigation level.

Furthermore, the comparison of the mean interaction effects of irrigation levels and foliar application of carbon nanoparticles on the fresh weight of fruit (Fig. 4) revealed that the highest fresh fruit weight (15.81 g) was obtained with 200 mg L^{-1} of nanographene under 100% FC, reflecting a 284.67% increase compared to the control. Conversely, the lowest fresh fruit weight (2.02 g) was observed with the application of 200 mg L^{-1} of nano-fullerene under 50% FC, showing a 76.89% reduction compared to the control.

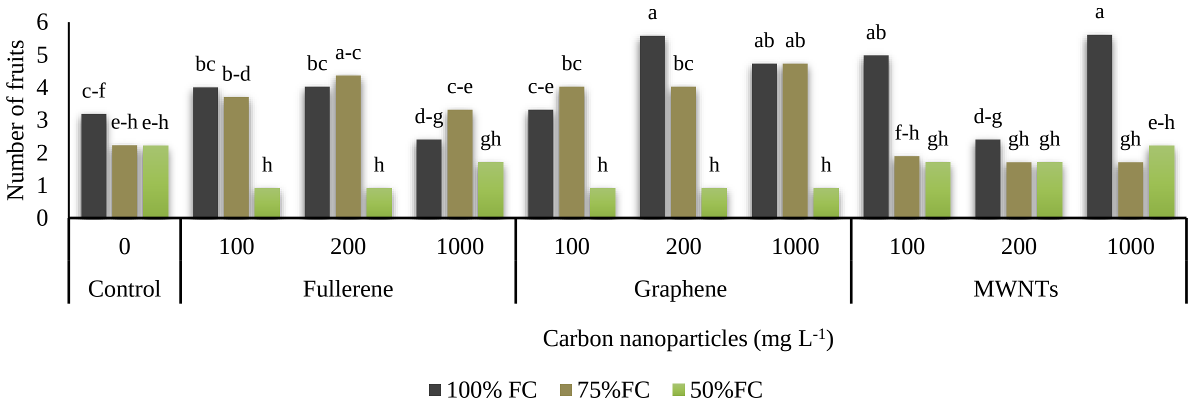

Fig. 3. Changes in the number of fruits on bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

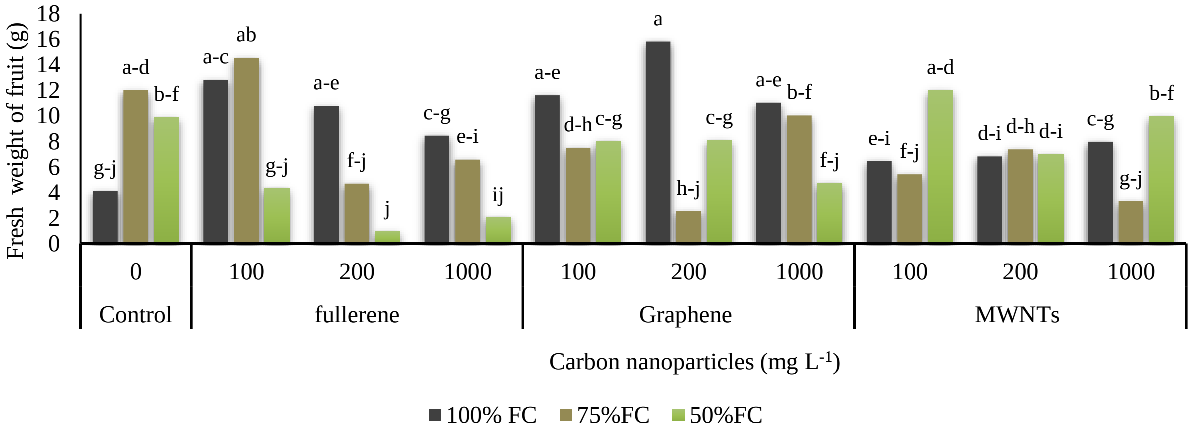

Fig. 4. Changes in the fresh weight of fruits in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

Membrane stability

In comparing the combined effects of irrigation levels and carbon nanoparticles application on membrane stability (Fig. 5), it was discovered that the highest stability (60.98%) was achieved with 1000 mg L^{-1} of nano fullerene and 100% FC irrigation, which was not significantly different from the control. Conversely, the lowest stability (16.43%) was observed with 1000 mg L^{-1} of nanographene and 50% FC irrigation, which was also not significantly different from the control.

Total chlorophyll and ratio of chlorophyll a to b

In evaluating the interaction effect of irrigation levels and foliar application of carbon nanoparticles on chlorophyll traits (Figs. 6 and 7), the highest total chlorophyll content was observed at an irrigation level of 100% field capacity (FC) with the application of 1000 mg L^{-1} of nano-fullerene (1.48 mg g^{-1} FW), reflecting a 23% increase compared to the control. The lowest total chlorophyll content was recorded at 75% FC with the foliar application of 200 mg L^{-1} nanographene (0.83 mg g^{-1} FW), representing a 27% decrease compared to the control.

Additionally, the highest chlorophyll a to b ratio was observed at 50% FC with the application of 100 mg L^{-1} nanographene (2.33), showing a 73% increase relative to the control. In contrast, the lowest ratio was found at 75% FC with the application of 100 mg L^{-1} of multi-walled nanotubes (0.58), indicating a 57% reduction compared to the control.

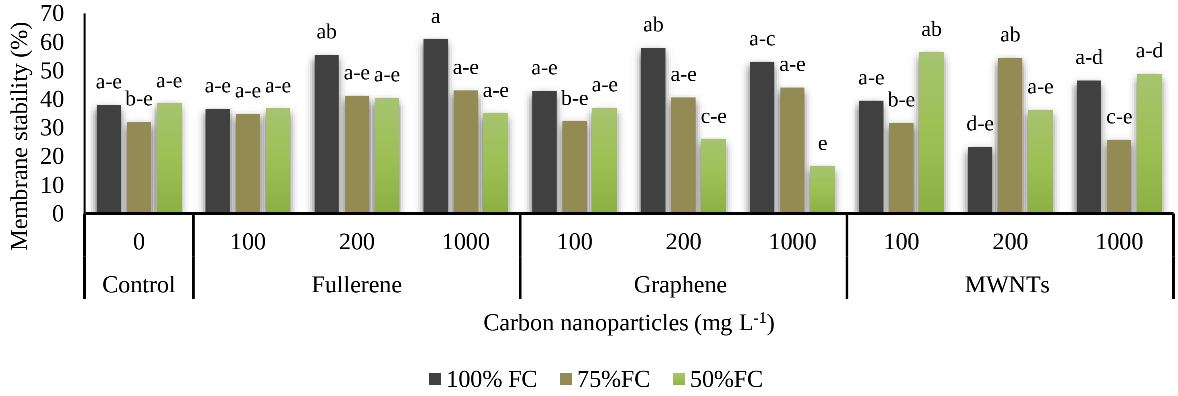

Fig. 5. Changes in membrane stability in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

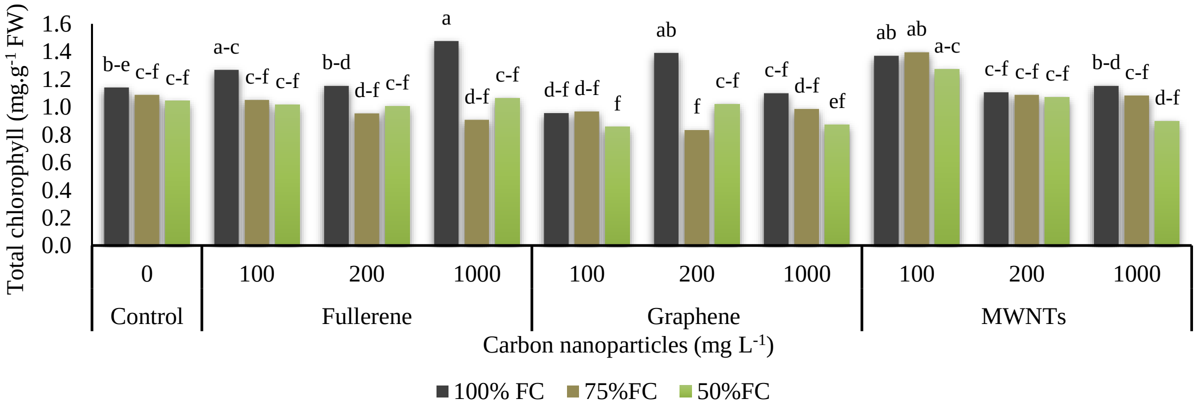

Fig. 6. Changes in total chlorophyll in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

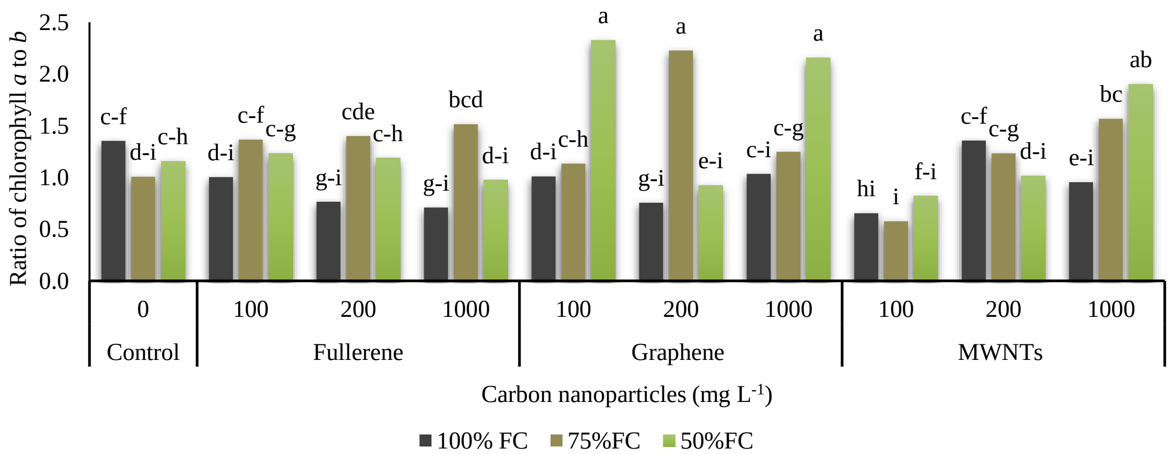

Fig. 7. Changes in the ratio of chlorophyll a to b in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

Gas exchange traits

The results of the comparison of the mean effect of carbon nanoparticle foliar spraying alone on the characteristic of carbon dioxide substomatal (Table 5) showed that the highest value of this characteristic was associated with the application of a concentration of 100 mg L^{-1} of nanographene (430.80 µmol CO_2 mol^{-1}). Which was not significantly different from the control. The lowest value was related to foliar spraying with a concentration of 200 mg L^{-1} of nano fullerene (269.77 µmol CO_2 mol^{-1}). Additionally, the highest rate of transpiration (Table 5) was observed in the treatment of 1000 mg L^{-1} of multi-walled nanotubes (3.29 mmol H_2O m^{-2} s^{-1}), while the lowest rate was related to the control (1.95 mmol H_2O m^{-2} s^{-1}).

Table 5. Mean comparisons of the physiological traits of bell pepper plants subjected to carbon nanoparticle foliar spraying treatments.

Treatments	Treatment levels	Carbon dioxide substomatal (µmol CO_2 mol^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})
	Control	387.90^{a}	1.95^{c}
Carbon nanoparticle foliar spraying	Graphene (100 mg L^{-1})	430.80^{a}	3.01^{ab}
	Graphene (200 mg L^{-1})	326.73^{ab}	3.42^{a}
	Graphene (1000 mg L^{-1})	386.25^{a}	2.21^{c}
	Fullerene (100 mg L^{-1})	424.63^{a}	2.31^{c}
	Fullerene (200 mg L^{-1})	269.77^{b}	3.19^{ab}
	Fullerene (1000 mg L^{-1})	337.34^{ab}	2.18^{c}
	MWNTs (100 mg L^{-1})	430.48^{a}	2.55^{bc}
	MWNTs (200 mg L^{-1})	345.93^{ab}	2.07^{c}
	MWNTs (1000 mg L^{-1})	345.93^{ab}	3.29^{a}

Non-identical letters indicate a significant difference at the 5% level among the means based on Duncan's test.

According to the results of the comparison of mean interaction effects of irrigation levels and carbon nanoparticle foliar spraying on the characteristics of photosynthesis rate and water vapor pressure difference (Figs. 8 and 9), the highest value of photosynthesis rate was achieved with the application of 1000 mg L^{-1} multi-walled nanotubes and an irrigation level of 100%FC (35.68 µmol CO_2 m^{-2} s^{-1}), which was 31.64% higher than the control). The lowest value of this trait was observed with the application of 200 mg L^{-1} multi-walled nanotubes and an irrigation level of 50% FC (139.50 µmol CO_2 m^{-2}), which was 58.36% less than the control. Additionally, the highest value of vapor pressure difference was obtained with the application of 200 mg L^{-1} nanofullerene and an irrigation level of 50% FC (3.32 kPa), which was 564% higher than the control). The lowest value of this trait was recorded with the application of 100 mg L^{-1} nanographene and irrigation levels of 100% FC and 75% FC (0.32 kPa), which was not significantly different from the control.

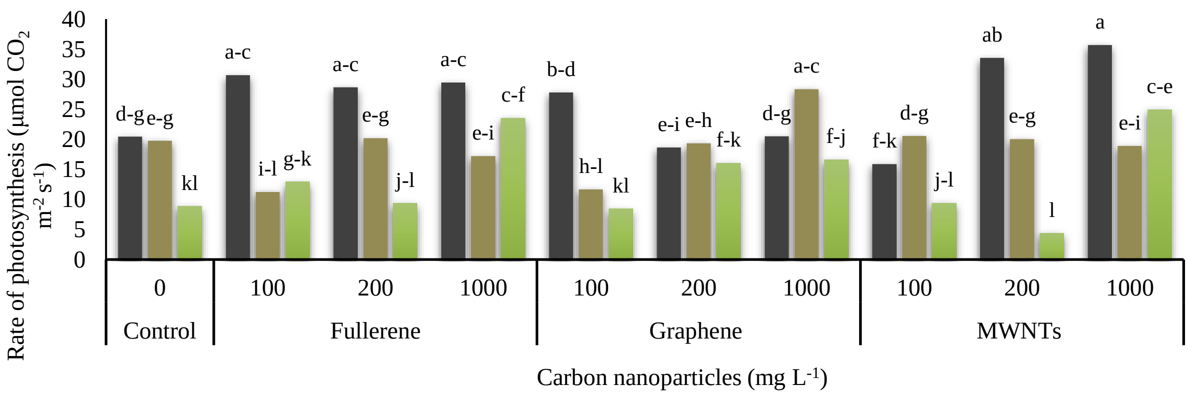

Fig. 8. Changes in the photosynthesis rate in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of P < 0.05, as determined by Duncan's test.

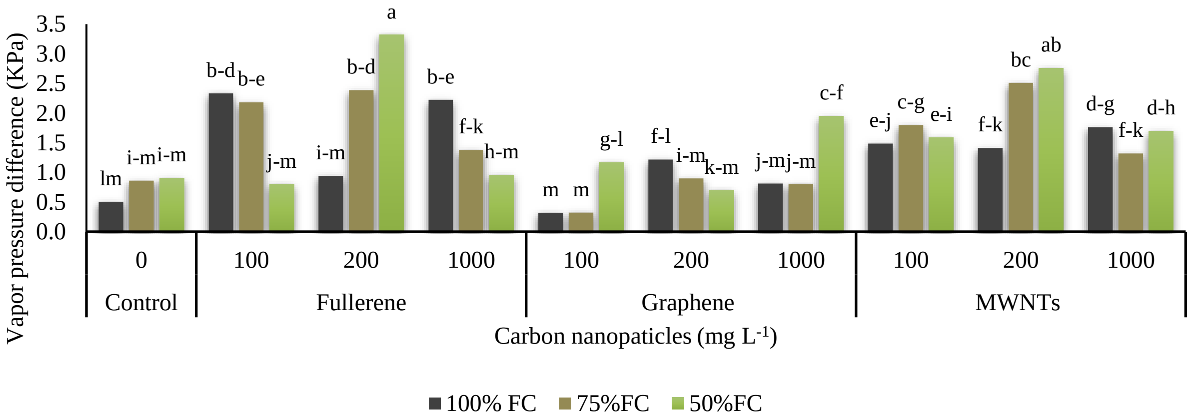

Fig. 9. Changes in the water vapor pressure difference in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

The comparison of mean interaction effects of irrigation levels and foliar spraying of carbon nanoparticles on photosynthetic water use efficiency (Fig. 10) showed that the highest amount of this attribute occurred by the application of 1000 mg L^{-1} of nanofullerene and an irrigation level of 50% FC (19.64 mol CO_2 mol^{-1} H_2O). This represented an increase of 67.4% compared to the control. The lowest amount was observed in the application of 100 mg L^{-1} nanographene, with an irrigation level of 75% FC (3.33 mol CO_2 mol^{-1} H_2O), which was not significantly different from the control.

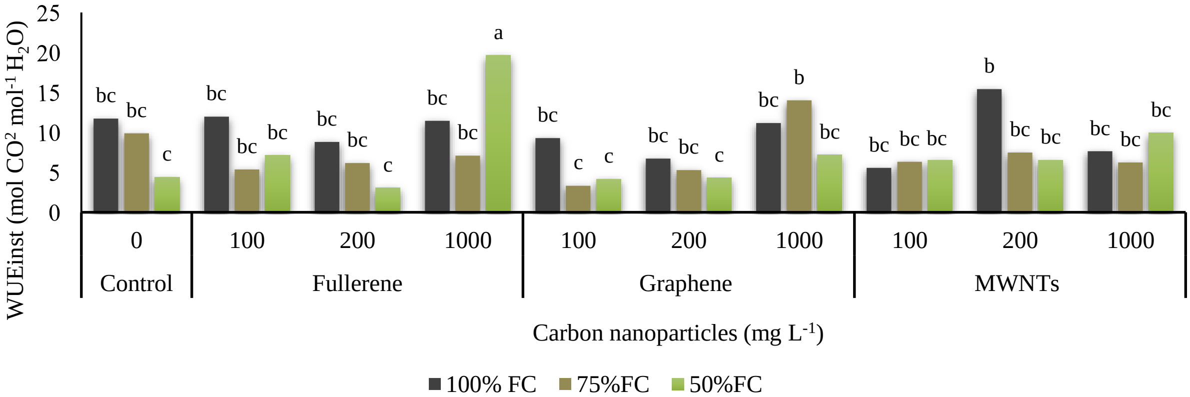

Fig. 10. Changes in photosynthetic water use efficiency in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

The application of 1000 mg L^{-1} of multi-walled carbon nanotubes at an irrigation level of 100% FC resulted in the highest carboxylation efficiency (0.082 mol CO_2 m^{-2} s^{-1}), which was not significantly different from the control. Additionally, the application of 200 mg L^{-1} of nanographene at an irrigation level of 50% FC had the lowest value for this attribute (0.015 mol CO_2 $m^{-2}s^{-1}$), which was also not significantly different from the control (Fig. 11).

When comparing the mean interaction effect of irrigation levels and the application of carbon nanoparticles on stomatal resistance (Fig. 12), the results showed that the highest value of this trait was associated with 200 mg L^{-1} multi-walled nanotubes and an irrigation level of 50% FC (0.0072 s m^{-2}). This represented a 140% increase compared to the control. This value was not significantly different from the application of a 200 mg L^{-1} nanofullerene concentration at the

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same irrigation level. The lowest value (0.0023 s m^{-2}), which was not significantly different from the control, was linked to foliar spraying of 1000 mg L^{-1} of multi-walled nanotubes with an irrigation level of 100% FC.

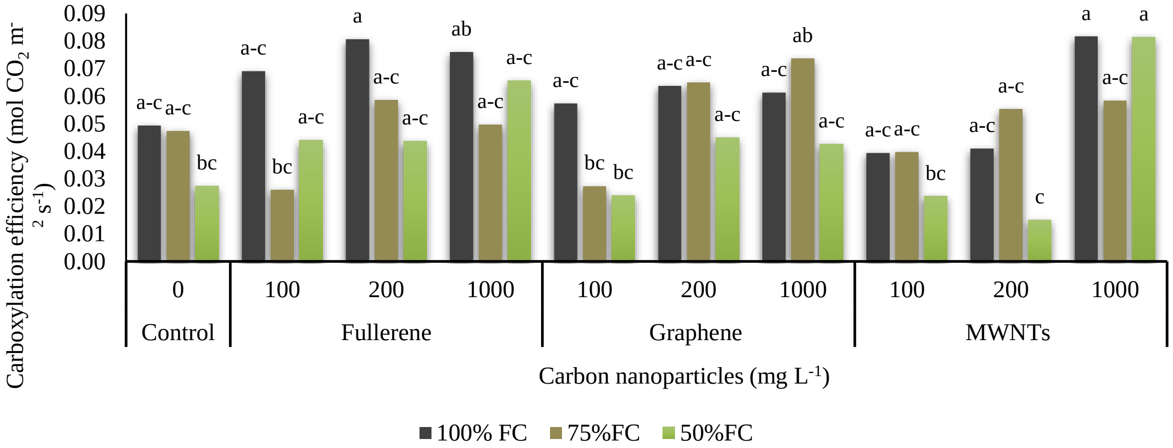

Fig. 11. Changes in carboxylation efficiency in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

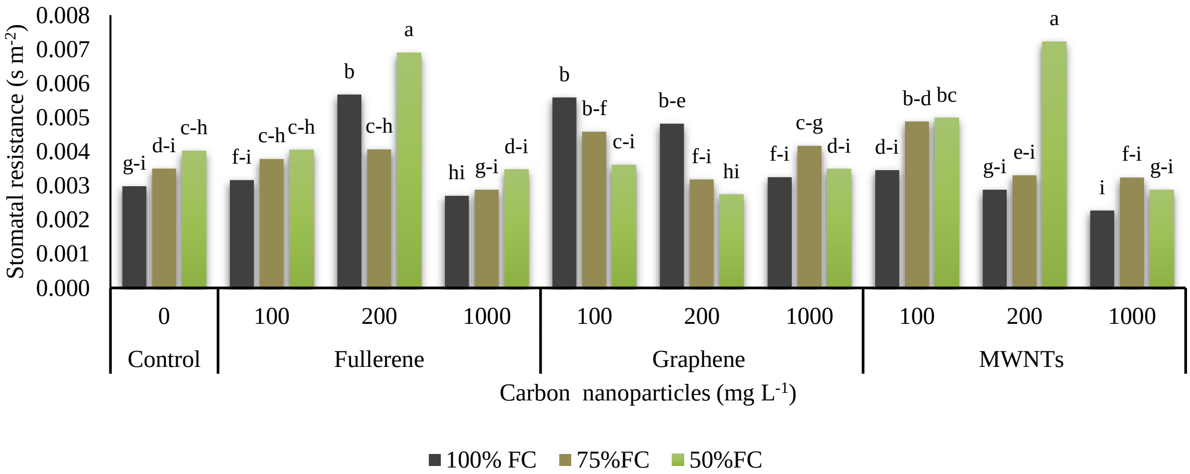

Fig. 12. Changes in stomatal resistance in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

Catalase and peroxidase enzyme assays

The results of the comparison of the mean interaction effect of irrigation levels and foliar spraying of carbon nanoparticles on the activity of catalase enzyme (Fig. 13) showed that the highest amount of this attribute was observed in the application of 1000 mg L^{-1} multi-walled nanotubes at 50% FC (1.49 µmol min^{-1} g^{-1} FW), representing an increase of 79.5% compared to the control. The lowest amount was found in the application of 200 mg L^{-1} nanographene at 75% FC (0.62 µmol min^{-1} g^{-1} FW), which was not significantly different from the control. Furthermore, the application of 100 mg L^{-1} multi-walled nanotubes at 100% FC resulted in the highest peroxidase enzyme activity (1.18 µmol min^{-1} g^{-1} FW), showing an increase of 151% compared to the control. Conversely, the application of 1000 mg L^{-1} nanofullerene at 100% FC caused the lowest value (0.07 µmol min^{-1} g^{-1} FW), which was not significantly different from the control for this trait (Fig. 14).

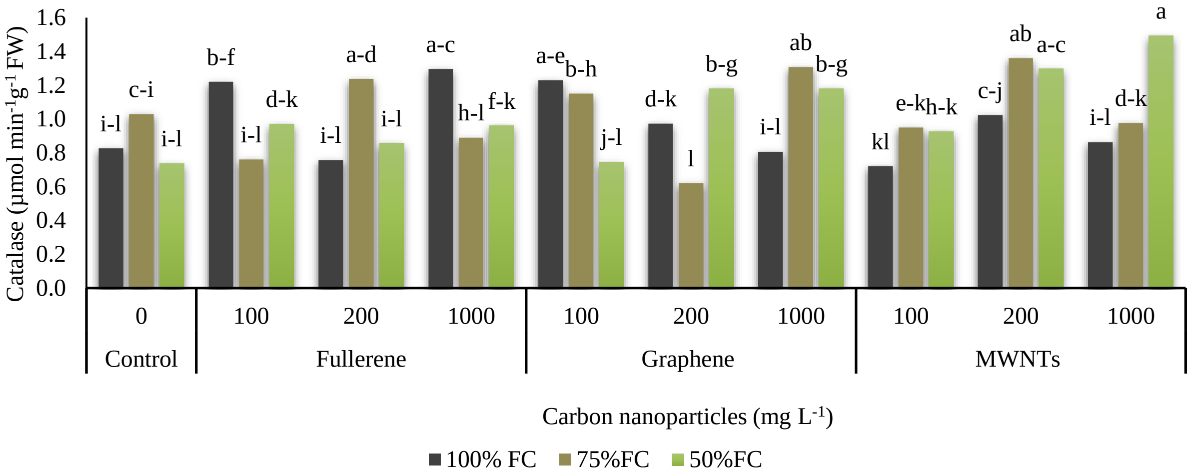

Fig. 13. Changes in the activity of the catalase enzyme in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

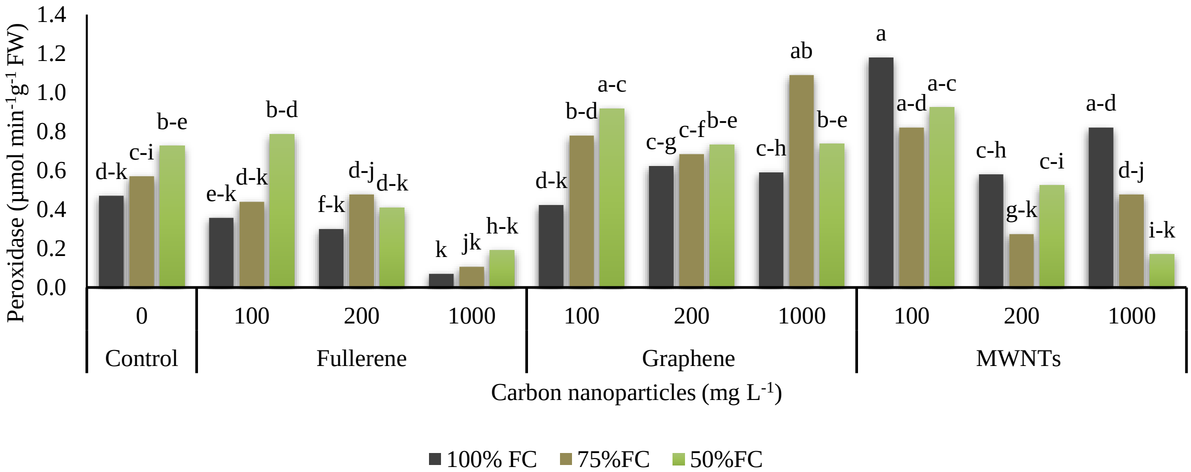

Fig. 14. Changes in the activity of the peroxidase enzyme in bell pepper plants exposed to diferent types of nanomaterials (fullerene C60, multi-walled carbon nanotubes, and graphene nanoplatelets) at varying concentrations (0, 100, 200 and 1000 mg L^{-1}) under different intensities of drought stress [(50%, 75%, and 100% (control) of feld capacity (FC) moisture)]. Bars with different letters indicate significant differences among the treatments used at a probability level of $P < 0.05$, as determined by Duncan's test.

Discussion

In the present study, the application of carbon nanoparticles helped moderate the effects of water-deficit stress on root dry weight. The response induced by carbon nanoparticles resembled the shade avoidance response (SAR) observed in *Arabidopsis*, which is potentially regulated by key pathways such as jasmonic acid, gibberellic acid, and auxin (Panigrahy et al., 2021). In previous research on mung beans, moderate concentrations of carbon nanoparticles (100 to 150 µM) enhanced total chlorophyll content (1.9), protein content (1.14), and plant biomass (fresh weight: 1.2; dry weight: 1.14), contributing to overall plant growth (Shekhawat et al., 2021).

Although the use of carbon nanoparticles in the current study did not mitigate the reduction in fruit number under stress conditions, foliar application of 200 mg L^{-1} nano-graphene and 1000 mg L^{-1} multi-walled nanotubes under full irrigation (100% FC) significantly increased fruit numbers compared to non-treated plants at the same irrigation level. The carbon nanoparticle-induced effects mirrored SAR traits, such as increased stem and root length, higher root numbers, expanded cotyledon area, elevated chlorophyll content, and enhanced total sugar levels (Panigrahy et al., 2021). Additionally, exposure to carbon nanotubes resulted in a 200% increase in tomato yield (Jha and Yadav, 2023).

This study also found that nanographene and nanofluorene were more effective than multi-walled nanotubes in enhancing fresh fruit weight, highlighting that the shape and size of carbon nanoparticles, despite their shared elemental composition, influence their efficacy. The impact of carbon nanoparticles varies depending on factors such as exposure conditions, nanoparticle type, dispersion state, and concentration (Jackson et al., 2013).

While the carbon nanoparticle concentrations used in this study could not fully mitigate stress-induced reductions in membrane stability and total chlorophyll content, the application of 1000 mg L^{-1} nanofullerene did increase chlorophyll levels. Furthermore, foliar treatments with various concentrations of graphene and 1000 mg L^{-1} multi-walled nanotubes significantly improved the chlorophyll a/b ratio, indicating the occurrence of oxidative stress.

Stress can reduce chlorophyll concentration in plants by inhibiting its production or accelerating its degradation through the enzyme chlorophyllase, as well as by promoting the photooxidation of chlorophyll via reactive oxygen species (ROS). This decline in chlorophyll b is more pronounced than in chlorophyll a, resulting in an increased ratio of chlorophyll a to b under stress conditions. This occurs because chlorophyll b, which is three times more abundant than chlorophyll a in the light-harvesting complexes of photosystem II, is more susceptible to stress-induced damage, leading to the destruction of a larger proportion of chlorophyll b (Oncel et al., 2000).

In studies comparing the effects of different concentrations of nanofullerene on the chlorophyll content of feverfew plants, the highest increase (23.6% above the control) was observed with foliar spraying at a concentration of 1000 mg L^{-1} (Ahmadi et al., 2020). Similarly, a concentration of 1000 mg L^{-1} of multi-walled nanotubes significantly increased the rate of photosynthesis under both stressed and non-stressed conditions. The application of carbon nanoparticles in corn also had a notable impact, enhancing plant height by 21.4%, shoot dry biomass by 27.1%, photosynthetic parameters, and improving soil chemical and biochemical properties (Xin et al., 2022).

In the present study, stress conditions increased the vapor pressure difference (VPD), and the application of carbon nanoparticles further intensified this effect. Specifically, foliar application of 200 mg L^{-1} fullerene under 50% FC irrigation significantly increased the water vapor pressure difference and stomatal resistance. An increased VPD indicates reduced plant transpiration. Stomata need to remain open at certain times to allow carbon dioxide uptake for photosynthesis, but under stress, they must close to minimize water loss and prevent wilting. Plants quickly adjust by closing their stomata in response to low relative humidity (high VPD) at a given temperature, while keeping them open when humidity is high (low VPD) (Alineaifard, 2014). Additionally, the application of 1000 mg L-1 nanofullerene significantly improved the efficiency of photosynthetic water use under 50% FC irrigation, highlighting its potential to enhance drought tolerance.

Furthermore, foliar spraying with 1000 mg L-1 of multi-walled nanotubes under the same irrigation conditions enhanced carboxylation efficiency. During moisture stress, plant survival becomes more crucial than optimal performance, causing the ratio of carbon dioxide absorption to transpiration to fluctuate. The difference in photosynthetic water consumption efficiency across various humidity regimes arises because drought stress affects photosynthesis and transpiration at different rates, leading to a significant variation in efficiency between moisture conditions (El Hafid et al., 1998). Plants can achieve high photosynthetic water-use efficiency either by increasing carbon assimilation or by reducing transpiration (Marco et al., 2000). Under mild stress conditions, this efficiency increases primarily due to a greater reduction in stomatal conductance compared to mesophyll conductance (Iqbal et al., 2021). The primary limitation to photosynthesis during stress is often a reduction in carboxylation efficiency (Barutcular et al., 2000).

The present study also found that the application of multi-walled nanotubes increased the activity of catalase and peroxidase enzymes, with nanoparticles displaying varied behavior at different irrigation levels. Antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, polyphenol oxidase, and ascorbate peroxidase play key roles in neutralizing reactive oxygen species in cells (Agarwal and Pandey, 2004). In a study examining the effects of ascorbic acid on drought tolerance in pepper (*Capsicum annuum* L.), it was reported that drought stress inhibited growth parameters such as fruit number, plant height, weight, yield, and total chlorophyll, while increasing catalase (CAT) and peroxidase (POD) activities in the leaves (Khazaei et al., 2020).

Carbon nanoparticles have been shown to enhance photosynthesis, crop growth, water uptake, antioxidant levels, and nutrient use efficiency (N, P, K) in plants (Mukherjee et al., 2016; Ghasempour et al., 2019; Zhao et al., 2021). Specifically, functionalized carbon nanoparticles improved relative water content, chlorophyll stability index, chlorophyll fluorescence (Fv/Fm), proline content, and antioxidant activity in chili peppers under drought stress, while reducing abscisic acid levels in the leaves (Alluqmani and Alabdallah, 2023). These findings align closely with the results of the present study.

Conclusions

In the present study, foliar application of carbon nanoparticles helped moderate the effects of water deficit stress on root dry weight. The application of 200 mg L^{-1} nanographene and 1000 mg L^{-1} multi-walled nanotubes under full irrigation (100% FC) increased the number of fruits compared to plants that did not receive nanoparticle treatments at the same irrigation level. Nano-graphene and nano-fullerene were more effective than multi-walled nanotubes in enhancing fruit fresh weight. Additionally, the application of 1000 mg L^{-1} nanofullerene increased chlorophyll content. Foliar application of various concentrations of graphene and 1000 mg L^{-1} multi-walled nanotubes significantly elevated the ratio of chlorophyll a to b. Furthermore, 1000 mg L^{-1} multi-walled nanotubes had a notable impact on increasing the rate of photosynthesis under both stress and non-stress conditions. Stress increased the vapor pressure difference (VPD), and the use of carbon nanoparticles further intensified this effect. For example, the foliar application of 200 mg L^{-1} fullerene under 50% FC irrigation significantly increased VPD and stomatal resistance. Moreover, 1000 mg L^{-1} nanofullerene significantly improved the efficiency of photosynthesis and water consumption under 50% FC irrigation. In addition, the foliar spraying of 1000 mg L^{-1} multi-walled nanotubes under the same irrigation conditions enhanced carboxylation efficiency. The application of multi-walled nanotubes also increased the activity of catalase and peroxidase enzymes, with the nanoparticles exhibiting varied effects across different irrigation levels in relation to these enzyme activities.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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