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SUNFLOWER (*HELIANTHUS ANNUUS* L.) RESPONSE TO DROUGHT STRESS CONDITIONS AND NANOPARTICLES THROUGH PHYSIOLOGICAL TRAITS

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SUMMARY

A field experiment took place during the 2024–2025 growing season on sunflower (*Helianthus annuus* L.) at the College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq. The said study aimed to evaluate the effects of water stress and foliar application of nano-titanium and nano-curcumin on the concentrations of enzymatic antioxidants (superoxide dismutase [SOD], peroxidase [POD], and catalase [CAT]) and non-enzymatic antioxidants—malondialdehyde (MDA). Subjection of sunflower plants to water stress was for five, 10, and 15 days, and for foliar application of nano-titanium concentrations at 50 and 100 mg L^{-1}, and after 72 hours with nano-curcumin concentrations at 25 and 75 mg L^{-1} along with control treatment. The results showed significant differences among studied factors on the physiological traits of the sunflower. Regarding drought stress, substantial differences showed the best results appeared with the longest irrigation interval due to the highest sunflower tolerance to environmental stress conditions. The water stress for a period of 15 days combined with nano-titanium (100 mg L^{-1}) and nano-curcumin (25 mg L^{-1}) provided the highest activity levels of SOD, POD, CAT, and MDA.

Keywords: Sunflower (*H. annuus* L.), nano-titanium and nano-curcumin, water stress conditions, peroxidase, catalase, curcumin, superoxide dismutase

Key findings: Sunflower (*H. annuus* L.), with the application of nano-titanium and nano-curcumin, demonstrated physiological response through increased activities of SOD, POD, and CAT, as well as MDA under water stress conditions.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most commonly grown crops worldwide due to its nutritional and economic value. It is a seasonal plant belonging to the family Asteraceae (Compositae) (Al-Kateb, 1988). The sunflower can greatly adapt and tolerate water deficit conditions and increased temperatures by improving the root system and reproductive growth to escape drought stress conditions. The growing of sunflowers is mainly for oil production because of their supreme oil percentage beginning at 40% (Petraru *et al.,* 2021), commonly used in human food (Hashim and Kakarash, 2024). In Iraq, sunflower is one of the primary oilseed crops, with some cultivars already developed. The sunflower oil, comprising fats, vitamin E, and mineral elements (especially copper and zinc), strengthens the human immune system (Omonov *et al.,* 2023).

The sunflower is also popular for its tolerance to multiple environmental stress factors (salts, drought stress, and phytotoxicity) because of its highest activity of antioxidant enzymes (SOD and CAT). The biotic and abiotic stress factors (drought, salinity, pathogens, phytotoxicity, and extreme temperatures) can have three classifications—mild, moderate, and severe (Yassin, 2001). Stomatal closure is one of the primary defense mechanisms when plants undergo drought stress conditions, which prevents loss of internal water but minimizes the gas exchange (Jaleel *et al.,* 2007). These plant responses result in decreased leaf water content and water potential and a reduced growth rate. With prolonged stress conditions, the photosynthesis and metabolic activities are inhibited, eventually resulting in plant death (Jaleel *et al.,* 2008). Drought resistance is a vital mechanism in plants that escape the plants for faster growth during the available soil moisture (Farooq *et al.,* 2014).

Hormonal regulation, especially managed by auxins, gibberellins, abscisic acid (ABA), and ethylene, is the key mechanism for supporting plant tolerance under drought stress conditions (Ullah *et al.,* 2018). In plant cells, the enhancement in ethylene and ABA concentrations can also facilitate the plants' adaptation to various stress conditions (Weyers and Paterson, 2001). Reactive oxygen species (ROS) reached quick production as an early response to drought and salt stress conditions. The ROS function as signaling molecules to activate enzymatic antioxidants (CAT, SOD, and POD), as well as non-enzymatic antioxidants (MDA, proline, tocopherols, and vitamin E) (Noctor *et al.,* 2000).

Recently, various elements and plant extracts have entailed formulation within the context of nanotechnology, as nanoparticles possess unique properties that induce substantial morphological and physiological variations in crop plants. Furthermore, the nanoparticle application enhances the nutritional and water uptake efficiencies and stimulates antioxidant enzyme activities in crop plants (Oleiwi *et al.,* 2024). Titanium is a naturally occurring element found in igneous and metamorphic rocks, is lightweight (non-toxic), and is considerably a vital element for agriculture (Skocaj *et al.,* 2011). Nano-titanium enhances photosynthesis and activates antioxidant enzyme activities, including CAT and POD. The most effective form of titanium in agriculture is TiO_2 (titanium dioxide nanoparticles), typically applied through foliar spray (Al-Khafaji *et al.,* 2024).

Furthermore, nano-curcumin has emerged as a strong antioxidant in the medical and agricultural fields, as it alters the process of producing free radicals (Bouta *et al.,* 2024). It serves as an aid to plant nutrients' use and reduces water stress with its phenolic compounds, which play a vital role in the plant's drought resistance (Al-Abboud *et al.,* 2024). The presented study aimed to evaluate the role of nano-titanium and nano-curcumin for alleviating water stress drought, spacing out irrigation intervals, and enhancing activity levels of enzymatic and non-enzymatic antioxidants in sunflowers (*H. annuus* L.).

MATERIALS AND METHODS

A field experiment on sunflower (*H. annuus* L.) commenced during the growing season of 2024–2025 at the College of Education for Pure

Sciences, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq. The seeds came from the local market during the agriculture period in September 2024. The following study aimed to analyze the physiological responses of sunflower plants under water stress conditions and also by receiving the treatments with two different nanoparticles (nano-titanium and nano-curcumin) in various concentrations. The experiment layout in a randomized complete block design had a factorial arrangement and three replications.

Treatments

Water stress durations were five, 10, and 15 days after each irrigation, in addition to the control treatment (having regular irrigation every five days). The two different nanoparticles used have different concentrations: nano-titanium (0, 50, and 100 mg L^{-1}) and nano-curcumin (0, 25, and 75 mg L^{-1}). The well-prepared experimental units totaled 81, which underwent leveling and planting. Each experimental unit was 1 m^2 × 1 m^2 arranged in four planting rows spaced 30 cm apart. For soil analysis, the soil samples taken before planting came from a depth of 5–30 cm and received evaluation in the laboratory at the University of Baghdad, Baghdad, Iraq. Fertilizer NPK application followed the recommended dose (100 kg ha^{-1}). Seeds of a local sunflower cultivar incurred sowing on September 16, 2024. The experimental field bore manual weeding, with random samples taken on November 20, 2024, to study the physiological parameters.

Preparation of solutions

Adding one gram of nano-titanium powder to distilled water brought the volume to one liter. The use of the dilution equation ($C_1V_1 = C_2V_2$) served to prepare up to 50 and 100 mg L^{-1} (for titanium) and 25 and 75 mg L^{-1} (for curcumin), as well as for the control using only distilled water. In the early morning, the sunflower leaves' spraying was at the 4–6 leaf stage with nano-titanium solutions. The control always received watering with distilled water. After 72 hours, the same plants underwent spraying with the nano-curcumin solutions, while the control again acquired watering with distilled water. Then, after 10 days, the randomly sampled leaves of plants succeeded in their collection for enzyme assays of enzymatic antioxidants (POD, SOD, and CAT) and non-enzymatic antioxidants (MDA) for the indicator of lipid peroxidation.

Determination of enzymatic and non-enzymatic antioxidants

Peroxidase (POD) activity assessment used the methodology of Nezih (1985). Superoxide dismutase (SOD) activity, as determined, employed the method of Beyer and Fridovich (1987). Catalase (CAT) activity testing was according to the general biochemical assay method for enzyme activity. The concentration of malondialdehyde (MDA) estimates (µg g^{-1}) followed the procedure of Carmak and Horst (1991).

Statistical analysis

All the recorded data entailed statistical analysis using the GenStat software package at a probability level of 0.05. The least significant difference ($LSD_{0.05}$) test used sought to compare and separate the treatment means for various studied traits (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

The results revealed a major influence of irrigation intervals on activities of the antioxidant enzyme peroxidase (POD) (Table 1). The 15-day irrigation interval recorded the highest mean value of POD activity (123.748 µg g^{-1}), whereas the 5-day irrigation interval recorded the lowest POD mean value (105.908 µg g^{-1}). Al-Da'mi (2018) and Gupta (2010) mentioned drought stress conditions could trigger an escalation in enzymatic antioxidant system activity, including POD, as a countermeasure against oxidative stress caused by reactive oxygen species (ROS). Similarly, POD could play an important role in maintaining the integrity of the plant cell walls under various stress conditions.

Table 1. Effect of drought stress, nano-titanium, and nano-curcumin spraying and their interaction on the total activity of peroxidase enzyme (µg g^{-1} fresh weight).

Drought stress	Nano-Curcumin - C (mg L^{-1}(	Nano-titanium spray concentrations - T (mg L^{-1})			C ×D
		0	25	75	
5	0	33.40	45.16	71.36	49.97
	25	92.25	139.48	152.23	127.99
	75	116.10	141.62	161.53	139.75
10	0	36.90	55.73	82.69	58.44
	25	91.62	142.64	150.84	128.37
	75	134.58	151.04	174.03	153.22
15	0	43.95	57.26	90.78	64.00
	25	103.68	160.66	191.54	151.96
	75	141.20	153.54	171.06	155.27
Means of titanium concentrations - T		88.19	116.35	138.45	
$LSD_{0.05}$	T effect = 0.0518				0.14
	D × C × T effect = 0.18				
D × T					
Drought stress		T concentrations (mg L^{-1})			Means D
		0	50	100	
5		80.58	108.75	128.37	105.90
10		87.40	116.47	135.86	113.34
15		96.28	123.82	151.13	123.74
$LSD_{0.05}$		0.093			0.075
C × T					
Curcumin (mg L^{-1})		T concentrations (mg L^{-1})			Means C
		0	50	100	
0		38.08	52.72	81.61	57.47
50		95.85	147.59	164.78	136.11
100		130.63	148.73	168.87	149.41
$LSD_{0.05}$		0.11			0.09

The results further disclosed a considerable increase in the POD enzyme activity regarding increasing concentration of nano-titanium (Table 1). With a raised nano-titanium concentration from 0 to 100 mg L^{-1}, the POD mean activity increased from 88.193 to 138.457 µg g^{-1}. These findings may be ascribable to the vital role of the foliar application of nano-titanium, as it can also increase the biomass, antioxidant ability, and photosynthetic ability to improve growth and reduce the negative effects of drought stress conditions (Bacilieri *et al.*, 2017; Soran *et al.*, 2021).

Moreover, a notable increase in mean POD activity with an increased concentration of nano-curcumin existed (Table 1). By increasing nano-curcumin concentration from 0 to 75 mg L^{-1}, the POD activity nearly tripled, from 57.476 to 149.416 µg g^{-1}. These results were greatly analogous to past findings that curcumin diminishes the effects of oxidative stress and induces antioxidant activity due to the phenolic compounds (Al-Abboud *et al.,* 2024).

Regarding superoxide dismutase (SOD), the results illustrated a significant impact of the irrigation intervals (Table 2). The mean SOD activity was largest when irrigation occurred every 15 days (210.10 µg g^{-1}) compared with the lowest mean obtained with an irrigation interval of five days (145.93 µg g^{-1}). Rabiei *et al.* (2015) shared this was likely because the water stress activates SOD activity, which, in turn, detoxifies superoxide radicals (O_2-) to H_2O_2 to avoid lipid peroxidation within chloroplast membranes as a defense mechanism. Simultaneously, singlet oxygen, additionally produced in mitochondria and peroxisomes, enhances the free radical reactions, further stimulating the antioxidant defense system (Munné-Bosch, 2005).

The findings indicated a significant increase in mean SOD activity with increased nano-titanium concentration, as mean SOD activity rose from 129.02 µg g^{-1} at 0 mg L^{-1} to

Table 2. Effect of drought stress, nano-titanium, and nano-curcumin spraying and their interaction on the total activity of SOD enzyme (μg g^{-1} fresh weight).

Drought Stress	Nano-Curcumin - C (mg L^{-1})	Nano-titanium spray concentrations - T (mg L^{-1})			C ×D
		0	25	75	
5	0	74.20	137.65	163.59	125.11
	25	84.93	145.79	167.82	132.84
	75	101.54	218.49	219.44	179.82
10	0	99.65	153.13	208.50	153.76
	25	121.77	184.21	205.45	170.47
	75	179.82	273.39	221.70	224.47
15	0	140.26	193.75	239.86	191.29
	25	149.91	215.67	383.55	216.38
	75	209.11	242.72	216.08	222.64
Means of titanium concentrations -T		129.02	196.08	214.00	
LSD$_{0.05}$	T effect = 0.73				1.71
	T × C × D effect = 2.33				
D × T					
Drought stress		T concentrations (mg L^{-1})			Means D
		0	50	100	
5		86.89	167.28	183.61	145.93
10		133.74	203.58	211.89	183.07
15		166.43	217.38	246.50	210.10
LSD$_{0.05}$		1.77			1.72
C × T					
Curcumin (mg L^{-1})		T concentrations (mg L^{-1})			Means C
		0	50	100	
0		104.70	161.48	203.98	156.72
50		118.87	181.89	218.94	173.23
100		163.49	244.86	209.08	209.14
LSD$_{0.05}$		1.16			0.60

214.00 μg g^{-1} at the nano-titanium concentration of 100 mg L^{-1} (Table 2). The reason for the SOD activity increase can be due to the improvement in photosynthetic efficiency by the nano-titanium, which diminishes the production of ROS by enzymatic antioxidant stimulation, protecting the chloroplasts (Khattak *et al.,* 2021). Furthermore, nano-titanium could stimulate the root activity, which further allows for greater mineral nutrient uptake, and an overall improvement ultimately enhanced the antioxidant enzyme activity (Soran *et al.,* 2021).

Likewise, the results revealed a marked elevation in mean SOD activity as nano-curcumin concentration increased (Table 2). The mean SOD activity surged from 156.72 to 209.14 μg g^{-1}, as the nano-curcumin concentration rose from 0 to 75 mg L^{-1}. Curcumin, being a robust antioxidant, and its increasing concentration subsequently boost effectiveness within the plants through various phenolic compounds, known to confer protection to the plants against drought stress conditions (Al-Abboud *et al.,* 2024).

According to CAT enzyme response, it was evident that irrigation intervals significantly affected the said enzyme activity level (Table 3). Irrigation every 15 days produced the highest mean CAT activity (0.4208 μg g^{-1}) compared with the 5-day irrigation regime, which had the lowest mean (0.3172 μg g^{-1}). The water stress conditions stimulate the production of free radicals, resulting in a rapid initiation of different defense mechanisms, such as closing of the stomata and increasing the level of abscisic acid to manage the plant's internal water loss. These also increased levels of enzymatic antioxidants, including CAT, to control the oxygen concentration within the cells (Asada, 1992; Al-Abboud *et al.,* 2024). Another possible reason could be the increased efficiency of the mechanisms mentioned above as photorespiration occurs, as well as the

Table 3. Effect of drought stress, nano-titanium, and nano-curcumin spraying and their interaction on the total activity of catalase (CAT) enzyme (µg g^{-1} fresh weight).

Drought stress	Nano-Curcumin - C (mg L^{-1})	Nano-titanium spray concentrations - T (mg L^{-1})			C × D
		0	25	75	
5	0	0.30	0.32	0.31	0.30
	25	0.32	0.32	0.32	0.32
	75	0.31	0.32	0.32	0.31
10	0	0.34	0.35	0.38	0.31
	25	0.39	0.41	0.43	0.36
	75	0.44	0.44	0.41	0.41
15	0	0.37	0.36	0.38	0.37
	25	0.41	0.44	0.46	0.44
	75	0.43	0.45	0.44	0.44
Means of titanium concentrations - T		0.37	0.38	0.38	
LSD$_{0.05}$	T effect = 0.0024				0.0046
	D × C × T effect = 0.0073				
D × T					
Drought stress		T concentrations (mg L^{-1})			Means D
		0	50	100	
5		0.31	0.31	0.31	0.31
10		0.39	0.40	0.40	0.40
15		0.41	0.41	0.43	0.42
LSD$_{0.05}$		0.0044			0.0035
C × T					
Curcumin (mg L^{-1})		T concentrations (mg L^{-1})			Means C
		0	50	100	
0		0.34	0.34	0.55	0.34
50		0.38	0.39	0.41	0.39
100		0.39	0.40	0.39	0.39
LSD$_{0.05}$		0.0042			0.0027

production of free radicals in the cells, which would, in turn, stimulate the level of antioxidant activity (Shabala, 2012).

With increasing concentrations of nano-titanium, the mean activity of CAT also enhanced (Table 3). The results enunciated that boosting the concentration of nano-titanium from 0 to 100 mg L^{-1} showed an elevated average of CAT activity from 0.3733 to 0.3877 µg g^{-1}. The observed effects revealed that either nano-titanium decreased the activity and inhibited the action of ROS (Munné-Bosch, 2005). Perhaps the nano-titanium alleviated the adverse effects of water stress by increasing enzymatic antioxidants, including CAT, with enhanced water and nutrient uptake and greater plant growth (Bacilieri *et al.,* 2017).

The increased mean CAT activity was evident with a higher concentration of nano-curcumin; as the integrity of the solution rose from 0 to 75 mg L^{-1}, the mean CAT activity enhanced from 0.3479 to 0.3996 µg g^{-1} (Table 3). These effects might be due to phenolic compounds in curcumin that stimulate the defense mechanism of the plant cells with higher concentration (Al-Abboud *et al.,* 2024).

For the malondialdehyde (MDA), the results displayed a significant impact of irrigation intervals (Table 4). The highest mean MDA resulted in the longest irrigation interval (15 days), whereas the lowest mean MDA (9.686 µg g^{-1}) occurred with the shortest interval (five days). The increases in MDA values may have originated from the extended water deficit conditions. In avoiding water deficit stress, the plant defense mechanism begins to produce more antioxidants to accelerate the vegetative growth and reproduction (Siddique *et al.,* 1993). The action of enzymatic and non-enzymatic antioxidants is synchronous; they work

Table 4. Effect of drought stress, nano-titanium, and nano-curcumin spraying and their interaction on total malondialdehyde (MDA) activity (μg^{-1}).

Drought stress	Nano-Curcumin - C (mg L^{-1})	Nano-titanium spray concentrations - T (mg L^{-1})			C ×D
		0	25	75	
5	0	8.76	10.64	10.36	10.01
	25	9.44	8.67	11.4	9.75
	75	8.95	8.75	10.34	9.38
10	0	9.65	8.21	10.67	9.51
	25	9.76	10.66	9.61	10.01
	75	9.13	10.60	9.71	9.81
15	0	9.48	9.38	9.90	9.59
	25	10.60	10.19	11.34	10.71
	75	9.52	10.57	9.76	9.95
Means of titanium concentrations - T		9.48	9.74	10.35	
LSD$_{0.05}$	T effect = 0.0319				0.03
	C × D × T effect = 0.08				

D × T

Drought stress	T concentrations (mg L^{-1})			Means D
	0	50	100	
5	9.05	9.35	10.65	9.68
10	9.51	9.82	10.00	9.78
15	9.87	10.05	10.33	10.08
LSD$_{0.05}$	0.048			0.024

C × T

Curcumin (mg L^{-1})	T concentrations (mg L^{-1})			Means C
	0	50	100	
0	9.93	9.84	10.70	10.15
50	9.30	9.41	10.31	9.67
100	9.20	9.97	9.97	9.71
LSD$_{0.05}$	0.048			0.020

together to eliminate the harmful effects of ROS and maintain cell balance. This mechanism reached activation in stress conditions, and the production of these compounds is continuous but within a balanced mechanism, as it depends on the stress conditions, the type of plant, and its ability to adapt (Fathi *et al.,* 2025).

The study results showed a marked increase in average MDA with elevated nano-titanium concentrations (Table 4). The MDA enhanced from 9.481 to 10.359 μg g^{-1} as nano-titanium concentration increased from 0 to 100 mg L^{-1}, likely due to improved mineral uptake and photosynthetic efficiency under environmental stress conditions (Vatankhah *et al.,* 2023; Omar *et al.,* 2023). On the contrary, the mean MDA significantly decreased from 10.151 to 9.676 μg g^{-1}, as nano-curcumin concentration increased from 0 to 25 mg L^{-1}. This could refer to the protection and low concentration of nano-curcumin had in reducing lipid peroxidation, as phenolic compounds found in curcumin protect the cell membranes (Rai *et al.,* 2015). Water stress conditions have different effects on the defense markers—SOD, POD, and CAT. Their influence on MDA rose through lipid breakdown. However, nano-curcumin has the effect of reducing lipid oxidation through phenolic compounds that play a significant role in drawing water across cell membranes, thus reducing the effects of hydration and inhibiting lipid oxidation. Regarding interactions, an interaction was notable, particularly between irrigation intervals and nano-titanium concentration. The largest interaction value (10.650 μg g^{-1}) appeared with the interaction of nano-titanium (100 mg L^{-1}) and the 5-day irrigation interval, while the lowest interaction value (9.052 μg g^{-1}) emerged with the interaction of nano-titanium (0 mg L^{-1}) and the 5-day irrigation interval.

CONCLUSIONS

The drought stress conditions and different concentrations of nano-titanium and nano-curcumin significantly affected the enzymatic (POD, SOD, and CAT) and non-enzymatic (MDA) antioxidants in sunflower (*H. annuus* L.). Regarding drought stress, remarkable differences existed, with the best results found with the longest irrigation interval due to the highest sunflower tolerance to environmental stress conditions. The results expressed that nano-titanium (100 mg L^{-1}) and nano-curcumin (25 mg L^{-1}) played an essential role in limiting the effects of water stress and improving antioxidant activity levels in sunflower plants.

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