Enzymes of Calendula officinalis L. as Affected by Foliar Application of Nano-nitrogen and Potassium Fertilizers, Under Water Stress Conditions

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Water stress is a major environmental factor that limits the growth and productivity of *Calendula officinalis* L. To alleviate its negative effects, recent approaches have increasingly focused on nano-fertilizers that enhance plant antioxidant defenses. This study therefore aimed to evaluate the effect of foliar application of nano-nitrogen (0, 2, and 4 mL L^{-1}) and nano-potassium (0, 2, and 4 g L^{-1}) fertilizers under two irrigation regimes (100% and 50% of field capacity) on the activity of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). The results revealed that irrigation at 50% field capacity significantly increased CAT, SOD, and POD activities compared with full irrigation. Enzyme activities decreased with increasing concentrations of nano-nitrogen and potassium fertilizers. The highest activities of all tested enzymes were observed under water stress without foliar application of nano-fertilizers (0 mL L^{-1} nano-N + 0 g L^{-1} nano-K + 50% irrigation), where CAT, SOD, and POD reached 0.42, 3.00, and 13.61 units mg^{-1} fresh weight, respectively. In contrast, the lowest enzyme activities were observed under full irrigation combined with the highest fertilizer levels (4 mL L^{-1} nano-N + 4 g L^{-1} nano-K + 100% irrigation), with CAT, SOD, and POD values of 0.31, 2.15, and 6.92 units mg^{-1} fresh weight, respectively. In conclusion, water stress enhanced the antioxidant defenses of *Calendula officinalis* L., while higher levels of nano-fertilizer slowed down enzyme activity.

Keywords

Nano-fertilizers, Water stress, Calendula officinalis, Abiotic stress, Nanomaterials, Antioxidant enzymes

RESEARCH PAPER

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Abstract

Water stress is a major environmental factor that limits the growth and productivity of *Calendula officinalis* L. To alleviate its negative effects, recent approaches have increasingly focused on nano-fertilizers that enhance plant antioxidant defenses. This study therefore aimed to evaluate the effect of foliar application of nano-nitrogen (0, 2, and 4 mL L^{-1}) and nano-potassium (0, 2, and 4 g L^{-1}) fertilizers under two irrigation regimes (100 % and 50 % of field capacity) on the activity of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). The results revealed that irrigation at 50 % field capacity significantly increased CAT, SOD, and POD activities compared with full irrigation. Enzyme activities decreased with increasing concentrations of nano-nitrogen and potassium fertilizers. The highest activities of all tested enzymes were observed under water stress without foliar application of nano-fertilizers (0 mL L^{-1} nano-N + 0 g L^{-1} nano-K + 50 % irrigation), where CAT, SOD, and POD reached 0.42, 3.00, and 13.61 units mg^{-1} fresh weight, respectively. In contrast, the lowest enzyme activities were observed under full irrigation combined with the highest fertilizer levels (4 mL L^{-1} nano-N + 4 g L^{-1} nano-K + 100 % irrigation), with CAT, SOD, and POD values of 0.31, 2.15, and 6.92 units mg^{-1} fresh weight, respectively. In conclusion, water stress enhanced the antioxidant defenses of *Calendula officinalis* L., while higher levels of nano-fertilizer slowed down enzyme activity.

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1. Introduction

Abiotic stresses such as drought are among the most critical challenges to global crop productivity, contributing to significant yield losses and threatening food security [1]. Water scarcity is a major issue in Iraq, where drought has become a severe limitation on agricultural development [2]. Further studies are needed to investigate the impact of water stress on the growth and productivity of calendula plants, particularly because *C. officinalis* is an important medicinal and ornamental species sensitive to environmental conditions [3].

Nutrient deficiencies exacerbate the effects of drought by reducing water and nutrient uptake, impairing turgor pressure, and increasing oxidative stress through excess production of reactive oxygen species (ROS) [3]. Nitrogen (N) and potassium (K) are two essential macronutrients that play vital roles in plant metabolism. Nitrogen supports protein synthesis, enzyme activity, and chlorophyll formation, while potassium regulates osmotic balance, stomatal function, and photosynthesis [4]. Several studies have shown that N and K fertilization improves growth and yield in various crops, but additional evidence is required to demonstrate their importance in *Calendula officinalis* [2].

In recent years, the use of nano-fertilizers has gained increasing attention as an innovative

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approach to improving nutrient efficiency and mitigating abiotic stresses. Nanomaterials have been reported to enhance plant growth, photosynthesis, and stress tolerance more effectively than conventional fertilizers [5,6], Nano-fertilizers are characterized by high efficiency in penetration, absorption, transport and metabolism in plant tissues if they are applied in small quantities and with smart targeting [7]. Furthermore, nanoparticles improve ROS detoxification by enhancing antioxidant enzyme activities [8,9]. However, further research is needed to clarify their effects on calendula plants under water-limited conditions.

Among plant defense mechanisms, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) are considered key biomarkers of stress tolerance [10]. CAT decomposes hydrogen peroxide, SOD converts superoxide radicals into hydrogen peroxide, and POD further detoxifies hydrogen peroxide into water [11]. Because of their central role in ROS detoxification [1], these enzymes were selected as the focus of this study, rather than broader growth or yield parameters, to provide a clear biochemical picture of the stress response in *Calendula officinalis*.

Accordingly, this study was conducted to evaluate the effects of foliar nano-nitrogen and potassium fertilizers on the antioxidant enzyme activities of *C. officinalis* under water stress conditions.

2. Materials and methods

2.1. Planting and treatments

The experiment was conducted in plastic pots at the College of Agriculture, University of Kerbala, during the winter season of 2023–2024, using non-saline sandy loam soil (Table 1). Each pot (40 cm height × 40 cm diameter) was filled with 25 kg of soil. Seeds of *Calendula officinalis* L. were sown on October 1, 2023, at a rate of 10 seeds per pot and later thinned to three plants per pot.

The study was arranged as a factorial experiment in a completely randomized design (CRD) with three replications. The factors were:

1) Irrigation was fixed at two levels: 100 % and 50 % of field capacity, not adjusted dynamically based on plant requirements
2) Nano-liquid nitrogen fertilizer (N) at 0, 2, and 4 mL L^{-1}.
3) Complex nano-potassium fertilizer (K) at 0, 2, and 4 g L^{-1}.

The nano-liquid nitrogen fertilizer and complex nano-potassium fertilizer were obtained from Fanavar Sepehr Parmis Fertilizer Company (Tehran, Islamic Republic of Iran). Control plants were sprayed with distilled water only. Phosphorus fertilizer (0.3125 g P pot^{-1} as calcium superphosphate) was incorporated before planting. Foliar sprays of nano-fertilizers were applied four times at four-week intervals until full flowering.

2.2. Enzyme assays

Fresh fully expanded leaves were sampled at the full flowering stage to determine antioxidant enzyme activity:

1) CAT activity was measured according to the method described by Ref. [12], which determines the decomposition rate of hydrogen peroxide at 240 nm. Results were expressed as units per mg fresh weight (FW).
2) SOD activity was estimated following the procedure of [13], based on inhibition of pyrogallol autoxidation at 420 nm. Results were expressed as units per mg FW.
3) POD activity was assayed according to Ref. [14], using guaiacol oxidation measured spectrophotometrically at 470 nm. Results were expressed as units per mg FW.

2.3. Statistical methods

All data were subjected to analysis of variance (ANOVA) using SAS software (version 9.3) [15].

Table 1. Some physical and chemical characteristics of the soil sample used in this study.[a]

Adjective		Value	Unit
pH		7.9	–
Electrical conductivity (EC)		1.28	$ds \cdot m^{-1}$
Organic matter		8.62	$gm \cdot Kg^{-1}$
	Ca^{2+}	5.5	C $mol \cdot Kg^{-1}$ Soil
Soluble cations	Mg^{2+}	3.8	C $mol \cdot Kg^{-1}$ Soil
	Na^{1+}	2.7	C $mol \cdot Kg^{-1}$ Soil
	K^{+}	0.41	C $mol \cdot Kg^{-1}$ Soil
	SO_4^{2-}	6.6	C $mol \cdot Kg^{-1}$ Soil
Soluble anions	HCO_3^{1-}	2.4	C $mol \cdot Kg^{-1}$ Soil
	CO_3^{2-}	Nil	C $mol \cdot Kg^{-1}$ Soil
	Cl^{-}	3.3	C $mol \cdot Kg^{-1}$ Soil
Available N		14	$gm \cdot Kg^{-1}$
Available P		13.6	$gm \cdot Kg^{-1}$
Available K		85.4	$gm \cdot Kg^{-1}$
	Sand	846	$gm \cdot Kg^{-1}$
Soil separators	Silt	83	$gm \cdot Kg^{-1}$
	Clay	71	$gm \cdot Kg^{-1}$
Texture Sandy loam			
Moisture at 10 kPa Tension: 9.6 %			
Moisture at 33 kPa Tension: 5.8 %			

[a] The soil analyses were conducted at the laboratories of the Faculty of Agriculture, University of Baghdad.

Mean differences were separated using the least significant difference (LSD) test at $p < 0.05$.

3. Results

3.1. CAT activity

Table 2 shows that CAT activity was significantly affected by water stress, nano-nitrogen, and nano-potassium. Irrigation at 50 % of field capacity (S_1) increased CAT activity compared with full irrigation (S_2), confirming the role of drought in stimulating antioxidant defense [11]. Among nano-nitrogen levels, the control treatment (N_0) gave the highest CAT activity, while higher concentrations (N_2, 4 mL L^{-1}) reduced it. Similarly, the absence of nano-potassium (K_0) resulted in greater CAT activity compared with K_1 and K_2.

The best treatment combination was $N_0K_0S_1$, which recorded the highest CAT value (0.42 units mg^{-1} FW). The lowest CAT activity was observed under $N_2K_2S_2$ (0.31 units mg^{-1} FW), where plants received the highest nano-fertilizer concentrations under full irrigation. These findings suggest that moderate stress without additional nano-fertilizer enhanced catalase activity, consistent with earlier observations that excessive nitrogen may reduce antioxidant activity [16,17].

3.2. SOD activity

The data given in Table 3 illustrate SOD activity increased significantly under water stress (S_1), reaching 2.88 units mg^{-1} FW compared with 2.49 units mg^{-1} FW at full irrigation (S_2). Foliar application of nano-nitrogen and nano-potassium reduced SOD activity in a concentration-dependent manner, with the highest values observed at N_0 and K_0.

The maximum activity (3.00 units mg^{-1} FW) was recorded at $N_0K_0S_1$, while the minimum (2.15 units mg^{-1} FW) occurred under $N_2K_2S_2$. The increase in SOD under drought stress indicates enhanced detoxification of superoxide radicals, whereas high levels of nano-fertilizers appeared to suppress enzyme activity, similar to results reported by Ref. [18].

3.3. POD activity

The results in Table 4 show POD activity was also enhanced by water stress (S_1), which increased enzyme activity to 10.64 units mg^{-1} FW compared with 8.34 units mg^{-1} FW at full irrigation (S_2). The absence of nano-nitrogen (N_0) and potassium (K_0) sprays gave the highest POD values, whereas increasing fertilizer levels reduced activity.

The best treatment was $N_0K_0S_1$, which produced the maximum POD activity (13.61 units mg^{-1} FW). The lowest activity was recorded at $N_2K_2S_2$ (6.92 units mg^{-1} FW). These results confirm that drought stress activates peroxidase, while excessive nano fertilization may reduce its effectiveness, in agreement with previous studies that reported decreased antioxidant enzyme activity under high nitrogen input [16,18].

Table 2. The effect of spraying with nano-nitrogen and nano-potassium fertilizers on the activity of the CAT enzyme (units mg^{-1} fresh weight) in C. officinalis *plants grown under two irrigation levels.*

Nano-nitrogen spraying (ml L^{-1}) N	Nano-potassium spraying (g L^{-1}) K	Irrigation levels (from field capacity value) S						N*K
		50 % S_1			100 % S_2			
0	0	0.42			0.37			0.40
	2	0.41			0.37			0.39
	4	0.41			0.36			0.38
2	0	0.41			0.35			0.38
	2	0.38			0.34			0.36
	4	0.38			0.32			0.35
4	0	0.39			0.35			0.37
	2	0.38			0.31			0.35
	4	0.38			0.31			0.34
S		0.39			0.34			
								N
N*S	0	0.41			0.37			0.39
	2	0.39			0.33			0.36
	4	0.38			0.32			0.35
								K
K*S	0	0.40			0.36			0.38
	2	0.39			0.34			0.37
	4	0.39			0.33			0.36
L.S.D 0.05		*N*	*K*	*S*	*N * K*	*N * S*	*K * S*	*N * K * S*
		0.0036	0.0036	0.003	0.0072	0.0066	0.0066	0.0102

Table 3. The effect of spraying with nano-nitrogen and nano-potassium fertilizers on the activity of the SOD enzyme (units mg^{-1} fresh weight) in C. officinalis *plants grown under two irrigation levels.*

Nano-nitrogen spraying (ml L-1) N	Nano-potassium spraying (g L^{-1}) K	Irrigation levels (from field capacity value) S						N*K
		50 % S_1			100 % S_2			
	0	3.00			2.70			2.85
	2	2.94			2.67			2.81
0	4	2.92			2.66			2.79
	0	2.92			2.56			2.74
	2	2.87			2.46			2.66
2	4	2.81			2.40			2.60
	0	2.91			2.51			2.71
	2	2.80			2.36			2.58
4	4	2.74			2.15			2.44
S		2.88			2.49			
								N
	0	2.95			2.67			2.81
N*S	2	2.86			2.47			2.67
	4	2.81			2.34			2.57
								K
	0	2.94			2.59			2.76
K*S	2	2.87			2.50			2.68
	4	2.82			2.40			2.61
L.S.D 0.05		*N*	*K*	*S*	*N * K*	*N * S*	*K * S*	*N * K * S*
		0.0273	0.0273	0.0223	0.0546	0.0496	0.0496	0.0769

4. Discussion

Across all three enzymes (CAT, SOD, POD), water stress (50 % FC) markedly increased activity relative to full irrigation, with the highest values recorded under $N_0K_0S_1$ (0.42, 3.00, and 13.61 units mg^{-1} FW, respectively) and the lowest under $N_2K_2S_2$ (0.31, 2.15, and 6.92 units mg^{-1} FW). These results indicate a pronounced drought-induced antioxidant response, with enzyme activity down-regulated when nano-N and nano-K were applied at higher rates.

Water stress is known to induce oxidative stress by enhancing the production of reactive oxygen species (ROS) (e.g., $O_2{\cdot}^-$ and H_2O_2) through stomatal closure, over-reduction of the photosynthetic electron chain, and increased photo respiratory fluxes. This typically results in higher antioxidant

Table 4. The effect of spraying with nano-nitrogen and nano-potassium fertilizers on the activity of the POD enzyme (units mg^{-1} fresh weight) in C. officinalis *plants grown under two irrigation levels.*

Nano-nitrogen spraying (ml L^{-1}) N	Nano-potassium spraying (g L^{-1}) K	Irrigation levels (from field capacity value) S						N*K
		50 % S_1			100 % S_2			
	0	13.61			9.33			11.47
	2	12.25			9.19			10.72
0	4	11.11			9.09			10.10
	0	10.68			9.08			9.88
	2	9.85			7.96			8.90
2	4	9.44			7.44			8.44
	0	10.01			9.07			9.54
	2	9.42			6.96			8.19
4	4	9.41			6.92			8.16
S		10.64			8.34			
								N
	0	12.32			9.20			10.76
N*S	2	9.99			8.16			9.07
	4	9.61			7.65			8.63
								K
	0	11.43			9.16			10.29
K*S	2	10.51			8.03			9.27
	4	9.98			7.82			8.90
L.S.D 0.05		N	K	S	N * K	N * S	K * S	N * K * S
		0.0447	0.0447	0.0365	0.0894	0.0812	0.0812	0.1259

enzyme activity. Plants up-regulate SOD (conversion of $O_2\bullet^-$ to H_2O_2) and CAT/POD (detoxification of H_2O_2) as frontline defenses, and our findings at 50 % FC clearly reflect this mechanism [11,19]. Thus, the observed increase under S_1 conditions corresponds to stress signaling and ROS detoxification.

In contrast, the reduced enzyme activities under higher nano-N and nano-K levels may be explained by two complementary mechanisms consistent with plant physiology:

(i) Stress mitigation hypothesis. Adequate potassium improves osmotic adjustment, stomatal regulation, and photosynthetic efficiency, thereby lowering ROS production and reducing the requirement for strong enzymatic up-regulation [4,20]. Similarly, foliar nitrogen may enhance chlorophyll/protein turnover and alleviate drought signals. In this scenario, reduced enzyme activity does not imply a weakened defense, but rather a lower induction due to attenuated stress.

(ii) Nitrogen-driven redistribution/redox effects. Elevated nitrogen can alter antioxidant enzyme activity and phenolic metabolism, likely by affecting carbon−nitrogen balance and redox allocation [16−18]. Since phenolics represent a major class of non-enzymatic antioxidants [21], increased nitrogen may limit phenolic accumulation and shift reliance toward enzymatic versus non-enzymatic defenses. Although phenolics were not measured in this study, it remains a plausible mechanism [22].

Taken together, the most parsimonious interpretation of our dataset is that nano-N and nano-K attenuated drought signaling and/or shifted redox allocation, thereby reducing the need for maximal CAT, SOD, and POD induction. While many studies report that nanoparticles enhance antioxidant enzyme activities under stress (often alongside growth or yield improvements) [5,6,8,9], our results differ. In our conditions, enzyme activities decreased with higher nano-fertilizer rates, but this can still be reconciled if nano-fertilization primarily reduced ROS generation (i.e., less stress → lower enzyme induction). Without direct ROS biomarkers, both interpretations remain valid.

5. Conclusions

This study demonstrated that irrigation at 50 % of field capacity stimulated the antioxidant defense system in *Calendula officinalis* L. by significantly increasing the activities of CAT, SOD, and POD. The highest values for all three enzymes were recorded under water stress without foliar application of Nano-nitrogen or nano-potassium fertilizers ($N_0K_0S_1$), indicating that drought alone triggered a strong enzymatic defense response. In contrast, increasing concentrations of nano-nitrogen and nano-potassium fertilizers reduced enzyme activities, suggesting that nano-fertilization moderated the drought-induced oxidative signal and thereby lowered the need for maximal enzymatic induction. Overall, these results highlight that moderate water stress can enhance antioxidant capacity in calendula, while careful management of nano-fertilizer applications is necessary to balance nutrient supply with the maintenance of natural defense mechanisms. This information is important for optimizing the cultivation of *C. officinalis* under water-limited conditions.

Ethical statement

This research involved only plant materials (*Calendula officinalis* L.) and did not include any experiments on humans or animals. All procedures complied with institutional and international ethical guidelines for research involving plant materials.

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Conflicts of interest

The authors declare that they have no competing interests.

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