



Research Article

Impact of Nanopotassium and Multi-Walled Carbon Nanotube Spray on Enzyme Activity in *Vigna radiata* L. Under Water Stress

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Abstract | A pot experiment was carried out at the College of Agriculture, University of Karbala, during the academic season 2023-2024 to investigate the effects of spraying complex nano potassium fertilizer and multi-walled carbon nanotubes, alone and combined, on the activity of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in the leaves of the mung bean plants (*Vigna radiata*) grown under water stress in Loamy sand soil. The experiment involved three factors: the first was spraying with complex nano potassium fertilizer at concentrations of 0, 2 and 4 gL⁻¹; the second was spraying with multi-walled nano carbon tubes at 0 and 0.1 gL⁻¹; and the third factor was irrigation at two levels 50% and 100% of the soil field capacity. Results showed that enzyme activities decreased significantly when plants were sprayed with nano potassium fertilizer, carbon nanotubes, and their combination. However, enzyme activities increased under water stress. These findings provide valuable insights for enhancing enzyme efficiency in mungbean under water limited environment.

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Introduction

Mung bean (*Vigna radiata* L.) is a green legume belonging to the Fabaceae family and is classified under the genus *Vigna* (Alkatib, 1988). It is a nutrient rich crop with numerous health benefits. Mungbean have been shown to help reduce the risk of chronic diseases, support heart health, lower high cholesterol, regulate blood pressure, improve digestion, and help maintain stable blood sugar levels (Zhang et al., 2022). Water stress is a type of environmental stress resulting from either a shortage or excess of

water in the surrounding of plants. However, when referring specifically to water deficiency without increased water availability, the term “drought stress” is commonly used (Yassin, 1992). Water scarcity has become a major challenge for living organisms, including humans, and is especially critical in regions like Iraq. Drought is now recognized as a major factor limiting plant growth and agricultural productivity. Because of this, understanding how plants respond to water stress is essential for improving crop resilience and performance (Nawaz et al., 2020; Al-Karawi, 2025).

Potassium and carbon are both vital elements that play key roles in plant physiology. Potassium, in particular enhances plant resistance to drought and salinity by improving photosynthetic efficiency, increasing carbohydrate content, expanding leaf area, and regulating stomatal behavior under stress. It also helps reduce water loss through transpiration and supports overall plant growth (Wang *et al.*, 2013). Using nano-fertilizers on leaves is a cutting-edge method to boost plant growth, raise yields, and enhance fruit quality. Nutrients are delivered with remarkable speed and are highly absorbed by the stomata. Because of their small size, the nanoparticles exhibit greater absorption efficiency. The plant absorbs them more easily, making them highly effective in cases of severe nutrient deficiency. It's best to apply in the morning. This enhances productivity and fosters growth. Its vegetative growth characteristics are superior to those of traditional fertilization (Alkarawi *et al.*, 2024; Alkarawi, 2025a).

Multi-walled carbon nanotubes (MWCNTs) are a unique type of nanomaterial known for their exceptional physical and chemical properties. These nano-scale carbon structures, often described as honeycomb-like tubular filaments, have been widely used across various scientific fields, including agriculture (Teradal and Jelinek, 2017; Roy and Sreejith, 2017; Alwan and Alkarawi, 2024). In agriculture, nano materials are utilized to enhance crop resilience to adverse environmental conditions such as drought and high temperatures, while also helping to reduce production costs (Al-Juthery *et al.*, 2021). Antioxidant enzymes play a crucial role in protecting plants against oxidative stress, which is commonly triggered by environmental stresses like drought. Among these enzymes superoxide dismutase (SOD) is the first line of defense against oxidative stress. It converts superoxide radicals into hydrogen peroxide and oxygen, thus protecting plant cells from damage. It has three main forms, Cu/Zn-SOD found in the cytoplasm and chloroplasts, Fe-SOD in chloroplasts, Mn-SOD in mitochondria (Sonowal *et al.*, 2025). Peroxidase (POD) is a redox enzyme that breaks down hydrogen peroxide (H_2O_2) into oxygen and water. It exists in several forms, including phenol peroxidase (which strengthens cell wall and defend against pathogens), ascorbate peroxidase (protects photosynthetic tissues), glutathione peroxidase (maintain redox balance in the cytoplasm (Hasanuzzaman *et al.*, 2018). Catalase is a heme

containing antioxidant enzyme found mainly in the peroxisomes. It helps breakdown hydrogen peroxide into water and oxygen, protecting key components of the photosynthetic machinery such as Rubisco and the PSII complex from oxidative damage. It also works in coordination with peroxidase to neutralize reactive oxygen species (ROS) (Movahedi *et al.*, 2024). This study aims to evaluate the effects of foliar application of complex nano-potassium fertilizer and multiwalled carbon nanotubes on the activity of antioxidant enzymes *Vigna radiata* plants under water stress conditions.

Materials and methods

A factorial experiment was carried out during the 2023-2024 agricultural season using a Completely Randomized Design (CDR) with four replications. The study involved three experimental factors:

Factor 1: Foliar application of complex nano-potassium fertilizer at three levels ($K_0 = 0$ g/L, $K_1 = 2$ g/L, $K_2 = 4$ g/L).

Factor 2: Foliar application of multi-walled carbon nanotubes (MWCNTs) at two levels ($C_0 = 0$ g/L, $C_1 = 0.1$ g/L)

Factor 3: Irrigation with river water at two levels of soil moisture ($S_1 = 50\%$ Field Capacity (FC), 100% FC)

The design resulted in a total of 48 experimental units. Mungbean (*Vigna radiata* L.) seeds were sown on July 1, 2023, in plastic pots (25 kg capacity) filled with sandy loam soil. Each pot initially contained 10 plants, which were later thinned to 3 plants per pot two weeks after emergence. To ensure uniform spraying and proper leaf coverage, 0.15 mL/L of a wetting agent (coloring matter) was added to the spray solution. Control treatments were sprayed with distilled water only. Urea fertilizer was applied in two equal doses, one week after seedling emergence, and one week after thinning, at a rate equivalent to 50 kg/ha. Calcium superphosphate was also applied once at the time of seedling emergence, at a rate of 50 kg/ha, following the recommendations of Ahmmed *et al.* (2021).

Some physical and chemical properties of the soil were analyzed based on the procedures described by Brady and Weil (2010), as presented in Table 1. The crop was harvested on November 15, 2024.

Table 1: Physical and chemical properties of the experimental soil.

Soil parameters		Values	Units
Soil reaction pH 1:1		7.9	
Electrical conductivity EC		1.28	dS m ⁻¹
Organic matter		8.62	1
carbonate minerals		440	g Kg ⁻¹
Organic carbon		5	
Gypsum		1.6	
CEC		13.95	Cmol kg ⁻¹
SAR		0.89	%
ESP		0.053	
Positive dissolved ions	Ca ²⁺	5.5	ml
	Mg ²⁺	3.8	equivalent
	Na ⁺	2.7	liter ⁻¹
	K ⁺	0.41	
Negative dissolved ions	SO ₄ ²⁻	6.6	
	CO ₃ ²⁻	14	
	HCO ₃ ⁻	14	
	Cl ⁻	3.3	
Available Nitrogen		14	mg Kg ⁻¹ soil
Available Phosphorus		13.6	
Available Potassium		85.4	
Sand		846	g Kg ⁻¹ soil
Silt		83	
Clay		71	
Soil texture		Loamy sand	
Moisture at 10 kPa tension:		9.6%	
Moisture at 33 kPa tension:		5.8%	

The analyses were performed at the central laboratory of the College of Agricultural Engineering Sciences, University of Baghdad.

Enzyme activity measurements

Superoxide dismutase (SOD) and Peroxidase (POD) activities in the plant leaves were determined according to the method described by Peskin and Winterbourn (2000). Catalase (CAT) activity was measured following the procedure of Hamza and Hadwan (2020).

Statistical analysis

All the collected data were statistically analyzed using GenStat (Payne et al., 2011). Differences between treatment means were assessed at a significance level of $p \leq 0.05$

Results and Discussion

Superoxide dismutase (SOD) enzyme activity

The effect of the experimental treatments on the activity of the SOD enzyme in mungbean leaves is illustrated in Table 2. Irrigation levels had a significant impact, with the highest SOD activity observed under

the 50% field capacity irrigation treatment (S1), reaching 2.99 U/g fresh weight (f.wt.), compared to full irrigation (S2). This increase reflects the response of plant to water stress, as SOD plays a key role in protecting cells from oxidative damage by preventing lipid peroxidation under drought conditions (Islam et al., 2021). Spraying with nano-complex potassium fertilizer also significantly affected SOD activity. The control treatment (K₀) recorded the highest enzyme activity at 3.05 U/g f.wt, while activities decreased in the K1 and K2 treatments to 2.85 and 2.79 U/g f.wt, respectively. Thios reduction may be due to nano-potassium interfering with the enzyme structure, altering its composition and thus inhibiting its activity (Islam et al., 2024). Additionally, nanoparticles can affect genes related to SOD production, such as CSD1 and MSD1, resulting in reduced enzyme synthesis (Alhasany et al., 2024).

Table 2: Influence of foliar applied nanopotassium and muti walled carbon nanotubes, and their interaction, on superoxide dismutase (SOD) activity in the leaves of *Vigna radiata* plants subjected to water deficit.

K* ^C	Irrigation level compared to soil moisture content at field capacity %				Nano K fertilizer concentration (g L ⁻¹) K		Carbon nanotube concentration (g L ⁻¹) C
	100 % S2		50 % S1				
3.10	2.96	3.24		K0		C0	
2.90	2.82	2.98		K1			
2.85	2.79	2.92		K2			
3.01	2.90	3.12		K0		C1	
2.80	2.74	2.86		K1			
2.75	2.63	2.82		K2			
	2.80	2.99		S Mean			
C Mean							
2.95	2.85	3.04		0		S*C	
2.84	2.75	2.93		1			
K Mean							
3.05	2.93	3.18		0		S*K	
2.85	2.78	2.92		1			
2.79	2.71	2.87		2			
C*K*S	S*K	S*C	K*S	S	K	C	LSD 0.05
0.0112	0.0092	0.0102	0.0092	0.0046	0.0056	0.0046	

The application of muti-walled carbon nanotubes (MWCNTs) also influenced SOD activity significantly. The highest activity was recorded in the control (C₀) at 2.95 U/g f.wt, compared 2.84 U/g f.wt in the treated (C₁) group. Nanocarbon is known to enhance drought tolerance by activating plant defense mechanism against reactive oxygen species (ROS)

and reducing oxidative stress. This can improve photosynthesis and cell membrane stability, which lowers ROS production and consequently reduces the need for SOD activity (Sarkar *et al.*, 2022a; Ogawa, 2023).

The combined effect of nano potassium and carbon nanotubes also showed significant results. The highest SOD activity occurred under the untreated control ($K_0 C_0$) at 3.10 U/g f.wt, whereas the lowest was at the combined highest treatment levels ($K_2 C_1$) with 2.75 U/g f.wt. The decline in SOD activity in these combined treatments likely results from disputed cell functions due to an imbalance between potassium and nanocarbon, which inhibits the enzyme activity (Islam *et al.*, 2024).

Moreover, the interaction between nano potassium and irrigation level significantly affected SOD activity. The highest value (3.18 U/g f.wt) was at ($K_0 S_1$), while the lowest (2.71 U/g f.wt) occurred at ($K_2 S_2$). The reduction in SOD with nano potassium under full irrigation may be due to the suppression of gene and protein expression responsible for this enzyme (Al-Falahi *et al.*, 2023). This interaction can increase ROS, disrupting ionic balance and damaging chloroplasts, which further reduces SOD levels (Ahmad *et al.*, 2025). Similarly, the interaction between carbon nanotubes and irrigation water levels showed a significant effect on SOD. The highest activity (3.04 U/g f.wt) was observed at ($C_0 S_1$), and the lowest (2.75 U/g f.wt) at ($C_1 S_2$). The decrease in SOD activity under these conditions is attributed to oxidative stress and cellular imbalance, which damage amino acids in the SOD enzyme and weaken photosynthesis (Dang *et al.*, 2023). Carbon nanoparticles can also inhibit defense signaling pathways such as NPR1, which regulate antioxidant enzymes (Prakash *et al.*, 2025). The triple interaction of all factors further influenced SOD activity. The highest enzyme activity (3.24 U/g f.wt) occurred at ($K_0 C_0 S_1$), and the lowest (2.63 U/g f.wt) at ($K_2 C_1 S_2$). The reduction in SOD activity under combined treatments is likely due to damage to mitochondria and plastid membranes—key sites of SOD production (Chandrashekar *et al.*, 2023). Nanoparticles can cumulatively inhibit gene expression by disrupting pathways like Mitogen Activated Protein Kinase (MAPK), which regulate antioxidant responses including SOD (Al-Semmak, 2025).

Peroxidase (POD) enzyme activity

The effect of the treatments on peroxidase (POD) in mungbean leaves is illustrated in Table 3. Irrigation level significantly influenced POD activity, with the highest value at 50% field capacity (S_1) reaching 7.88 U/g f.wt, compared to full irrigation (S_2). POD activity increases under water stress as part of the plant antioxidant defense system, breaking down hydrogen peroxide into water and oxygen, and aiding lignin synthesis to strengthen cell walls and reduce water loss (Raheja *et al.*, 2025).

Table 3: Effect of foliar applied nanopotassium and multi walled carbon nanotubes, and their interaction, on peroxidase (POD) activity in the leaves of *Vigna radiata* grown under water stress conditions.

K°C	Irrigation level compared to soil moisture content at field capacity %				Nano K fertilizer concentration (g L ⁻¹) K		Carbon nanotube concentration (g L ⁻¹) C
	100 % S2		50 % S1				
7.16	6.44	7.88		K0		C0	
6.51	6.18	6.85		K1			
6.33	5.92	6.74		K2			
6.92	6.32	7.52		K0		C1	
6.24	5.84	6.65		K1			
6.05	5.78	6.32		K2			
	6.08	6.99		S Mean			
C Mean							
6.66	6.18	7.15		0		S*C	
6.40	5.95	6.83		1			
K Mean							
6.38	6.38	7.70		0		S*K	
6.01	6.01	6.75		1			
5.85	5.85	6.53		2			
C*K*S	S*K	S*C	K*S	S	K	C	LSD
0.0178	0.011	0.0123	0.011	0.0055	0.0068	0.0055	0.05

Spraying with nano-complex potassium fertilizer significantly affected POD activity, with the highest enzyme activity recorded in the control treatment (K_0) at 7.04 U/g f.wt. Enzyme activity decreased in K_1 and K_2 treatments to 6.38 and 6.19 U/g f.wt, respectively. This decline may be due to disturbances in the uptake of essential elements like iron and selenium, which are cofactors for POD, as well as inhibition of antioxidant gene pathways such as WRKY, leading to reduced enzyme synthesis (Ahanger *et al.*, 2021; Alharbi *et al.*, 2024).

The effect of carbon nanotubes on POD activity was also significant. The control (C_0) showed highest

activity (6.66 U/g f.wt) than the treated group (C1) with 6.40 U/g f.wt. The decrease in enzyme activity may be due to structural damage to the enzyme active sites caused by nanocarbon, as well as disruption of the MAPK pathway that controls POD gene expression (Mousavi *et al.*, 2025).

Table 4: Influence of nano-potassium and carbon nanotubes on catalase (CAT) activity and their interaction in *Vigna radiata* leaves under water stress conditions.

K*C	Irrigation level compared to soil moisture content at field capacity %		Nano K fertilizer concentration (g L ⁻¹) K	Carbon nanotube concentration (g L ⁻¹) C
	100 % S2	50 % S1		
0.44	0.42	0.45	K0	C0
0.41	0.40	0.43	K1	
0.41	0.39	0.42	K2	
0.43	0.41	0.44	K0	C1
0.40	0.38	0.41	K1	
0.39	0.37	0.41	K2	
	0.39	0.43	S Mean	
C Mean				
0.42	0.40	0.43	0	S*C
0.40	0.39	0.42	1	
K Mean				
0.43	0.42	0.45	0	S*K
0.41	0.39	0.42	1	
0.40	0.38	0.41	2	
C*K	S	S*K	S*C	K*S
0.0016	0.001	0.0011	0.001	0.0005
			0.0006	0.0005
				0.05

The interaction nano potassium and carbon nanotubes had a notable impact on POD activity. The highest enzyme activity was recorded at (K₀ C₀) with 7.16 U/g f.wt, while the lowest occurred at (K₂ C₁) with 6.05 U/g f.wt. This reduction may be caused by structural damage to POD caused by nanoparticle penetration and disruption of signaling pathways regulating enzyme production (Sarkar *et al.*, 2022a; Islam *et al.*, 2024). The dual interaction between nano potassium and irrigation level significantly affected POD activity. The highest value was at (K₀ S₁) with 7.70 U/g f.wt, and the lowest at (K₂ S₂) with 5.85 U/g f.wt. The decrease under combined stress is likely due to oxidative damage from accumulated hydrogen peroxide, which impairs the enzyme active site, as well as inhibition of key transcription factors such as VrWRKY53 involved in POD synthesis (Ahanger *et al.*, 2021; Al-Falahi *et al.*, 2023).

Similarly, the interaction between carbon nanotubes and irrigation water levels showed a significant effect. POD activity was highest at (C₀ S₁) with 7.15 U/g f.wt and lowest at (C₁ S₂) with 5.95 U/g f.wt. The reduction in POD activity may result from damage to the enzyme tertiary structure due to nanoparticle infiltration, along with inhibition of transcription factors NAC and bZIP that regulate peroxidase genes (Dang *et al.*, 2023; Ahmad *et al.*, 2025). The triple interaction of all factors also significantly influenced POD activity. The highest activity was observed at (K₀ C₀ S₁), with 7.88 U/g.f.wt, while the lowest occurred at (K₂ C₁ S₂) with (5.78) U/g f.wt. The reduced enzyme activity under combined treatments is likely due to membrane damage in cells and organelles like plastids, leading to structural impairment of the enzyme and decreased function (Al-Semmak, 2025).

Catalase (CAT) enzyme activity

The effect of different treatments on the activity of the catalase (CAT) in mung bean leaves. The results indicate a significant impact of irrigation levels on enzyme activity. The highest CAT activity (0.45 katal/g f.wt) was observed under water limited conditions (S1), compared to fully irrigated treatment (S2). This increase in activity under drought stress is likely a defense mechanism to neutralize hydrogen peroxide and reduce oxidative damage caused by reactive oxygen species (ROS) (Sarkar *et al.*, 2022a).

Spraying with nano-complex potassium fertilizer also significantly influenced CAT activity. The control treatment (K₀) recorded the highest enzyme activity at 0.43 katal/g f.wt, whereas enzyme levels declined in the K₁ and (0.41 katal/g f.wt and K₂ (0.40 katal/g f.wt), treatments. This reduction may be attributed to increased ROS production caused by nano-potassium, which can damage the enzyme active site and reduce its efficiency (Table 4). Additionally, the disruption of gene expression through stress related pathways such as MAPK, and inhibition of transcription factors bZIP and NAK, may further contribute to the reduction in CAT activity (Al-Falahi *et al.*, 2023; Islam *et al.*, 2024).

Carbon nanotube treatments also had a significant effect. The untreated group (C₀) showed higher CAT activity (0.42 katal/g f.wt), than the treated group (C₁), which reached 0.40 katal/g f.wt. The reduced enzyme activity under MWCNT application could be due to decreased CO₂ uptake, impaired photosynthesis, and lower ATP production, all of which are essential

for enzyme synthesis and repair. Nanotubes may also disrupt regulatory gene pathways such as VrMAPK3, reducing CAT enzyme activation (Sarkar *et al.*, 2022b; Ogawa, 2023).

The interaction between nano potassium and carbon nanotubes also significantly affected CAT activity. The highest value was recorded under the control ($K_0 C_0$) at 0.44 katal/g f.wt, while the lowest 0.39 katal/g f.wt occurred at the highest treatment levels ($K_2 C_1$). The decline in enzyme activity is likely due to oxidative stress and ROS accumulation, which disrupts cellular ionic balance and damages the enzyme heme active site. Disruption of MAPK signaling may further reduce CAT gene expression (Islam *et al.*, 2024; Al-Semmak, 2025). A significant interaction was also observed between potassium treatments and irrigation levels. The combination of low potassium and water stress ($K_0 S_1$) produced the highest CAT activity (0.45 katal/g f.wt), while the lowest (0.38 katal/g f.wt) was at ($K_2 S_2$). This drop may result from excess ROS and H_2O_2 in chloroplasts, leading to degradation of the enzyme iron center and suppressed gene expression due to inhibited MAPK pathway signaling (Maduraimuthu *et al.*, 2023; Rady *et al.*, 2023).

The interaction between carbon nanotubes and irrigation level also had a significant impact. The highest enzyme activity (0.43 katal/g f.wt) occurred under ($C_0 S_1$), while the lowest (0.39 katal/g f.wt) was at ($C_1 S_2$). Reduced enzyme activity is likely due to cellular damage caused by nanotube penetration, especially in peroxisomes where CAT is located. Additionally, disruption in calcium signaling and transcription factor inhibition (e.g., bZIP) could be contributing factors (Dang *et al.*, 2023; Prakash *et al.*, 2025). Finally, the triple interaction of all factors showed significant influence on CAT activity. The highest value (0.45 katal/g f.wt) was recorded in the ($K_0 C_0 S_1$) treatment, while the lowest (0.37 katal/g f.wt) occurred under ($K_2 C_1 S_2$). This decline is likely due to compounded stress effects, including MAPK pathway suppression, ionic imbalance, and increased ROS production in plastids, all of which impair enzyme synthesis and activity (Al-Semmak, 2025; Sonowal *et al.*, 2025).

Conclusion

1. The activity of antioxidant enzymes (SOD, POD,

CAT) increases under water stress conditions, indicating their role in mitigating oxidative damage caused by drought.

2. Increasing the concentration of nano-complex potassium fertilizer lead to a noticeable reduction in the activity of all studied antioxidant enzymes, suggesting a dose dependent inhibitory effect.
3. Application of multi walled carbon nanotubes negatively affected the activity of the antioxidant enzymes, likely due to interference with metabolic and regulatory pathways.
4. Both dual and triple interactions between nano potassium, carbon nanotubes, and irrigation levels had significant impact on enzyme activity, highlighting the importance of understanding the combined effects of abiotic stress and nanomaterial application.

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Novelty Statement

This study reveals a new understanding of how nano-potassium fertilizer and multi-walled nanotubes affect the antioxidant defense system in mungbean plants under water stress. It shows that while mild stress boosts enzyme activity, higher levels of these nanomaterials can actually reduce the plant's natural defenses. These findings offer useful insights for managing nanomaterial use in drought-affected crops.

Author's Contribution

Muhammad T. B.I. Algazali: Developed the concept and overall theory, practical applications, and executed hands-on experiments. Qais H. Al Semmak: offered supervision, technical input, and help with article management. The manuscript belongs to the first author, who is a PhD student. Two authors writers collaborated on the manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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