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Research Article

The effect of spraying multi-walled carbon nanotubes and nano potassium fertilizer on the content of proline, abscisic acid, and chlorophyll in the leaves of the Vigna radiata plant under water stress.

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Abstract:

To investigate the effects of spraying complex nano potassium fertilizer and multi-walled carbon nanotubes, as well as their interactions, on the content of proline, abscisic acid, and chlorophyll content in the leaves of *Vigna radiata* plants growing under water stress conditions in sandy loam soil during the winter of 2023–2024, a pot experiment was conducted at the College of Agriculture, University of Kerbala. experiment consisted of three components: involved applying complex nano potassium fertilizer at concentrations of 0, 2, and 4 g per liter^{-1}; the second involved applying of multi-walled carbon nanotubes at concentrations of 0 and 0.1 g L^{-1} ,the third was irrigation at two levels 50% and 100% of soil's field capacity .The experiment followed a completely randomized design (C.R.D.) with four replications, the factorial experiment includes (48) experimental units. The results of the study showed a significant decrease in the activities of proline and abscisic acid and a significant increase in the chlorophyll content of the leaves when spraying with complex nano-potassium fertilizer and multi-walled carbon nanotubes and their interactions when irrigating at a level of 100% of the field capacity value of the soil, While the opposite was shown when irrigating at 50% from the field capacity value of the soil. The results showed an increase in the concentration of
proline and abscisic acid, while the chlorophyll content decreased at the level of 50% of the field capacity value of the soil at (S_1).

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Introduction: Mung bean is a green legume of the genus Cowpea in the Fabaceae family [1] , and its scientific name is *Vigna radiata*, Mung beans contain many nutrients, and other numerous health benefits, the most important of which include reducing the risk of chronic diseases, improving heart health, lowering high cholesterol levels, controlling blood pressure levels, enhancing the digestive process, and maintaining on the sugar levels in blood [2].

Water stress is a type of environmental stress that refers to the lack or excess of water in a plant's environment, On this basis, the term "drought stress" is used for drought stress resulting from a lack of water without an increase in it [3].

One of the problems facing living organisms, including humans, in our lives is the issue of water scarcity. Drought has become a factor that limits and restricts plant growth and development, especially in our country, Iraq. Since water stress is one of the important factors that limit the production of crops, so the plant's ability to tolerate this stress must be studied. Stresses that affect in the plants, such as water stress, negatively impact in the ability of crops [4].

Potassium and carbon are essential elements that play important physiological roles in plants. Potassium enhances the plant's resistance to drought and salinity, and aids in increasing leaf area by enhancing the efficiency of photosynthesis increasing the plant's carbohydrate content. It also stimulates plant growth, reduces transpiration rate, and regulates the opening and closing of stomata under stress conditions [5].

Multi-walled carbon nanotubes are an essential member of the nanomaterial family due to their unique physical and chemical properties. Nanocarbon is also used in many scientific applications, including agricultural sciences [6]. [7] , defined carbon nanotubes as disc-shaped filaments of nano-carbon resembling honeycombs. Nanomaterials are used in agriculture to reduce agricultural production costs. And increasing plant defenses to mitigate harsh conditions such as high temperature, water stress, etc. [8].

Proline maintains osmotic balance by acting as an effective osmotic buffer, which helps balance the water potential within the cell and maintain its volume and stability in the event of water deficiency, It also works as a powerful antioxidant, removing free radicals that accumulate during environmental stress and cause severe damage to plant cells. It also contributes to the stability of cell membranes, protecting them from damage resulting from environmental stress, Proline also regulates gene expression and cellular responses that help plants adapt to harsh environmental conditions [9].

Proline is an amino acid, its chemical formula is (C5H9NO2). It has high solubility in water and consists of a pyrrolidine ring linked to a carboxyl group, which makes it effective in building plant proteins, maintaining on the cell membrane, and resisting of oxidative stress [10].

Abscisic acid (ABA) is a water-insoluble plant hormone with the chemical formula ($C_{15}H_{20}O_4$). Produced in the leaves , when exposed to water stress, Abscisic acid (ABA) reduces water loss through transpiration under drought conditions. It also produces proteins that regulate plant growth and thus resist oxidative stress and adapt to environmental stress conditions. Abscisic acid concentration increases under water stress and drought conditions. Abscisic acid plays several roles under water stress conditions, including closing stomata and thus reducing water loss through transpiration, which keeps the plant moist for as long as possible, It also regulates gene expression to help the plant tolerate drought, stimulates root growth to absorb the largest amount of water, and inhibits the growth of stems and leaves to reduce the surface area exposed to water loss, It also stimulates bud dormancy to conserve energy and water resources until plant conditions improve [11].

Chlorophyll is the essential green pigment in plants, algae, and some bacteria. Chlorophyll molecules consist of a porphyrin ring. It contains magnesium at its center and a hydrophobic phytol chain [12].

It is responsible for absorbing light energy and converting it into chemical energy during the process of photosynthesis. It is essential for the continuation of life on Earth, as it produces oxygen and fixes carbon dioxide [13]. Its types are chlorophyll (a), whose chemical formula is C55H72O5N4Mg, and chlorophyll (b), whose chemical formula is C55H70O6N4Mg, It absorbs light with different wavelengths ranging from (460 - 650), Chlorophyll has several functions, including absorbing blue and red light with high efficiency, converting light energy into chemical energy, and acting as a free radical (ROS) inhibitor in cooperation with carotenoids, Chlorophyll also transfers energy to the reaction centers of the photosynthetic system [14].

Water stress leads to a decrease in chlorophyll content in plants due to the closure of stomata and reduced uptake of carbon dioxide needed for photosynthesis, which leads to a decline in photosynthesis and a reduction in energy production, Water deficiency also affects the expression of genes responsible for chlorophyll synthesis, leading to a decline in enzyme activity and disruption of chlorophyll production, thus reducing the chlorophyll content in the leaves. Water stress also leads to a decrease in light absorption, as chlorophyll is the pigment responsible for absorbing light energy. When it decreases, this means a weakening of the plant's ability to absorb the light needed to complete the photosynthesis process [15].

This study was conducted to identify on effect of spraying nano- potassium fertilizer and multi-walled carbon nanotubes (MWCNTs) the content of proline, abscisic acid and chlorophyll content in the mung bean plant (*Vigna radiata*).

Materials & methods:

A factorial experiment was conducted during the 2023-2024 agricultural season according to a Complete Randomized Design (C.R.D). This experiment included three factors with four replications. The first factor is spraying with complex nano potassium fertilizer at three levels (K_0, K_1, K_2) with concentrations (0 g, 2 g, 4 g) of complex nano potassium fertilizer in sequence. The second factor is spraying with multi-walled carbon nanotubes (MWCNT) at two levels, C_0 and C_1, with concentrations of (0 g, 0.1 g) multi-walled carbon nanotubes, respectively. The third factor was represented by irrigation with river water at an amount of 50% of the soil moisture content at field capacity (S_1), and irrigation with river water at an amount of 100% of the soil moisture content at field capacity (S_2) . The experiment included (48) experimental units. Mung bean seeds were planted on 1/7/2023 AD in plastic pots, the capacity of one pot is (25) kg of sandy loam soil, at a rate of (10) plants per pot. The plants were thinned to (3) plants per pot after two weeks from when seedlings emerged. Some physical and chemical properties of the study soil were studied according to the methods mentioned by [16], and shown in Table (1). Add 0.15 ml/L from coloring matter as a spreading material to achieve good spraying until completely wetted on the plant leaves. The control treatment was sprayed with distilled water only. Urea fertilizer was added in two doses, the first after one week from seedling emergence and the second after one week from thinning the plants at a rate of 50 kg ha^{-1}, also added calcium superphosphate fertilizer at once when the seedlings emerged, at a rate of 50 kg ha^{-1} [17]. The plants were harvested on 15/11/2024 AD.

Proline concentration was measured according to the method of Bates et al., (1973). 0.5 g of fresh leaves were crushed with 10 ml of sulfosalicylic acid (3%) in a ceramic mortar. A central electric motor was started at 10,000 rpm for one minute to remove the precipitate. The mixture was filtered through Whathman's No. 1 filter paper, after which 3 ml of the filtrate was mixed with 3 ml of Ninhydrin acid and 3 ml of glacial acetic acid in test tubes and placed in a water bath at 95°C for half an hour. Then the tubes were cooled at laboratory temperature, and 4 ml of toluene was added to them and the mixture was shaken for 20 seconds. After that, the red toluene layer was measured with a spectrophotometer and then the concentration of proline acid was calculated at a wavelength of (520 nanometers) [18].

The concentration of abscisic acid in mung bean was measured according to the method of Nuray and Topcuoglu (2002), where it was completely prepared as used in measuring the acid with a degree of 100 ml of a mixture of methanol, chloroform and ammonium hydroxide and then kept at a resistance of (-20) °C until the matching process was carried out. Then the acidity of the solution is adjusted to pH 2.5 using drops of hydrochloric acid, then 3 ml of Ethyl Acetate is added to complete the extraction process. After that, the optical density of the upper layer is measured to estimate abscisic acid at a wavelength of (263) nanometers. [19].

The chlorophyll content in the plant leaves of the mung bean plant was measured for each experimental unit using the (OPTI-SCIENCES) device located in the laboratories of the College of Education for Pure Sciences, where it was used in the field in the College of Agriculture. Measurements were taken for all the treatments under study, which were (48) treatments, and the measurement was done according to the method of (Reynolds et al., 1998) [20].

The data were statistically analyzed using the statistical program Genstat, [21].

The comparison between the coefficients was at the probability level of 0.05.

Table -1: Some physical and chemical properties of the soil sample used in agriculture .

Adjective		Value		Unity
Soil reaction pH 1:1		7.9		—
Electrical conductivity EC		1.28		Desi Siemens M^{-1}
Organic matter		8.62		gm Kg-1
carbonate minerals		440		gm Kg^{-1}
Organic carbon		5		
Gypsum		1.6		
CEC		13.95		centimole charge kg^{-1}
SAR		0.89		%
ESP		0.053		%
positive dissolved ions	Ca^{2+}	5.5		ml equivalent $liter^{-1}$
	Mg^{2+}	3.8		
	Na^{+}	2.7		
	K^{+}	0.41		
negative dissolved ions	SO_4^{2-}	6.6		
	CO_3^{2-}	14		
	HCO_3^{-}	14		
	Cl^{-}	3.3		
Ready-made nitrogen		14		mg Kg^{-1} soil
Ready-made phosphorus		13.6		
Ready potassium		85.4		
soil texture type		sand	846	gm Kg^{-1} soil
		Alluvial	83	
		Clay	71	
sand Loamy				
Moisture at 10 kPa tension: 9.6%				
Moisture at 33 kPa tension: 5.8%				

analyses were conducted in the central laboratory at the College of Agricultural Engineering Sciences, University of Baghdad.

Results & Discussions:

1. Proline: The results in Table (2) showed the significant effect of water stress on the proline Concentration, the results of the statistical analysis indicated an increase in the concentration of proline when irrigated at a level of 50% of the value of the field capacity of the soil, the highest value was achieved at the (S_1), reaching 78.08 mg kg^{-1}, Compared to the proline value when irrigated at 100% of the soil field capacity value, it reached 66.60 mg kg^{-1} at the (S_2) . Proline increases under stress conditions to balance osmotic pressure and maintain photosynthetic enzymes, thus reducing ROS accumulation under water stress conditions [22].

The effect of spraying with nano potassium on the proline trait was significant, as the results indicated a decrease in its percentage at (K_1) at a concentration of 2 gm of nano potassium, reaching 65.12 (mg kg^{-1}). The potassium levels decreased more at (K_2) with a concentration of 4 gm of nano potassium, reaching 62.12 (mg kg^{-1}) compared to the highest value at (K_0) treated with distilled water only, reaching 83.78 (mg kg^{-1}). This is because nano potassium improves the efficiency of physiological processes in plants and regulates osmotic pressure, thus improving the absorption of water and nutrients to enhance the efficiency of photosynthesis, which leads to a decrease in proline accumulation [23].

The effect of spraying with multi-walled carbon nanotubes had a significant effect on the proline property, as its percentage decreased in treatment (C_1) at a concentration of 0.1 g of nano carbon, reaching 67.33 (mg kg^{-1}) compared to the highest value in treatment (C_0), which amounted to 35.73 (mg kg^{-1}). This is because multi-walled carbon nanotubes (MWCNTs) enhance the activity of the RUBISCO enzyme, which improves growth and thus reduces the plant's need for proline. Carbon nanotubes also increase light absorption and facilitate electron transfer, which increases the production of ATP and NADPH. Thus, the need for proline accumulation decreases, leading to a decrease in proline concentration in plant cells [24] and [25].

As for the interaction between nano potassium and MWCNTs, the K_1C_1 treatment achieved the lowest value for proline, reaching 62.00 (mg kg^{-1}). The proline value decreased further in the (K_2C_1) treatment, reaching 60.00 (mg kg^{-1}) compared to the highest value in the (K_0C_0) treatment, which amounted to 87.57 (mg kg^{-1}). The interaction improves photosynthetic enzymes, electron transport and energy production, which reduces proline accumulation [26].

Table -2: Effect of spraying with nano potassium and multi-walled carbon nanotubes on proline Concentration (mg kg^{-1}) in leaves of *Vigna radiata* plant under water stress conditions.

K * C	Irrigation level compared to soil moisture content at field capacity (%) S		Nano potassium fertilizer concentration (g/L^{-1}) K	Carbon nanotube concentration (g L^{-1}) C
	S_2	S_1		
87.57	78.32	96.80	0	0
68.25	63.80	72.70	1	
64.25	62.70	65.80	2	
80.00	75.40	84.60	0	1
62.00	60.60	63.40	1	
60.00	58.80	61.20	2	
	66.60	74.08	S effect rate	
C effect rate				
73.35	68.28	78.43	0	S * C
67.33	64.93	69.73	1	
K effect rate				
83.78	76.87	90.70	0	S * K
65.12	62.20	68.05	1	
62.12	60.75	63.50	2	

S * K * C	S * K	S * C	K * C	S	K	C	L.S.D 0.05
0.3702	0.2296	0.2554	0.2296	0.1148	0.1406	0.1148	

The table indicated that the binary interaction between nano potassium and water stress had a significant effect on the proline trait, as the proline percentage decreased at K_1 with a concentration of 2 g of complex nano potassium fertilizer and reached 68.05 (mg kg^{-1}), and its percentage decreased further at K_2 with a concentration of 4 g of nano potassium and reached 63.50 (mg kg^{-1}) compared to the highest value at (K_0) which amounted to 90.70 (mg kg^{-1}). The reason for this is that nano potassium improves ionic balance and osmotic pressure, which reduces the need for proline. It also enhances the efficiency of photosynthesis, which reduces the plant's dependence on proline as an antioxidant [27] and [28].

As for the results of the interaction between multi-walled carbon nanotubes (MWCNTs) and water stress, the results indicated a significant effect on the proline property, as the proline percentage decreased at (C_1) at a concentration of 0.1 g of nanocarbon. Its value reached 69.73 (mg kg^{-1}) compared to the highest value at (C_0) which amounted to 78.43 (mg kg^{-1}). MWCNTs increase light absorption and enhance the activity of carotenoids and chlorophyll, thus improving the efficiency of photosynthesis. As a result, the plant does not need proline, which reduces its accumulation in plant cells [29]. This is consistent with what was confirmed by [30].

The results of the triple interaction between potassium nanoparticles, multi-walled carbon nanotubes (MWCNTs) and water stress showed a significant effect on the proline property, as the proline value decreased at the triple interaction ($K_2C_1S_2$) to reach 58.80 (mg kg^{-1}). Compared to the highest value of proline at the triple interaction ($K_0C_0S_1$) which reached 96.80 (mg kg^{-1}), This is because enhances the efficiency of photosynthetic enzymes (RUBISCO, which leads to stimulating the activity of the enzyme ProDH responsible for proline decomposition, and reducing its accumulation [31]. This leads to a decrease in proline, and this was agreed upon by [32].

2 . Abscisic acid (ABA):

The results in Table (3) showed the effect of spraying with nano potassium and multi-walled carbon nanotubes on the property of abscisic acid (ABA) was significant, as the results of the statistical analysis indicated an increase in (ABA) under water stress conditions at a level of 50% of the value of the field capacity of the soil. The highest value was achieved at the (S_1), which reached 36.97 micrograms g^{-1}, compared to the value of (ABA) under irrigation at a level of 100% of the field capacity value of the soil at the (S_2) , which reached 31.81 micrograms g^{-1}. ABA increases under water stress conditions due to the activity of the enzyme cis - Epoxycarotenoid-9 Dioxygenase (NCED) , which is responsible for the synthesis of ABA, which leads to an increase in its concentration under drought conditions. This is in agreement with [33].

Table -3: Effect of spraying with nano potassium and multi-walled carbon nanotubes on the Abscisic acid (micrograms/g) trait in the leaves of *Vigna radiata* plant exposed to water stress.

K * C	Irrigation level compared to soil moisture content at field capacity (%) S 100% S_2	50% S_1	K Nano potassium fertilizer concentration (g/L^{-1})	C Carbon nanotube concentration ($g\ L^{-1}$)
39.36	36.28	42.44	0	0
35.81	33.38	38.24	1	
33.50	30.67	36.45	2	
37.42	34.54	40.26	0	1
30.65	28.54	32.76	1	
29.55	27.42	31.68	2	
	31.81	36.97	S effect rate	
C effect rate				
36.24	33.44	39.04	0	S * C
32.54	30.18	34.90	1	
K effect rate				
38.39	34.43	41.35	0	S * K
33.23	30.96	35.50	1	
31.55	29.09	34.06	2	

K * C S *	S * K	S * C	K * C	S	K	C	L.S.D 0.05
2.111	0.6775	0.7540	0.6775	0.3386	0.4147	0.3386	

Show the table above effect of spraying with nano potassium had a significant effect on the (ABA) trait, as its percentage decreased at (K_1) with a concentration of 2 grams of nano potassium and reached 33.23 micrograms g^{-1}. Its percentage also decreased further at (K_2) and at a concentration of 4 grams of nano potassium, reaching 31.55 micrograms g^{-1}. Compared to the highest value at (K_0) treated with distilled water only, which amounted to 38.39 micrograms g^{-1}, The decrease in abscisic acid when sprayed with nano-potassium is attributed to several mechanisms. These include enhancing water and nutrient absorption, improving cellular water status, and reducing dehydration signals responsible for abscisic acid (ABA) synthesis. Furthermore, inhibition of the 9-Cis-epoxycarotenoid dioxygenase (NCED) gene, which is responsible for ABA synthesis, which leads to a decrease in ABA accumulation [34] and this is consistent with [35].

The results also showed that spraying with multi-walled carbon nanotubes had a significant effect on the (ABA) trait, as its percentage decreased in treatment (C_1) at a concentration of 0.1 g of nano carbon, reaching 32.54 micrograms g^{-1}. Compared to the highest value at (C_0) treated with distilled water only, which amounted to 36.24 micrograms g^{-1}. The reason for this is that MWCNTs inhibit the enzymes responsible for ABA synthesis, such as Abscisic Aldehyde Oxidase 3 (AAO3), and improve the internal water condition and thus maintain the carbon metabolism rate, which leads to a decrease in ABA accumulation [36].

As for the interaction between nano potassium and multi-walled carbon nanotubes (MWCNTs), the treatment (K_1C_1) achieved the lowest value for (ABA), which reached 30.65 micrograms g^{-1}. The value of (ABA) decreased more in the (K_2C_1) treatment, as it reached 29.55 micrograms g^{-1}. , compared to the highest value in the (K_0C_0) treatment, which amounted to 39.39 micrograms g^{-1}, This is because the interaction between nanomaterials inhibits the NCED3 gene, which is the primary catalyst for abscisic acid synthesis, thus reducing the concentration of ABA [37].

The results of the interaction between nano potassium and water stress also showed a significant effect on the (ABA) trait, as the (ABA) percentage decreased at K_1 at a concentration of 2 gm of nano potassium and reached 35. micrograms g^{-1}. The percentage of ABA decreased more at K_2 with a concentration of 4 gm of nano potassium and reached 34.06 micrograms g^{-1} compared to the highest value at (K_0) which amounted to 41.35 micrograms g^{-1} . Potassium improves the delivery of water and nutrientsand improves the condition of cellular water. It also improves water balance and reduces dehydration signals, which leads to a decrease in ROS accumulation, and thus a decrease in ABA accumulation [38].This is consistent with what was confirmed by [39].

The results of the binary interaction between multi-walled carbon nanotubes (MWCNTs) and water stress showed a significant effect on the (ABA) trait, as the (ABA) percentage decreased at C_1 at a concentration of 0.1 g of nano carbon, reaching 34.90 micrograms g^{-1} , compared to the highest value at (C_0), which amounted to 39.04 micrograms g^{-1}. MWCNTs inhibit genes responsible for ABA synthesis such as 9-Cis-epoxycarotenoid dioxygenase (NCED) and Abscisic Aldehyde Oxidase 3 (AAO3), which leads to decreased ABA accumulation [40].

The results of the triple interaction between nano potassium, multi-walled carbon nanotubes (MWCNTs) and water stress showed a significant effect on the (ABA) trait, as the value of (ABA) decreased at $K_2C_1S_2$, reaching 27.42 micrograms g^{-1}, compared to the highest value of (ABA) at ($K_0C_0S_1$), which reached 42.44 micrograms g^{-1}. The reason for this is that the triple interference works to inhibit the NCED genes responsible for ABA synthesis and also works to stimulate the CYP707A genes responsible for ABA decomposition. It also improves the efficiency of water and nutrient absorption to reduce oxidative stress damage, which reduces the accumulation of abscisic acid [41]. This was agreed with [42].

3 . chlorophyll content

The results shown in (Table 4) showed the effect of water stress on the chlorophyll content was significant, as the results of the statistical analysis indicated a decrease in chlorophyll content under water stress conditions at a level of 50% of the field capacity of the soil at the (S_1) , as its value reached 25.62 Spad units, compared to the chlorophyll content value at irrigation at a level 100% of the soil field capacity value at the (S_2) , it reached 32.08 Spad units. This is due to inhibition of the activity of enzymes involved in chlorophyll synthesis, such as Protochlorophyllide reductase, and increased chlorophyll decomposition by the enzyme Chlorophyllase under drought conditions. Chlorophyll content also decreases due to disturbances in carbon metabolism caused by oxidative stress, as the accumulation of ROS causes damage to the green material under drought conditions, thus reducing chlorophyll content [43].

The results of spraying with nano potassium showed a significant effect on the chlorophyll content, as its percentage increased at (K_1) with a concentration of 2 grams of nano potassium and reached 29.50 Spad units, and its percentage increased more at (K_2) with a concentration of 4 grams of nano potassium, as it reached 32.65 Spad units compared to the lowest value at (K_0) treated with distilled water only, as it reached 24.41 Spad units. Nano potassium works to reduce the increased accumulation of ROS in the plant when the plant is exposed to drought conditions by stimulating and activating chlorophyll synthesis enzymes, which leads to protecting the chlorophyll content from damage. This leads to a reduced risk of oxidative stress and thus an increased chlorophyll content and improving the efficiency of carbon metabolism [44]. Nano potassium also regulates the opening and closing of stomata, which improves water use efficiency under stress conditions and thus maintains photosynthetic activity and increases chlorophyll synthesis [45].

Table - 4: Effect of spraying with nano potassium and multi-walled carbon nanotubes on the chlorophyll content of leaves of *Vigna radiata* plant exposed to water stress.

C * K			Irrigation level compared to soil moisture content at field capacity (S)%				K Nano potassium fertilizer concentration (g/L^{-1})	C Carbon nanotube concentration ($g\ L^{-1}$)
			%100 S_2		%50 S_1			
23.03			24.50		21.57		0	0
29.10			33.00		25.20		1	
31.50			36.00		27.00		2	
25.80			27.00		24.60		0	1
29.90			34.00		25.80		1	
33.80			38.00		29.60		2	
			32.08		25.62		S effect rate	
C effect rate								
27.87			31.16		24.59		0	S * C
29.83			33.00		26.66		1	
K effect rate								
24.41			25.75		23.08		0	S * K
29.50			33.50		25.50		1	
32.65			37.00		28.30		2	
S * K * C	S * K	S * C	K * C	S	K	C	L.S.D 0.05	
1.2502	0.8625	0.7754	0.8625	0.3877	0.4748	0.3877		

As the table above indicated, spraying with multi-walled carbon nanotubes had a significant effect on the chlorophyll trait, as its percentage increased in treatment (C_1) with a concentration of 0.1 g of nano carbon and reached 29.83 Spad units, compared to the lowest value in treatment (C_0) with distilled water only, which reached 27.87 Spad units, The reason for this is that MWCNTs protect chlorophyll from damage by improving the absorption of chlorophyll synthesis elements such as nitrogen and magnesium and enhancing the activity of photosynthetic enzymes such as RUBISCO. Which leads to reducing oxidative stress and protecting plastid membranes [46], and nanocarbon also stimulates growth hormones responsible for chlorophyll synthesis such as auxins and gibberellins [47] and [48].

As for the binary interaction between nano potassium and multi-walled carbon nanotubes (MWCNTs), the treatment (K_1C_1) achieved a significant effect, as the chlorophyll value increased to 29.90 Spad units, it increased more in the treatment (K_2C_1) and reached 33.80 Spad units compared to the lowest value in the treatment (K_0C_0) which reached 23.03 Spad units.

The interaction between nanomaterials reduces the activity of the chlorophyllase enzyme, which is responsible for chlorophyll decomposition, leading to increased chlorophyll and improved photosynthesis efficiency, also nano- binary interference activates chlorophyll synthesis enzymes such as Mg-chelatase, and enhancing the absorption of elements necessary for chlorophyll synthesis such as Mg and Fe, which leads to an increase in the chlorophyll content in plant leaves [49].

The results of the interaction between nano potassium and water stress also showed a significant effect on the chlorophyll trait, as the chlorophyll percentage increased at K_1 with a concentration of 2 grams of nano potassium and reached 25.50 Spad units, and increased more at K_2 with a concentration of 4 grams of nano potassium, as it reached 28.30 Spad units, compared to the lowest value at (K_0), which reached 23.08 Spad units under drought conditions. The reason for this is that nano-potassium stimulates the action of proteins that form chlorophyll, which increases the chlorophyll content [50]. Potassium also activates the gene responsible for chlorophyll synthesis, Glutamyl-tRNA reductase 1 (HEMA1) [51].

The results of the interaction between multi-walled carbon nanotubes (MWCNTs) and water stress indicated a significant effect on the chlorophyll property, as the chlorophyll percentage increased at (C_1) at a concentration of 0.1 grams of nanocarbon, reaching 26.66 Spad units compared to the lowest value at (C_0) of 24.59 Spad units. MWCNTs improve the absorption of water and essential elements of chlorophyll and activate enzymes and genes responsible for chlorophyll synthesis, which leads to reducing oxidative stress and thus increasing the accumulation of chlorophyll content [52] and [53]. MWCNTs also improves hormonal balance and protects photosynthetic systems, which is consistent with what [54], confirmed.

The results of the triple interaction between nanopotassium, MWCNTs and water stress also showed a significant effect on the chlorophyll property, the chlorophyll value increased at the triple interference ($K_2C_1S_2$) and reached 38.00 Spad units compared to the lowest chlorophyll value at the triple interference ($K_0C_0S_1$) which reached 21.57 Spad units. This is because the triple interaction improves the efficiency of water and nutrient absorption, and improves the enzymes of chlorophyll synthesis, which leads to an increase in chlorophyll content [55]. The triple interaction also improves the ionic balance through the absorption of some nutrients, including (Mg, K), and regulating of hormones, which leads to an increase in the chlorophyll content. This is consistent with what was confirmed by [56].

Conclusions:

The results of the study showed the following conclusions:

1. Proline and abscisic acid concentrations increase with decreased soil moisture content and plant exposure to water stress.
2. Decrease in chlorophyll content with decrease in soil moisture content and exposure of the plant to water stress.
3. The effect of nanopotassium on the activity of proline and abscisic acid, as their concentrations decreased with increasing spraying levels of complex nanopotassium fertilizer, while the chlorophyll content in plant leaves increased.
4. Multi-walled carbon nanotubes affected the activity of proline and abscisic acid, as decreasing their concentrations when sprayed with nanocarbon, while increasing the chlorophyll content in plant leaves.
5. The two- and three-way interaction of the study factors had a significant effect on the effectiveness of proline, abscisic acid, and chlorophyll content.

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