Egypt. J. Chem. **Vol. 67,** No. 8 pp. 1 - 12 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

IMPACT OF CALCIUM CARBONATE AND CHITOSAN AS SIGNAL MOLECULE ON MODULATING THE NEGATIVE EFFECTS OF DROUGHT STRESS ON PEANUT (*Arachis hypogaea* L.)

A.B. BAKRY[1], D.M. SABRA[1*], A.S.M. YOUNIS[1]and MERVAT SH. SADAK[2]

[1]*Field Crops Research Department, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt*

[2]*Botany Department, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt. .*

Abstract

Signal molecules such as calcium (Ca^{2+}) and chitosan play an essential role in alleviating drought negative impacts on various plants. To investigate the effects of calcium carbonate (Ca^{2+}) and chitosan at concentrations of 0, 20, 40, and 60 mgL^{-1}on growth, some biochemical and physiological aspects, also, seed yield and quality of peanut in water stress. The current work was performed in two field trials at two summer seasons of 2020 and 2021 in the National Research Centre (NRC) experimental farm, Al Nubaryia district, El-Behaira Governorate, Egypt. The results showed that, growing peanut plants under low irrigation water ($I_{75\%}$) resulted in significant reduction in growth criteria (shoot length, branches number /plant, shoot fresh and dry weight/plant), photosynthetic pigments components and endogenous indole acetic acid levels as well as yield components (branches & pods number /plant, pods & seeds yield /plant, biological yield/plant,100-seeds weight, pod & seed yield (kg/feddan), as well as comparing byuntreated plants ($I_{100\%}$). Reversal magnitude was reported when normal irrigation treatment I_{100} was applied. Normal irrigation improved significantly root length, fresh & dry weight as well as total soluble sugars (TSS) and proline contents. Calcium carbonate and chitosan exogenous treatments at various concentrations enhanced peanut growth & yield. Additionally, increased the nutritional contents in seeds such as carbohydrates, protein, and oil percentages. In conclusion, calcium carbonate and chitosan have an improving influence on mitigating negative impacts of drought stress on peanut plant productivity under new sandy soil, exogenous applications of 60 mgL^{-1} $CaCO_3$ or chitosan under water stress increased peanut productivity by 21.1 and 39.4% and saving 25% from irrigation water consumption compare with the control of 100 % well watered.

Keywords: Peanut, drought stress, signal molecules, calcium carbonate, chitosan, growth, seed yield.

Key findings: Calcium carbonate and chitosan foliar application lead to mitigate drought stress through improving the physiological characteristics of peanut, which ultimately reflected on plant productivity and chemical properties of the seeds.

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Introduction

Water deficiency is one of the major environmental stressors that alter plants´ physiology, biochemistry, morphology & molecular variations. To adapt the adverse climatic stresses, plants use various physiological mechanisms. Plant development is significantly hampered by water stress that causes stunting, wilted leaves, chlorosis, and yellowing. Furthermore, water deficiency harms photosynthetic pigments, alters cell structure and function, impairs metabolism, slows down nutrient uptake and transportation rate, increases the amount of energy the plant uses to grow, reduces growth, reduces lifespan, decreases tolerance, and even death (Nan *et al*., 2018). Moreover, Plants have evolved several of defenses against drought stress, including avoiding strategies, and adaptive processes. These systems can increase plant longevity by preventing oxidative damage, likestomata conductivity, osmotic equilibrium, antioxidant generation, also hormone synthesis unaffected by drought stress (Tayade *et al.*, 2018). Peanut (*Arachis hypogaea* L.) is an important oil seed and staple plant grown mostly in semiarid areas of the world (El-Metwally *et al*., 2022). The higher percentage of unsaturated edible oil (48–50%), easily absorbed protein (26–28%), half of the required vitamins, and one-third of the critical minerals make peanut seed more valuable (Bakry*et al*., 2020). According to a recent study, the only abiotic stress, as drought stress that caused the world production of peanuts to decrease by around 6 million tonnes per year (Sadak *et al.*, 2023). Numerous investigations were documented the detrimental impacts of drought on biomass production & pod yield (Bakry *et al*., 2012; Elewa *et al.*, 2017). Various methods are employed for reduction the effects of drought & raise peanut plant tolerance. One of these methods involves applying foliar treatments of various natural

*Corresponding author e-mail: deyaa_nrc@yahoo.com.; (**Deyaa Eldin M. Sabra**).

Receive Date: 28 December 2023, Revise Date: 14 March 2024, Accept Date: 23 April 2024

DOI:10.21608/EJCHEM.2024.255562.9105

substances, such as bio-regulators, vitamins, & nutrient components, like calcium (Ca^{2+}) is one of these nutrients, it serves a micro and multifunction activity in biochemical and physiological processes (Sadak *et al*., 2023a). Calcium is involved in so many crucial processes, including cell membrane stability & cell wall stabilization walls, activity of important enzymes & interactions with phytohormones, it is crucial microelement to growth and development of different crops. Additionally, it acts as a secondary messenger under biotic stressors (Sadak, 2016). Calcium is involved in the regulatory process that activate for adjusting salinity negative effects (Zehra*et al.*, 2012). Calcium gains an important role under stress conditions as it provides stress protection via the regulation of the different biochemical and physiological processes of cell walls. (Nofal *et al.*, 2011; Sadak and Talaat, 2021). Chitosan, among carbohydrate groups made of a glucose cycle with a free amino group, is another natural growth promoter. It is a low-cost, ecofriendly, & biodegradable substance with a variety of uses in agriculture. The primary function of chitosan in agricultural applications is to boost plant immunity and defend crop items towards microbes (Bakhoum *et al*., 2020). There have also been many studies on how chitosan affects plant development, growth, and productivity. Foliar treatments with chitosan enhanced the growth & productivity of sunflower, sweet pepper, and radish plants (Abdallah *et al.*, 2020a and 2020b and Sadak and Talaat, 2021).

Keeping in view, the important beneficial role of calcium and chitosan are reducing the effect of plants under drought stress, the current investigation aims to record the physiological impact of Ca^{2+} and chitosan in alleviating drought adverse influences on the growth and production of peanut crops in sandy soil.

MATERIALS AND METHODS

Experimental procedure: Two field trials were done throughout the summers of 2020 and 2021 in the National Research Centre (NRC) experimental farm, Al Nubaryia district, El-Behaira Governorate, Egypt (latitude 30º 30' 1.4' 'N, longitude 30º19' 10.9" E, and 21 m+mean sea level. Soil samples to a depth of 30 cm were taken before each experiment, and were examined using the established, documented methods of (Carter and Gregorich 2006) organic matter content, a $CaCO_3$ content of 2.4%, an electrical conductivity of 0.13 mhos/cm^3, and the availability of 18.0 ppm of N, 18.0 ppm of P, 104 ppm of K, and 0.05 ppm of Zn). Sand composed 94.7% of the soil, which also had the following properties: a pH of 8.6, 0.8%

The experiments designed in split plot in three replications, where the water irrigation quantities (WIQ) 100% and 75%, were located in main plots and calcium carbonate and chitosan with (0, 20, 40, and 60 mgL^{-1}) concentrations were randomly treated in sub-plots and carried out twice after 30 and 45 days from sowing date. The plot area was 10.5 m^2 consist of five rows (3.5 m length and 60 cm between rows). Variety Gize-6 of the peanut-certified seeds (*Arachis hypogaea* L.) was obtained from the Oil Crops Research Section of the Field Crops Research Institute of the Agricultural Research Center in Giza, Egypt. It was inoculated with the appropriate rhizobium bacteria inoculants shortly before planting, in both seasons, peanut seeds were sown in the first week of May. During the preparation of the seedbed, phosphorus fertilizer in the form of calcium superphosphate (15.5% P_2O_5) was supplied at a rate of 60 kg P_2O_5/feddan. 50 kg/fed of potassium sulphate (48% K_2O) was used. At a rate of 30 kg N/fed, ammonium sulphate (20.6% N), nitrogen fertilizer was supplied in two equal parts: the first half at planting and the second after 30 days after sowing.

Water Irrigation Requirements:

According to Allen *et al.,* (1989) the Penman Monteith equation and crop coefficient were used to determine water quantity needed for irrigation. A sprinkler irrigation system applied an average of 2500, and 1875, m^3 $fed.^{-1}$ of irrigation water every season, (100 % and 75%) respectively, (Keller and Karmeli, 1975).

The following equation was used to determine the irrigation water amounts:

$$IWR = (ET^o * Kc * 4.2) * 1.2$$

Where, IWR: irrigation water requirement (m^3/fed.), Kc: crop coefficient, ET^o: reference evapotranspiration (mlm/ day), 4.2: for feddan, 1.2: leaching requirement.

During the growth season, all relevant cultural practices were adopted uniformly.

Growth parameters: A sample plant was taken after 60 days from sowing to measure some of the morphological parameters such as Shoot length (cm), Number of branches and leaves/plant, shoot fresh and dry weight (g)/plant, Root length (cm), root fresh weight (g)/plant.

Yield parameters: A Sample of five plants was taken in each plot at harvest time (120 days from the sowing date), data on seed yield characters were recorded as follows:

Plant height (cm), Number of branches/plant, Numberof pods/plant, Biological yield/plant (g), Pod yield/plant (g), and Seed yield/plant (g). The Whole plot was harvested and the pods were air dried to calculate: Pod yield (kg/feddan), Seed

yield (kg / feddan), Oil yield (kg/feddan), and protein yield (kg/feddan).

Biochemical estimation:

Photosynthetic pigments: was determined by Lichtenthaler and Buschmann (2001) approach, the total amounts of chlorophyll a, b, and carotenoids of fresh peanut leaves were calculated. 80% acetone was used to grind leaves tissue in a mortar and pestle. Optical densities (O. D.) were measured at 662, 645 and 470 nm by spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). In mgg^{-1} FW, photosynthetic pigment levels were indicated.

Indole acetic acid content*:* Fresh samples were weighed out and extracted three times at 0°C using 85% cold methanol (v/v). The mixed extracts were collected, and then cold methanol was used to dilute them to a specified volume. Afterward, combine 1 ml of the extract with 4 ml of the para-dimethylamino benzoic acid (1 g in 50 ml HCL, 50 ml Ethanol), leaving 60 minutes at 30-40^{0}C. 530 nm was used to spectrophotometrically quantify the developing color (Gusmiaty *et al.,* 2019).

Total phenol content: The extract was prepared as an IAA extraction before being used to 0.5 ml of Folin, shaken, and let to stand for 3 minutes, then each tube was filled with distilled water, agitated, and 1 ml of saturated sodium carbonate before being left to stand for 60 minutes. Using a spectrophotometer, O. D. was measured at 725 nm as indicated by (Gonzalez *et al*., 2003).

Total soluble sugars (TSS): By soaking dry tissue for a whole night in 10 ml of 80% (v/v) ethanol at 25°C with regular shaking & centrifuging at 600g, total soluble carbohydrates (TSS) were extracted. To prepare for the measurement of soluble carbohydrates, the supernatant was evaporated till totally dry, and then dissolved in a known volume of distilled water. TSS was measured by using a SPEKOL Spectrocololourimeter VEB Carl Zeiss by reacting 0.1 ml of ethanolic extract with 3.0 ml newly produced anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in a boiling water bath for ten minutes (Chow and Landhausser, 2004).

Proline: Proline was measured by using the technique indicated (Versluses, 2010). Proline extract, acid ninhydrin, and glacial acetic acid were all added, they were then left for one hour in a boiling water bath and then an ice bath. With the use of a SPEKOL Spectrocololourimeter VEB Carl Zeiss, the absorbance was measured at 520 nm. Using a known concentration of genuine proline, a standard curve was produced

Total carbohydrate: Total carbs were measured according to (Albalasmeh*et. al.,* 2013). A test tube containing a certain weight (0.2–0.5 g) of the dry plant was filled with 10 ml of sulphuric acid (1N). After being sealed, the tube spent the night at 100°C in the oven. Then the mixture was filtered into a 100 ml measuring flask and filled to the proper level with distilled water. A test tube containing an aliquot of 1 ml of sugar solution was treated with 1 ml of 5% aqueous phenol solution and then 5.0 ml of concentrated sulphuric acid to quantify the total sugars using colorimetry. After being vigorously shaken for ten minutes, the tubes were submerged in a water bath heated to 23–30 ºC for 20 minutes. (Shimadzu's UV 1201) spectrophotometer was used to measure the optical density of the generated color at 490 nm.

Protein: Bovine serum albumin as a reference was determined the total protein according to Pedrol and Tamayo (2001). Two grams of material ground in a mortar with five milliliters of phosphate buffer (pH 7.6) were used, and then the homogenate was centrifuged at 8000 rpm for 20 minutes, various samples' supernatants placed in different tubes. After making the volume of each sample in each tube equal to phosphate buffer solution, the extraction was kept in the freezer at 40 degrees Celsius for subsequent analysis, then 30 µl of the extraction samples were removed and mixed individually with 70 µl of distilled water. Each tube's total capacity was now 3 ml. The absorbance at 600 nm measured compared to reagent blank after a 5-minute incubation period at room temperature. The relationship between protein concentration (g) and absorbance (600 nm) was estimated using a standard curve.

Oil contents: According to (Das *et al.,* 2002), the powdered seeds are agitated overnight with isopropanol and chloroform to extract the oil from peanut seeds (1:1), the solvent evaporated. The dissolved total oils were refining by washing with 1% aqueous saline solution after the lipid residue picked up in chloroform: methanol (2:1 v/v) solution and given a folch wash. Chloroform mixed with the pure oil solution was used to rinse the aqueous phases. After the chloroform expelled, the entire batch of pure oil was weighed.

Statistical Analysis

The statistical analysis of split plot design variance done on the data. Given that the trend was similar throughout both seasons, the homogeneity test using Bartlet's test used to integrate the analyses of the two seasons. Using SPSS software, the LSD test was calculated to compare the means at $P < 0.05$. (SPPS Institute Inc. 2008; Steel *et al.,* 1997).

RESULTS

Effect of Water irrigation:

Various morphological aspects (shoot length (cm), number of branches/plant, fresh, and dry weight (g)/ shoot, root length (cm), and root fresh weight (g) in Table (1). The results stated significant differences among the two irrigation quantities in this work. While, water deficit ($I_{75\%}$) significantly reduced shoot length, number of branches/plant, fresh and dry weight / shoot. Furthermore, $I_{75\%}$

increased significantly root length and fresh weight of rootin comparison with control plants. The damaging influences of drought on peanuts grown under sandy soil were illustrated as photosynthetic components suppression. Data in (Table 1) show that, water irrigation quantity (WIQ) $I_{75\%}$ significantly decreased the component of photosynthetic pigments in comparison with plants irrigated by 100% WIQ. Results indicated with 75% WIQ reduced chlorophyll by 10.14%, chlorophyll by 10.44%, carotenoids by 7.10%, and total pigments by 9.76%, The indole acetic acid (IAA) concentration of the peanut leaves was dramatically reduced when irrigation water was reduced from 100% to 75%, although overall peanut phenolic content was significantly enhanced in comparison to unstressed plants. Endogenous IAA content decreased from 59.666 to 40.087 (µg/100g fresh weight) while phenolic contents increased from 48.559 to 59.289 (mg/100 dry weight) under $I_{100\%}$ and $I_{75\%0}$ respectively. The components of the plant defense mechanism discovered in this research were osmoprotectants(total soluble sugars TSS and proline concentrations, as shown in (Table 1). In comparison to control plants ($I_{100\%}$), TSS and prolinelevels of peanut leaves increased significantly when WIQ was reduced to ($I_{75\%}$), however, TSS and proline increased by 21.9% and 28.28%, respectively, under $I_{75\%}$ WIQ. Water irrigation quantities influence peanut yield, its components also nutritional contents in seeds are presented in (Table 1), a water deficit of 75% leads to a significant decrease yield components such as plant height (cm), biological yield /plant (g), number of branches, and pods/plant, pods and seeds yield/plant (g), 100 seeds weight (g), pods and seeds yield (kg/fed) in comparison to $I_{100\%}$ (WIQ) treated plants. The percentages of reductions were 33.10% and 44.38% in pods and seeds yield (kg/fed) compared with full irrigation (I_{100}). Regarding to the nutritional seeds contents such as carbohydrates %, oil % and protein %. Water deficit caused a significant decrease in the above mentioned parameters in comparison to fully irrigated plants ($I_{100\%}$), (Table 1). The reductions in percentages were 9.96, 5.96, 11.22, 47.76, and 50.87% in the percentage of carbohydrates, protein and oil, in addition to oil yield and protein yield respectively in comparison to fully irrigated controls.

Effect of foliar treatment of $CaCO_3$ and chitosan:

Regarding the foliar application of different signaling molecules $CaCO_3$ and chitosan with different levels of influence on the morphological characteristics of peanut plant, our data of Table (2) stated that either $CaCO_3$ or chitosan different concentrations (20, 40, and 60 mgL^{-1}) led to a significant increments of all studied morphological characteristics when compared by control plants (0). Furthermore, 40 mgL^{-1} of either $CaCO_3$ or chitosan was the most effective treatment on the morphological characteristics, except for branches number/also, shoot fresh and dry weight the most effective $CaCO_3$,treatment was 60 mgL^{-1}, and root length, the level was 20 mgL^{-1}. While chitosan with 60 mgL^{-1} was most effective in shoot fresh weight and root length as compared with the other levels (Table 2). The obtained results in Table (2) stated that, exogenous treatments with $CaCO_3$ or Chitosan with (20, 40 and 60 mgL^{-1}), level causeda significant increase in photosynthetic pigments constituents comparison to the untreated plants. Chitosan treatments were more effective than $CaCO_3$, where caused increase in different photosynthetic pigment. Moreover, results clearly indicated that, 40 $mggL^{-1}$ was greater than other used levels in improving IAA or phenolic contents (Table 2). The effect of the used antitranspirant compounds $CaCO_3$ or Chitosan with different concentrations on TSS and proline contents of peanut plants are stated in (Table 3). $CaCO_3$ or Chitosan caused a significant gradual increase in TSS or proline contents especially at 40 mgL^{-1} it shows the highest increases compared to the contents of TSS and proline which decreases (Table 2). It is clear that; Chitosan with 40 mgL^{-1} was more effective compared with other treatments, it caused 17.80% and 33.87% in TSS and proline contents, while, $CaCO_3$ caused 12.46% and 25.95%, respectively compared with control. Variations of seed yield, its related characters and nutritional value in peanut seeds treated by different concentrations of $CaCO_3$ or Chitosan with (0, 20, 40 and 60 mgL^{-1}) are indicated in (Table 2). Results revealed that, the exogenous treatments affected marked and a significant improves in different yield components such as total carbohydrates%, protein%, and oil%, in additional, oil and protein yields (kg/fed). The obtained results showed that 40 mgL^{-1} of either $CaCO_3$ or Chitosan was superior on the other used levels on increasing most of yield, its components and different nutritional value studied. Except Chitosan treatment with 60 mg/L^{-1} gave the highest pods and seed yields (kg/fed) in comparison to other levels. L^{-1} was the impacted treatment comparison to other tested levels of $CaCO_3$ or Chitosan. The variations in endogenous IAA and phenolic contents of peanut as affected by various $CaCO_3$ or Chitosan (20, 40 and 60 mgL^{-1}) exogenous treatments are showed in Table 2. Foliar treatment of either $CaCO_3$ or Chitosan with different levels increased significantly IAA and phenolic levels in comparison to control. The treatment of 40 m

Table 1. Impact of water irrigation quantities on peanut plant grown in sandy soil conditions. (Combined Data of two season 2020 and 2021)

Characters		Water irrigation quantities (m3/fed)		LSD 5%
		100 %	75 %	
Shoot length (cm)		32.38	24.05	0.97
No. of Branches/ plant		10.71	6.00	0.45
Shoot Fresh wt. (g)		96.18	43.31	2.36
Shoot Dry wt. (g)		40.05	28.19	1.47
Root Length (cm)		13.00	15.95	0.43
Root Fresh wt. (g)		1.87	2.28	0.10
Chlorophyll a	(µg/100g fresh weight)	976.46	877.45	1.72
Chlorophyll b		654.92	586.55	1.98
Carotenoids		304.00	282.41	1.28
Total Chlorophylls		1935.38	1746.41	2.73
IAA (µg/100g fresh weight)		59.666	40.087	0.25
Phenols (mg/100g fresh weight)		48.559	59.289	0.28
TSS (mg/100 g dry weight)		24.858	30.302	0.103
Proline (mg/100 g dry weight)		43.394	55.666	0.161
Plant Height (cm)		44.62	36.76	1.23
Biol. Yield/ Plant (g)		122.08	52.27	3.61
No. of Branches /Plant		12.71	7.95	0.55
No. of Pods /Plant		50.29	28.33	1.37
Pods Yield /Plant (g)		67.06	31.34	5.05
Seed Yield /Plant (g)		51.28	20.07	2.33
100 Seeds wt. (g)		67.52	41.76	2.48
Pod Yield (kg/fed)		1126.05	753.30	7.53
Seed Yield (kg/fed)		701.91	390.42	6.33
Total carbohydrates%		15.92	14.35	0.52
Oil%		43.76	41.15	0.41
Protein %		17.65	15.67	0.26
Oil yield (kg/fed)		308.20	160.99	4.16
Protein yield (kg/fed)		124.59	61.21	2.11

Table 2. Impact of $CaCO_3$ or Chitosanat (0, 20, 40 and 60 mg/L) on peanut plant grown in sandy soil conditions (Combined Data of two seasons 2020 and 2021)

Characters		Control	CaCO3 (mg/L)			Chitosan (mg/L)			LSD 5%
			20	40	60	20	40	60	
Shoot length (cm)		17.83	28.17	30.33	30.33	29.83	32.67	28.33	2.17
no. of Branches / plant		5.50	7.33	8.17	9.50	8.50	9.50	10.00	0.94
Shoot fresh wt. (g)		40.09	58.32	72.91	73.30	71.00	93.91	78.68	4.69
shoot dry wt. (g)		19.40	30.70	38.65	40.17	33.02	43.93	32.95	2.89
Root length (cm)		10.33	16.83	15.83	15.17	13.17	14.50	15.50	1.83
Root Fresh wt. (g)		1.47	2.21	2.40	2.05	1.97	2.30	2.13	0.18
Chlorophyll a	(µg/100g fresh weight)	878.45	915.90	945.73	921.55	935.67	965.91	925.49	0.88
Chlorophyll b		578.64	618.93	638.05	619.68	629.85	637.34	622.68	3.48
Carotenoids		278.63	288.22	300.60	286.33	295.81	306.08	296.77	2.37
Total Chlorophyll		1735.71	1823.04	1884.38	1827.56	1861.33	1909.33	1844.94	4.01
IAA (µg/100g fresh wt.)		36.993	47.245	53.415	47.708	50.305	58.550	54.923	0.50
Phenols (mg/100g fresh wt.)		41.338	49.455	58.468	52.503	53.788	63.330	58.588	0.76
TSS (mg/100 g dry wt.)		25.155	26.908	28.290	27.038	28.140	29.633	27.898	0.258
Proline (mg/100 g dry wt.)		41.323	46.733	52.045	48.808	49.643	55.318	52.845	0.402
plant height (cm)		28.50	39.67	44.33	43.83	43.83	44.67	40.00	2.06
Biological yield/ plant (g)		44.59	64.93	79.45	94.80	84.46	133.48	108.53	8.54
no. of branches /plant		6.67	9.50	9.33	11.33	11.00	12.83	11.67	1.60
no. of pods /plant		26.33	34.00	45.33	38.17	38.83	47.33	45.17	4.55
pod yield /plant (g)		21.82	42.44	47.61	33.73	67.10	66.63	65.06	9.65
seed yield /plant (g)		14.34	26.33	34.60	25.13	49.28	51.59	48.47	4.04
100-seeds wt. (g)		39.38	44.08	63.77	50.98	61.64	62.43	60.21	4.18
Pod yield (kg/fed.)		532.15	785.02	939.72	1093.20	942.65	1074.10	1210.88	13.79
Seed yield (kg/fed.)		306.71	488.82	533.65	686.64	483.79	555.77	767.76	8.19
Total Carbohydrates %		14.33	15.13	15.61	14.88	15.35	15.69	14.97	0.17
Oil %		41.27	41.88	42.76	42.33	42.39	43.58	42.99	0.05
protein		15.78	16.54	17.25	16.60	16.44	17.25	16.74	0.03
Oil yield (kg/fed.)		127.74	206.81	229.96	294.46	206.31	243.90	332.97	12.44
Protein yield (kg/fed.)		49.07	82.19	93.69	115.84	80.46	97.79	131.26	5.13

Table 3. Impact of $CaCO_3$ and Chitosan (0, 20, 40 and 60 mg/L) and two irrigation levels (100 % to 75 %) on seed, oil and protein yields and their components of peanut plants grown in sandy soil conditions. (Combined Data of two seasons 2020 and 2021).

IWR %	Treatments (mg/L)		Plant Height (cm)	Bio. Yield/ Plant (g)	No. of Branches /plant	No. of Pods /Plant	Pods Yield /Plant (g)	Seed Yield /Plant (g)	100-Seeds wt. (g)	Pod Yield (kg/fed.)	Seed Yield (kg/fed.)	Oil Yield (kg/fed.)	Protein Yield (kg/fed.)
100	Control		37.3	64.2	7.3	35.3	33.6	21.87	42.5	656.0	405.7	172.20	66.79
	CaCO3	20	41.3	97.7	11.7	42.3	52.8	41.97	53.8	889.0	659.8	284.39	114.26
		40	46.7	110.3	11.7	53.7	67.0	50.37	88.6	1141.1	709.6	310.60	128.97
		60	47.0	143.6	15.3	52.3	44.2	34.95	66.2	1356.8	886.0	392.05	155.31
	Chitosan	20	47.7	116.7	12.7	51.0	92.9	70.94	73.7	1106.7	581.9	253.89	101.28
		40	48.0	178.7	15.3	60.0	84.9	69.22	72.8	1329.5	702.1	313.98	130.26
		60	44.3	143.3	15.0	57.3	93.9	69.65	75.0	1403.3	968.3	430.26	175.26
75	Control		19.7	25.0	6.0	17.3	10.0	6.81	36.2	408.3	207.6	83.28	31.36
	CaCO3	20	38.0	32.2	7.3	25.7	32.1	10.68	34.4	681.0	317.9	129.24	50.12
		40	42.0	48.6	7.0	37.0	28.2	18.83	38.9	738.3	357.5	149.32	58.41
		60	40.7	46.0	7.3	24.0	23.3	15.31	35.7	829.6	487.3	196.87	76.36
	Chitosan	20	40.0	52.2	9.3	26.7	41.3	27.62	49.6	778.6	385.6	158.72	59.64
		40	41.3	88.2	10.3	34.7	48.3	33.95	52.0	818.7	409.7	173.83	65.32
		60	35.7	73.7	8.3	33.0	36.2	27.29	45.4	1018.5	567.2	235.69	87.27
LSD 5%			3.25	9.55	1.46	3.63	13.36	6.18	6.57	19.92	7.23	3.45	2.16

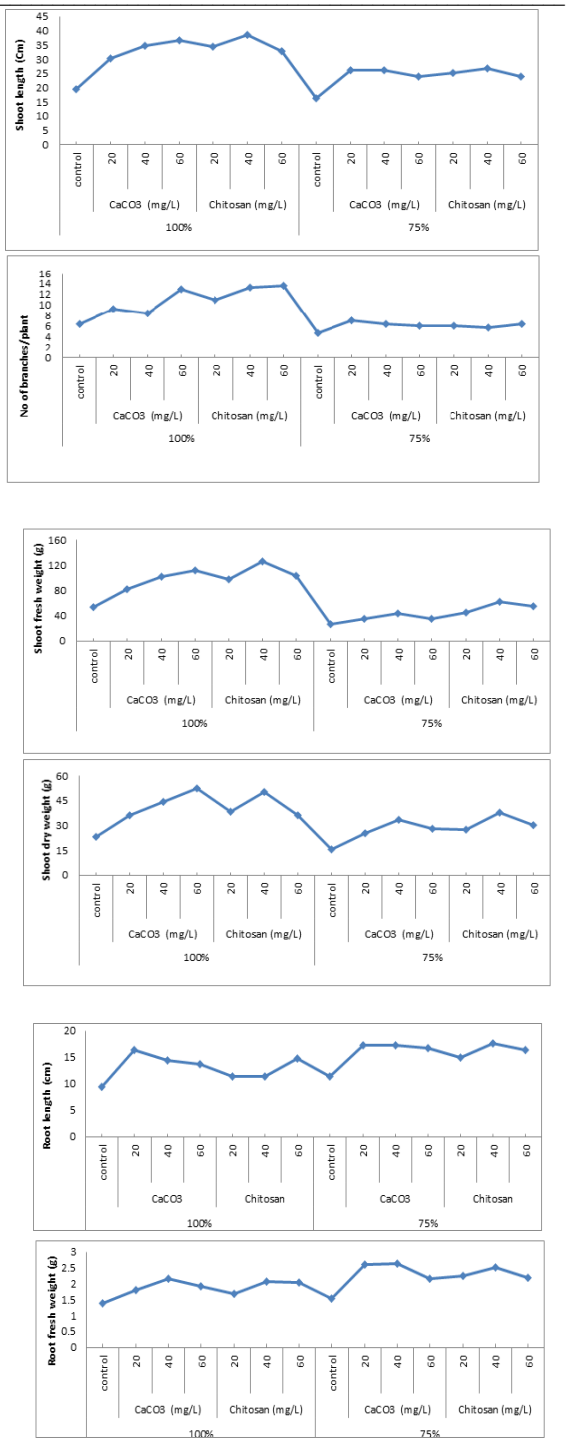
Effect of interaction of water irrigation with foliar treatment of $CaCO_3$ and chitosan:

Regarding to interaction influence of exogenous application with the two signaling molecules $CaCO_3$ and Chitosan with different levels on peanut plants growing under different water irrigation levels $I_{100\%}$ and $I_{75\%}$. The obtained results of (Figure. 1) clearly showed that $I_{75\%}$WIQ decreased significantly different morphological characteristics of the peanut plant. Meanwhile, various concentrations of either $CaCO_3$ and chitosan (20, 40 and, 60 mgL^{-1}) significantly improved the morphological characteristics of peanuts compared with unstressed plants under $I_{100\%}$. Furthermore, under $I_{75\%}$ different treatments of $CaCO_3$ and Chitosan could mitigate the stress influences caused by drought on different growth criteria compared with control. On peanut plants, the harmful impacts of drought-induced stress were characterized as a reduction of photosynthetic contents. Chlorophyll a, chlorophyll b, and carotenoids concentrations, and total pigments, were all significantly reduced when irrigation water was decreased (Figure. 2). Various levels of $CaCO_3$ and Chitosan foliar treatments on peanut plants led to a significant increase in a number of the components that make up the photosynthetic pigment under the two irrigation levels that were utilized (normal 100% and drought 75%). Data also, demonstrated that, at both water irrigation levels, foliar treatment with 40 mg of either $CaCO_3$ or Chitosan increased Chlorophyll a, chlorophyll b, carotenoids, and total pigments of peanut plants more effectively than control (I_{100} and I_{75}). Indole acetic acid (IAA) concentrations dropped significantly when irrigation water was reduced from 100 % to 75 %, although overall phenolic contents significantly increased. Furthermore, findings in (Figure. 3) demonstrated that different concentrations of $CaCO_3$ or chitosan applications significantly improved IAA endogenous levelsand produced greater significant increments of phenolic level of peanut plants in comparison to control. Water irrigation quantities (I_{100} and I_{75}) and $CaCO_3$ or chitosan applications (0, 20, 40 & 50 mgL^{-1}), varied $CaCO_3$ or chitosan foliar applications significantly increased IAA and phenolic levels of peanut in the two water stress (Figure. 3). The plant resistance system components for alleviating the negative influence of drought are known as osmolytes (total soluble sugars (TSS) and proline); (Figure. 4). Peanut contents of TSS and proline improved significantly due to lowering WIQ (I_{75}) compared with normal irrigation. $CaCO_3$ or chitosan exogenous treatment significantly increased gradually the above-mentioned osmolytes contents. Under I_{100} and I_{75} WIQ irrigation, treatment with $CaCO_3$ or chitosan highlighted these different levels elevated TSS and proline contents compared to control. As shown in Table 3 and Figure 5, water deficit at 75% induced a significant decrease in yield and its components, while, $CaCO_3$ and chitosan application caused a significant increase in different yield parameters of the peanut plant. However, treatment with $CaCO_3$ and chitosan alleviated the negative effects of drought while also, increasing seed yield

productivity and its related constituents of peanut plants.

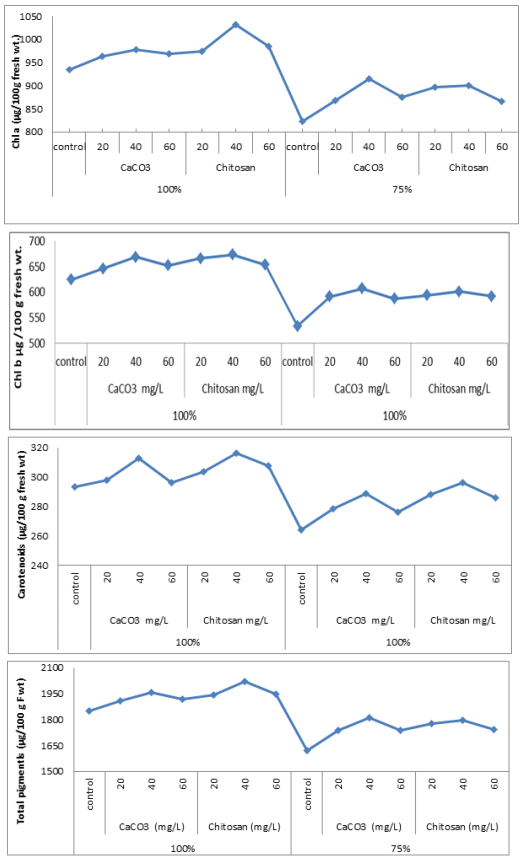
DISCUSSION

Drought is among the most significant abiotic destruction variables that inhibit growth, and production of many plant species as peanut. In this study, drought stress exhibited a negative effect on productivity parameters Tables (1 & 3 and Figure 1). These findings support the information collected on different plants by Sadak and Bakry (2020); Sadak *et al*., (2020a); Abd –Elhamid *et al.*, (2021); Bakhoum *et al.*, (2022). Drought stress inhibits cell division and growth by down regulation of cycling-dependent protein kinase expression, which results in a decrease in the branches No of peanut (Table 1). Additionally, water deficiencies cause stomatal closure, which is mediated by abscisic acid and affects several of metabolic processes, including a decrease in tissue water content (Sadak, 2016). As a result, plant growth and development are inhibited. Furthermore, an increase in transpiration rate over water received by the plant may be the cause of this negative effect shown in the tissue of stressed peanuts. In contrast hand, water deficit enhanced root growth, and fresh and dry weight. According to (Bakoum and Sadak, 2022), plants cultivated in severe water shortage circumstances had more root biomass than controls. However, peanut avoids the effects of reduced cell leaf area or fallen leaves due to their deep, thick root system. Deeper roots in particular were demonstrated as advantageous in plant survival in water stress. In addition, the first signs of dryness caused peanut plants to grow, longer roots and produce more fresh weight as peanut cells started to redirect assimilates from the stem and use them for root growth to improve water uptake (Sadak *et al*., 2019). Water deficiency led to a decrease in photosynthetic pigment constituents (Table 1 and Figure 2), which discusses to chlorophyll break down as a result of ROS negative impact (Sadak *et al.*, 2020b). Conferring to other reviews on some plant species, photosynthetic pigments reduce in response to drought (Sadak *et al*., 2019). The accumulation of ROS during water deficit inhibited the formation of many components of photosynthetic pigments and reduced the activity of the photosynthetic electron transport chain in apples. Additionally, this decreased impact is caused by diffusion restriction on the closure of stomata and decreased rubisco concentration, showing co-dominance and biochemical restriction of the stomata under drought circumstances, which can alter CO_2 assimilation rates (Sadak and Bakry 2020).



LSD at 5%: for Shoot length: 2.58, Number of branches/plant: 1.18, Shoot fresh weight: 6.24, shoot dry weight: 3.89, root length: 1.15 and root fresh weight: 0.25

Figure 1.Impact of $CaCO_3$ and Chitosan (0, 20, 40 and 60 mg/L) and two water irrigation quantities (100% and 75%) on growth criteria of peanut plants grown in sandy soil conditions (Combined Data of two season 2020 and 2021)

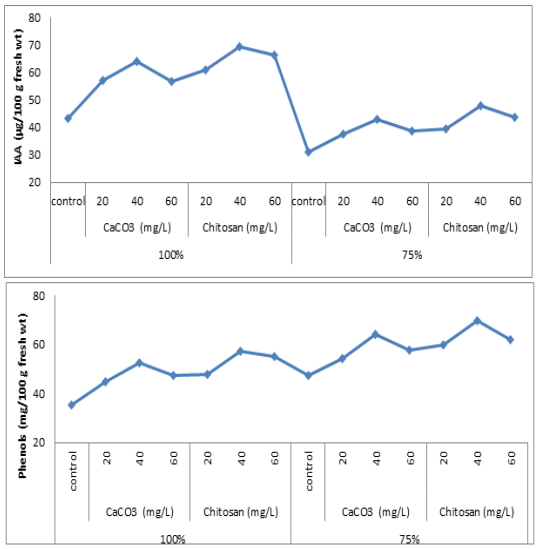


LSD at 5%: for chlorophyll-a: 4.55, chlorophyll-b: 5.23, carotenoids: 3.39 and total pigments: 7.23

Figure 2. Impact of $CaCO_3$ and Chitosan (0, 20, 40 and 60 mg/L) and two water irrigation levels (100 % to 75 %)on photosynthetic pigments (µg/100 g fresh weight) of peanut plants grown in sandy soil conditions. (Combined Data of two seasons 2020 and 2021).

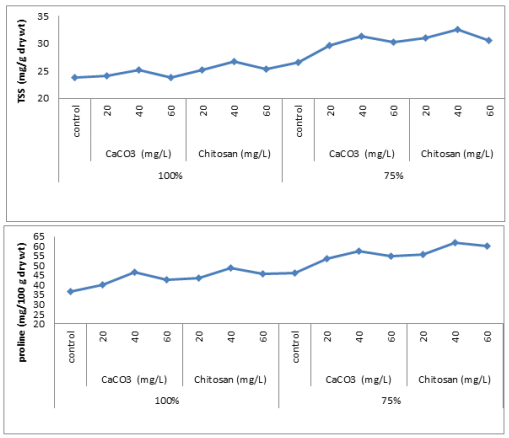
A known naturally occurring auxin, indole-3-acetic acid (IAA), is a bio-regulator produced by plants, fungi, and bacteria. IAA is critical in controlling plant growth and development. Reduced irrigation water use (I_{75}) reduced vegetative development characteristics were associated with lower indole acetic acid (IAA) concentration of peanuts (Table 1 and Figure. 3). Improved IAA breakdown and transformation to an inactive form may be responsible for the decreased IAA content under drought.

In response to drought stress (I_{75}), peanut leaf phenolic content increased (Table 1 and Figure 3). This rise in phenol levels has the power to lessen reduced effects of drought. Plants produce greater phenolics because of many biochemical systems being disturbed by water shortage. Increased contents of ROS are usually accompanied of variations in net carbon acquisition in plants under drought stress, and these changes have a substantial influence on the signaling methods of secondary metabolites, notably polyphenols (Sachdev *et al.*, 2021).



LSD at 5%: for IAA: 0.65, phenols: 0.73

Figure 3. Impact of $CaCO_3$ and Chitosan at (0, 20, 40 and 60 mg/L) and two water irrigation levels (100 % to 75 %) on IAA (µg/100 g fresh weight) and phenols (mg/100 g fresh weigh) of peanut plants grown in sandy soil conditions. (Combined Data of two seasons 2020 and 2021)

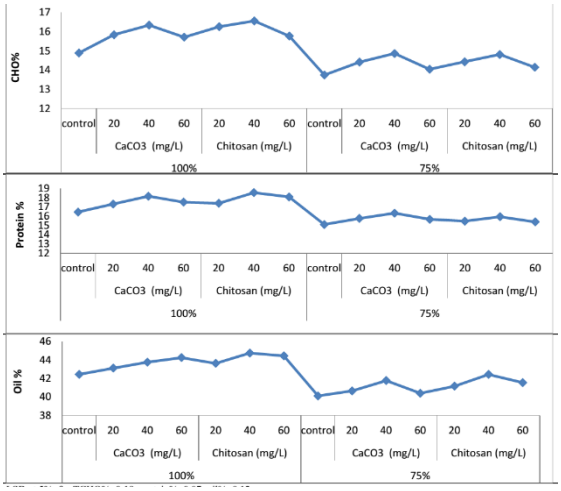


LSD at 5%: for TSS: 0.273, proline: 0.425

Figure 4. Impact of $CaCO_3$ and Chitosan (0, 20, 40 and 60 mg/L) and two irrigation levels (100 % to 75 %) on TSS and proline (mg/100 g dry weigh) of peanut plants grown in sandy soil conditions. (Combined Data of two seasons 2020 and 2021)

Additionally, phenolics are essential antioxidants and scavengers of ROS. Osmolytes accumulation is among plants' powerful defensive mechanisms against environmental challenges, especially drought stress. Together with $CaCO_3$ and chitosan, these suitable solutes assist in maintaining cell turgor by adjusting the osmotic pressure and functioning as ROS scavengers (Sadak *et al.*, 2020). In the present investigation, peanuts subjected to water stress had increased levels of

osmolytes, such as proline and TSS (Table 1 and Figure 3). The results of this study support those by Elewa *et al*., (2017); Bakhoum *et al*., (2022) they found quinoa and lupine plants, respectively, improved TSS and proline during water stress, and



this accumulation is positively linked with resistance to drought stress.

LSD at 5%: for TCHO%: 0.19, protein%: 0.07, oil%: 0.12

Figure 5. Impact of $CaCO_3$ and Chitosan (0, 20, 40 and 60 mg/L) and two water irrigation levels (100 % to 75 %)on carbohydrate, protein and oil percentages of peanut seeds plant grown in sandy soil conditions. (Combined Data of two seasons 2020 and 2021)

Drought stress resulted in considerable reductions in the carbohydrate, protein, and oil produced by peanut seeds, which harmed their nutritional value. In other plant species, similar results were also found by Sadak *et al*. (2015) and Rady *et al*., (2015), carbohydrate, protein, and oil levels of some plant species, respectively, had reduced. The declines in carbohydrate content are primarily caused by a decline in growth indicators and photosynthetic pigments (Table 1 and Figure 2). Because of their direct connection to physiological activities including photosynthesis, translocation, and respiration, also changes in carbohydrates in the seeds produced are extremely important (Sadak and Ramadan, 2021). Stress from drought lowers the amount of chlorophyll in leaves, which lowers photosynthetic activity. As a result, less carbohydrates may accumulate in mature leaves and fewer carbohydrates may be transported from the leaf to maturing seeds (Elewa *et al*., 2017; Abdallah *et al.,* 2020a 2020b and 2020c). Regarding the improvement of peanut growth and production $CaCO_3$ has been shown to have (Table 2 and Figure1) (Table 2 and Figure 2). Similar findings in wheat were seen (Sadak *et al*., 2020b; Sadak and Talaat, 2021). The promotive impact of Ca^{2+} might be due to its position as a vital secondary messenger involved in signaling pathways connected to several defensive mechanisms brought on by abiotic stress (Tuteja, 2009). Additionally, Ca^{2+} can improve plant water status by protecting membranes from lipid peroxidation and oxidative stress brought on by drought this is because calcium is a crucial component of plant cell walls and is essential for cell growth and division. Regarding photosynthetic pigments, it has been demonstrated that foliar Ca^{2+} treatments improved the photosynthetic pigment of peanut leaves on a variety of plant species, including pepper (Yang *et al.,* 2016) and wheat (Sadak *et al.,* 2020b). These positive effects might be linked to Ca^{2+} ability to protect cellular structures from dehydration damage by preserving osmotic stability of the cytoplasm in plants (Bakry *et al.*, 2012; Sadak and Talaat, 2021). Under either typical irrigation or drought regimes, different Ca^{2+} concentrations led to significant increases in endogenous IAA. $CaCO_3$ has been shown to help wheat plants produce more IAA, according to Sadak and Talaat (2021). These increases might be the result of $CaCO_3$- induced impact, which significantly improves IAA production and transport while decreasing IAA oxidase activity.

Treated pea plants with calcium caused the accumulation of phenolic compounds, and Sharma *et al*. (2020) discovered that the amount and the activity of phenolic used in its metabolism dramatically improved with Ca treatment, demonstrating the importance of calcium's effect on phenolic content.

Compared to controls, TSS and proline levels significantly increased when $CaCO_3$ at various doses were applied in this investigation. The previous investigations by Sadak *et al.* (2020b); Sadak and Talaat (2021) confirmation that the obtained results on TSS and proline of peanut plants. These increases may support plants in controlling their cells' osmotic potential, which would enhance water uptake and transport during water deficit. Additionally, proline protects cell components, and various enzymes from oxidative damage, also acts as a free radical scavenging (Rady *et al*., 2015).

It was knew that chitosan foliar application enhanced growth and yield by making plants more resistant to various types of stress, which relevant to the promotional impact of applying different concentrations of chitosan to the leaves of peanut plants to improve growth and productivity, in addition to being a very efficient biomolecule, chitosan also regarded among the bio-regulators and signal molecules, farther this promotion function improved nitrogen transportation in leaves & the activities of nitrogen metabolism enzymes, which led to an increase in growth and development (Ramadan *et al*., 2020;Sultana *et al*., 2017). Treatment with chitosan causes changes in the regulation of protein metabolism with an increase in different storing proteins and hormone metabolism, as well as the over-expression of

genes used in photosynthesis. As a result of the chitosan treatments, more pigments used in photosynthesis produced. These improvements in cytokinin levels that encouraged chlorophyll production or the enhanced availability of amino compounds released from chitosan might be the causes of these increases. According to Behboudi *et al.* (2018) barley photosynthetic pigments improved by chitosan application. Additionally, various chitosan applications may enhance the IAA levels in peanut plants. These improvements might carried by chitosan induction of auxin-related gene expression, increased IAA production and translocation, and lowered IAA oxidase activity (Bakhoum *et al*., 2020; Amany *et al*., 2020).

The TSS and proline levels of peanut plants treated with chitosan clearly increased, according to data reported in (Table 2), pigments may causes of the promoting impact of chitosan treatment on the carbohydrate contents of seeds produced (Table 2). These increments in the protein and carbohydrate level might be result of enhanced photosynthetic activity (*Arachis hypogaea* L.) that improved the transport of sugars from leaves to produced seeds.

CONCLUSIONS

According to the results, it could be concluded from this study that the experimented treatments of $CaCO_3$ or chitosan applied had an important effect on the water stressed of peanut plants grown under sandy soil. The results showed that the seed yield productivity decreased when the plants subjected to drought stress, while the exogenous applications of 20, 40, or 60 mgL^{-1} $CaCO_3$ increased seed yield by 62, 46.1 and 67.7% under well watering, while under water stress it increased by 53.1, 47.2 and 78.2 % compare with control, where chitosan treatments at (20, 40, and 60 mg/L) increased seed yield/ fed by 19.9, 50.9 and 80.1 % under well watering, while under water stress the seed yield/fed increased by 36.5, 52.4 and 87.8 % compare with control. In conclusion, exogenous applications of 60 mgL^{-1} $CaCO_3$ or chitosan under water stress increased peanut productivity by 21.1 and 39.4% and saving 25% from irrigation water consumption compare with the control of 100 % well watered, this was due to photosynthetic pigment components, IAA, and osmoprotectants increased.

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