



## The Synergistic Effect of Aspirin and Brassinolide Hormone in Some Enzymatic and Non-enzymatic Antioxidants of Caraway Plant (*Carum carvi*) Exposed to Water Stress

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### Abstract

The experiment was conducted to study the effect of water stress (5,10,15) days and interaction with spraying Aspirin (ASA) (0,5,10) mg.L<sup>-1</sup> and brassinolide (BL) growth regulator (0,0.5,1) mg.L<sup>-1</sup> on some biochemical and enzymatic and non-enzymatic antioxidants (Salicylic acid concentration, Carotene concentration, Phytosterols, Glutathione Peroxidase (GPX) Enzyme Activity, Glutathione (GSH) concentration) and the Free radicals scavenging activity for Caraway plant *Carum carvi*, the experiment performed with factorial Randomized Block Complete Design (RBCD), with three replicates and 81 experimental units the area of one of them 2m<sup>2</sup>.

The Results Showed:

- The effect of severe water stress for 15 days was negative effect and decreased the means of studying characters.
- Increasing in the GPX activity and GSH means under Water stress and spraying with (Aspirin, Brassinolide)
- The means of free radicals scavenging activity decreased with water stress.
- The effect of aspirin and brassinolide was positive to increase of study characters with the influence of concentrations under water stress.
- The role of aspirin and brassinolide were positive to decrease of passive effect of water stress also the triple interaction between the severe stress at 14 days and then spraying the aspirin and hormone was positive to decrease the effect of severe stress.

**Keywords:** (Water stress, Aspirin, Brassinolide, *Carum carvi*).

### Introduction

Salicylic Acid (Aspirin Derivative) is one of the newly discovered endogenous plant hormones. It has an important and effective role in stimulating plants to resist biological and environmental stress, including water stress. Its most important physiological role is to inhibit ethylene synthesis and control the movement of stomata and has an inhibition effect to the efficacy of Abscisic acid (ABA)[1] and has the ability to control the metabolism of the amino acid and protect by resistance Systemic acquired resistance

(SAR) [2] aspirin can be important non-enzymatic antioxidants and which have a role in the scavenging Oxygen Species (ROS)[3], Steroid hormones are known to be specialized only in animals and are responsible for many animal biologic activities and very low concentrations with high physiological effect[4]. However, the idea of steroid hormones within the plant metabolites was not included and was excluded because the steroid hormones are secreted by special glands, The World Grove

in 1979 discovered the presence of steroid hormones in Canola (*Brassica napus* L) plant one of Brassicaceae family brassinolide was isolated from the pollen and that the name of the brassinosteroids was derived from the Latin [5]. Brassinosteroid has been shown to be real phytosterols with an effective role with very low concentrations [6]. Recently, their effects are similar to those of animal steroids. The treatment of *Eruca Sativa* from the Brassicaceae family has an effect on increasing male hormone levels [7].

Medical plants are a great economic resource that cannot be underestimated as they are versatile and the most important in the pharmaceutical and drug industry. Caraway is one of the aromatic medicinal plants [8]. It contains a number of essential oils, which are secondary metabolites as well as many other compounds. Recent studies have shown that the plant has a natural anti-oxidant capacity and is against the most important and most serious diseases are cancer [9].

The properties of the active compounds of caraway is the regulation of sugar in the blood and substitute for a number of drugs organized for sugar as tests have shown an increase in the release of insulin in the blood as well as being an organizer of the functions of liver and pancreas [10] the water stress is a global problem because lack of water resources with economic impacts and large-scale social repercussions [11].

## Materials and Methods

The experiment was carried out in the gardens and laboratories of Al-Farahidi University for the 2017-2018 season located at latitude 33.40 and longitude 44.40 and at 23 meters above sea level.

The soil of the garden was prepared and its chemical and physiological properties were estimated according to the method described by [12] as in Table (1), supplemented with the addition of NPK 15:15:15 and at 100 kg/ha-1 [13].

The experiment was designed according to the Randomize Complete Block (Design) (RCBD) design as a global experiment ( $3 \times 3 \times 3$ ). The experiment was divided into three replicates each containing 27 experimental units. The experimental unit area ( $2 \text{ m}^2$ ).

Process of planting the seeds (local variety) was done on 11/11/2017 after sifting and testing the proportion of germination 98%.

Water stress coefficients

The duration of water stress was determined by the method of blocking irrigation

- Irrigation every 5 days (control treatment).
- Irrigation every 10 days.
- Irrigation every 15 days.

Prepare the standard aspirin and brassinolide solution by dissolving 1 g of each powder in 1 liter of distilled water and then 5 and 10 mg.-1 concentration for aspirin and 0.5 and 1 mg L<sup>-1</sup> for brassinolide

## Biochemical Studies

- Estimation of Salicylic acid concentration ( $\mu\text{g. gm}^{-1}$ ) [14].
- Estimation of Phytosteroids ( $\mu\text{g. gm}^{-1}$ ) [15].
- Estimation of Carotene concentration ( $\mu\text{g. ml}^{-1}$ ) [16].
- Estimation of DPPH free radicals scavenging activity (%) [17].
- Estimation of Glutathione peroxidase Enzyme (GPX) U.mg.p<sup>-1</sup> [18].
- Estimation of Glutathione concentration nm.mol.mg<sup>-1</sup> [19].

The results were statistically analyzed according to the statistical program (SAS) and the use of the least significant difference (L.S.D) to compare the coefficients of the coefficients at the level of probability 0.05 [20]

## Results and Discussion

The results showed in Table (1) that the intensity of water stress significantly affected the decrease in the mean of salicylic acid concentration 15-day period showed a significant decrease of 41.97% compared to 5 days, The results showed that when sprayed with aspirin the mean and the concentration increased when spraying with at a concentration of 10 mg.L<sup>-1</sup> The mean of salicylic acid concentration increased to 24.65  $\mu\text{g.mg}^{-1}$  compared with 22.31  $\mu\text{g.mg}^{-1}$ .

There was a significant effect of the spray of brassinolide when increasing the concentration from 0 to 1 mg.L<sup>-1</sup> the mean of this effect increased significantly from 21.13 to 24.95 µg. Mg<sup>-1</sup>. The results showed that there were significant differences in the interaction between the periods of water stress and aspirin, with a concentration of 10 mg.L<sup>-1</sup> and under the influence of 14 days of stress the mean was 18.02 compared with 8.89 µg.mg<sup>-1</sup> at the same time and at 0 mg.L<sup>-1</sup> concentration. The results showed a significant increase in the mean of this characteristic in the interaction between aspirin and brassinolide, the results of the

triple interaction confirmed that aspirin spray and brassinolide hormone and the higher concentrations had a positive effect in reducing the effect of water stress. and spray with aspirin, hormone and concentrations of 10 and 1 mg.L<sup>-1</sup> The value was 18.89 µg.mg<sup>-1</sup> compared to 14.31 µg.mg<sup>-1</sup> at the same 15-day period and not to spray both aspirin and hormone The results showed that the intensity of stress led to a decrease in concentrations of endogenous salicylate and this is due to the inhibition of the metabolism of organic acids, pathway synthesis of salicylic acid [21].

**Table 1: Effect of Aspirin and brassinolide hormone in the endogenous concentration of salicylic acid (µg.mg<sup>-1</sup>)**

water stress X aspirin concentrations	BL (mg/L <sup>-1</sup> ) Brassinolide Con.			Aspirin ASA(mg/L <sup>-1</sup> )	Water stress (days)
	1	0.5	0		
28.07	29.55	28.45	26.22	0	5
28.95	30.00	29.89	26.95	5	
29.54	30.72	30.59	27.31	10	
22.87	24.94	23.20	20.46	0	10
25.23	26.56	26.35	22.79	5	
26.78	28.73	28.34	23.26	10	
16.21	17.23	16.99	13.71	0	15
16.64	17.92	17.70	14.31	5	
17.60	18.89	18.72	15.20	10	
2.279	24.95	24.47	21.13	Brassinolide mean	
	0.312 brassinolide effect			L.S.D(0.05)	
	0.938 triple interaction				
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			(days) Water periods	
	1	0.5	0		
28.85	30.09	29.56	26.82	5	
24.96	26.75	25.96	22.17	10	
16.74	18.02	17.80	14.41	15	
0.321	1.480			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			aspirin Concentrations (mg/L <sup>-1</sup> )	
	1	0.5	0		
22.31	23.91	22.88	20.13	0	
23.61	24.83	24.65	21.35	5	
24.65	26.12	25.89	21.93	10	
0.312	6.559			L.S.D(0.05)	

The results of Table (2) showed that the increase in the severity of the water stress affected significantly in the increase mean concentration of phytosterols. 15day period showed a significant decrease from 24.09 to 11.52 µg. gm<sup>-1</sup>. While spraying with aspirin at a concentration of 10 mg. The mean concentration of phytosterols increased significantly to 21.48 µg. gm<sup>-1</sup> compared to 16.83 µg. The results confirmed the presence of significant effect on the spray the brassinolide increase the mean concentration of phytosterols raise the concentration of the hormone from 0 to 1 mg.L<sup>-1</sup>. The average this trait was significantly increased from 15.26 to 21.94 µg. gm<sup>-1</sup>, as well as the results, proved that there were significant differences

at the interaction between the periods stress and aspirin concentrations as at the concentration of 10 mg.L<sup>-1</sup> under the influence of 15 days of stress has increased the average and this trait was significantly increased compared with the same period of stress and lack of aspirin spraying, in addition to the effect of 15 days of stress, the mean of was significantly increased to 6.951 compared to 6.47 µg. gm<sup>-1</sup>. When the 15 day stress period and not spray the brassinolide, the results showed that there was a significant difference in the triple interaction between the stress periods and both aspirin and brassinolide were exposed at 15 days of stress. The concentration of aspirin and hormone concentrations was increased by

2.20mg.L<sup>-1</sup>. The concentration of the phytosterols increased to 21.94 µg. Gm<sup>-1</sup> compared with 3.75 µg. gm<sup>-1</sup> at the 14 day stress period and the concentration was 0 mg. L<sup>-1</sup> for aspirin and brassinolide hormone, the reason for the inhibition concentration of phytosterols is that stress is one of the types

of oxidative stress that affects the oxidation through the enzyme Oxidase either due to the increase of the concentration of phytosterols concentrations of aspirin spray due to the role of antioxidant effect to Oxidase [22].

**Table 2: Effect of Aspirin and brassinolide hormone in the phytosterols concentration (µg. gm<sup>-1</sup>)**

water stress X aspirin concentrations	BL (mg/L <sup>-1</sup> ) Brassinolide Con.			Aspirin ASA(mg/L <sup>-1</sup> )	Water stress (days)
	1	0.5	0		
22.80	24.48	23.47	20.44	0	5
23.84	25.55	24.51	21.46	5	
25.63	27.87	26.56	22.47	10	
19.52	22.39	20.37	15.81	0	10
22.27	25.00	23.46	18.35	5	
24.03	27.00	25.70	9.37	10	
8.16	11.88	8.85	3.75	0	15
11.59	15.01	12.94	6.81	5	
14.80	18.29	17.25	8.84	10	
3.867	21.94	20.35	15.26	Brassinolide mean	
	0.436 brassinolide effect			L.S.D(0.05)	
	1.308 triple interaction				
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			(days) Water periods	
	1	0.5	0		
24.09	25.97	24.85	21.46	5	
21.94	24.80	23.18	17.84	10	
11.52	16.95	13.01	6.47	15	
0.436	2.705			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			aspirin Concentrations (mg/L <sup>-1</sup> )	
	1	0.5	0		
16.83	19.58	4.52	13.33	0	
19.23	21.85	5.60	15.54	5	
21.48	24.39	5.98	16.90	10	
0.436	7.215			L.S.D(0.05)	

The results showed in Table (3) that the increase in the intensity of water stresses significantly affected the decrease in the carotene concentration. 15 day period showed a significant decrease of 13.85% compared with 5 days. When sprayed with aspirin with a concentration of 10 mg.L<sup>-1</sup> carotene increased to 37.38 compared with 35.31 µg. ml<sup>-1</sup> when not sprayed aspirin, The results showed a significant effect of the spray of brassinolide with the concentration increased from 0 to 1 mg.L<sup>-1</sup>. The mean of this effect increased significantly from 53.58 to 37.05 µg. ml<sup>-1</sup> The results also showed significant differences in the interaction between stress periods and aspirin concentrations.

Under the influence of 15 days of stress, this means was 34.35 µg. ml<sup>-1</sup> compared with 32.16 µg. ml<sup>-1</sup> at the same stress period and non-spraying of aspirin, brassinolide spraying behaved similarly to aspirin by reducing the effect of stress. The mean concentration of carotene increased under the

15 day stress period when spraying with 1 mg.L<sup>-1</sup> the concentration was 33.97 µg. ml<sup>-1</sup> compared to the same period and at 0 mg. L<sup>-1</sup> of the hormone, which was 32.42% The results showed significant differences in the interaction between aspirin and brassinolide. The results of the triple interaction showed that aspirin, brassinolide and higher concentrations had a positive effect in reducing the effect of water stress. The low concentration of carotene is due to water shortage in the plant tissue [23]. The process of fission of the water molecule is the basis for photosynthesis and the intensity of the stress results in the degradation of chlorophyll due to increased concentration of free radicals from chloroplast thylakoids [24].

The role of the aspirin in reducing the impact of water stress is the main reason is the mechanism of action and behavior of aspirin as an is a non-enzymatic antioxidant at the same time as this unique characteristic of this salicylic acid with important in the

plant's tolerance to water when it is anti-oxidative oxidation is combined with amino acids In the pigment of carotene [25].

**Table 3: Effect of Aspirin and brassinolide hormone in Carotene concentration ( $\mu\text{g. ml}^{-1}$ )**

water stress X aspirin concentrations	BL ( $\text{mg/L}^{-1}$ ) Brassinolide Con.			Aspirin ASA( $\text{mg/L}^{-1}$ )	Water stress (days)
	1	0.5	0		
37.69	38.77	38.28	36.02	0	5
38.94	39.28	38.90	38.63	5	
39.46	39.81	39.53	39.03	10	
36.07	37.21	36.92	34.07	0	10
37.44	37.74	37.49	37.10	5	
38.34	38.70	38.27	38.05	10	
32.16	33.50	32.96	30.00	0	15
33.52	33.75	33.58	33.23	5	
34.35	34.65	34.34	34.54	10	
1.084	37.05	36.70	35.58	Brassinolide mean	
	0.116 brassinolide effect			L.S.D(0.05)	
	0.348 triple interaction				
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL ( $\text{mg/L}^{-1}$ )			(days) Water periods	
	1	0.5	0		
38.70	39.29	38.91	37.90	5	
37.28	37.88	37.56	36.41	10	
33.34	33.97	33.36	32.42	15	
0.116	1.326			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL ( $\text{mg/L}^{-1}$ )			aspirin Concentrations ( $\text{mg/L}^{-1}$ )	
	1	0.5	0		
35.31	36.50	36.06	33.36	0	
36.64	36.93	36.66	36.32	5	
37.38	37.72	37.38	37.04	10	
0.116	2.930			L.S.D(0.05)	

The results of Table (4) showed that the increase in the intensity of water stresses significantly affected the decrease in the free radicals scavenging activity. A period of 15 days was observed for a significant period of time (7.96%) compared to 5 days. With aspirin at a concentration of  $10 \text{ mg.L}^{-1}$  the free radicals scavenging activity by 43.48% compared to 40.76% when the aspirin was not sprayed, the results showed a significant effect of spraying of brassinolide in the increase of this effect, when the concentration increased from 0 to  $1 \text{ mg.L}^{-1}$ , the mean of this effect increased significantly from 40.29 to 43.24%. The results also showed significant differences in the interaction between stress periods and aspirin concentrations with a concentration of  $10 \text{ mg.L}^{-1}$ .

Under the influence of 15 days of stress, the average grade increased to 39.74% compared to 35.85% during the same stress period and not to spray aspirin In addition, the spraying of the brassinolide hormone increased the mean level at 15 days and sprayed with  $1 \text{ mg.}$  The average increased from 35.19 to 39.93% in the same period, but at  $0 \text{ mg.L}^{-1}$  concentration of brassinolide. aspirin and brassinolide have significant differences and

increased concentration of two factors The results of the triple interference of aspirin spray and growth regulator brassinolide and higher concentrations have a positive effect on increasing the values of the character even under the influence of stress at the time of stress 15 days and aspirin and hormone concentrations 10 and  $1 \text{ mg. L}^{-1}$  on the sequence increased the value of this attribute to 43.24% compared with 31.26% At the same period of 14 days do not spray both aspirin and brassinolide, One of the reasons for the decline is due to the loss of plastids and their physiological function and Accumulation of toxic free radicals with inhibition of free radicals scavenging activity[26], Spray the aspirin to reduce the effect of stress as the component role of antioxidant and acts as a catalyst for a large number of antioxidant enzymes, especially the enzyme glutathione

Peroxidase GPX and CAT catalyze where these enzymes role in the transformation of hydrogen  $\text{H}_2\text{O}_2$  toxic resulting from the impact of stress and converted to  $\text{H}_2\text{O}$  water molecules[27] The results showed that the hormone brassinolide has a positive effect in reducing the effect of water stress and associated stress. The mechanism of steroid action controls the cellular control of the



concentration of Abscisic acid and control of the closure, opening of stomata and transpiration and maintaining the water content of plant tissue [28].

**Table 4: Effect of Aspirin and brassinolide hormone in the free radicals scavenging activity (%)**

water stress X aspirin concentrations	BL (mg/L <sup>-1</sup> ) Brassinolide Con.			Aspirin ASA(mg/L <sup>-1</sup> )	(days) Water
	1	0.5	0		
40.79	41.51	41.44	39.41	0	5
41.50	41.95	41.58	40.95	10	
41.74	42.26	41.99	40.97	20	
45.62	47.82	47.13	42.39	0	10
47.37	48.45	47.67	46.62	10	
47.74	38.69	48.05	46.73	20	
35.85	38.69	37.60	31.26	0	15
38.58	40.08	39.14	36.50	10	
39.74	41.02	40.40	37.80	20	
2.279	43.24	42.78	40.29	Brassinolide mean	
	0.785 brassinolide effect			L.S.D(0.05)	
	2.355 triple interaction				
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			(days) Water periods	
	1	0.5	0		
41.34	41.91	41.67	40.45	5	
46.91	47.87	47.61	45.25	10	
38.05	39.93	39.05	35.19	15	
2.279	1.969			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			aspirin Concentrations (mg/L <sup>-1</sup> )	
	1	0.5	0		
40.76	42.52	42.06	37.69	0	
42.08	43.28	42.80	41.36	5	
43.48	43.91	43.48	41.84	10	
0.785	4.938			L.S.D(0.05)	

The results of Table (5) showed that the increase in the stress resulted in a significant increase in the average activity of the GPX Enzyme. When the irrigation spacing ranged from 5 to 15 days the mean effectiveness increased from 28.08 to 83.70 U.mg.p<sup>-1</sup>. Aspirin spraying significantly increased GPX enzyme activity at concentrations of 5 and 10 mg<sup>-1</sup> to 43.08 and 48.41 U.mg.p<sup>-1</sup> respectively, compared with non-spray treatment of 40.76 U.mg.p<sup>-1</sup>. The spraying of Brassinolide significantly increased the average activity of the enzyme glutathione peroxidase at a concentration of 1 mg / L<sup>-1</sup> 56.36 U.mg.p<sup>-1</sup> compared to the 0 concentration of Brssinolide, the results of the double interaction between irrigation periods and aspirin spraying showed significant differences at 15 days and concentration of 0 mg.L<sup>-1</sup> of aspirin. The average of Glutathione concentration was 56.38 U.mg.p<sup>-1</sup> but significantly increased when spraying aspirin at concentration 1 mg.L<sup>-1</sup> to 65.12 U.mg.p<sup>-1</sup> under the same irrigation period of 15 days , the results

showed that the effect of the double interaction between the irrigation periods and the brassinolide a significant increase in the average effectiveness of the Glutathione peroxidase enzyme activity when the concentration of the brassinolide from 0 to 1 mg.L<sup>-1</sup>, under 10 days stress the GPX enzyme activity increased from 50.82 to 57.56 U.mg.p<sup>-1</sup> while at 15 days from 56.82 to 64.44 U.mg.p<sup>-1</sup>, The results of the triple interaction showed significant differences in the increasing of the GPX enzyme activity under the concentrations of aspirin and brassinolide with the irrigation spacing. The irrigation duration was 15 days and the concentrations 1 and 0.5 mg/L<sup>-1</sup>, The reason for the increasing GPX enzyme activity as an anti-oxidant reaction resulting from the activity of oxidizing enzymes and free radicals with the effect of water stress [29] the increasing in GPX enzyme activity under the effect of aspirin and brassinolide is due to the role of the hormone in increasing the rate of free radicals [30].

**Table 5: Effect of Aspirin and brassinolide hormone in Glutathione Peroxidase Enzyme activity (U.mg.p<sup>-1</sup>)**

water stress X aspirin concentrations	BL (mg/L <sup>-1</sup> ) Brassinolide Con.			Aspirin ASA(mg/L <sup>-1</sup> )	Water stress (days)
	1	0.5	0		
24.08	28.08	23.50	20.66	0	5
28.84	30.50	28.78	27.23	5	
31.34	32.94	31.67	29.41	10	
49.45	52.21	50.03	46.12	0	10

55.27	58.94	54.32	52.56	5	15
58.31	61.54	59.64	53.76	10	
56.38	60.41	56.62	52.09	0	
61.12	64.32	61.91	57.11	5	
65.12	68.58	65.52	61.24	10	
3.77	50.83	48.00	44.46	Brassinolide mean	
	1.01 brassinolide effect			L.S.D(0.05)	
	3.04 triple interaction				
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			(days) Water periods	
	1	0.5	0		
28.08	30.50	27.98	25.47	5	
54.29	57.56	54.67	50.82	10	
60.88	64.44	61.36	56.82	15	
1.01	3.767			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			aspirin Concentrations (mg/L-1)	
	1	0.5	0		
43.08	46.90	43.38	39.62	0	
48.41	51.25	48.34	45.64	5	
51.59	54.36	52.27	48.14	10	
1.01	18.37			L.S.D(0.05)	

The results of Table (6) showed that the effect of irrigation periods significantly increased the concentration of glutathione which increased significantly during 10 days of irrigation. The mean concentration was 419.47 nm.mol.mg<sup>-1</sup> while the irrigation was 15 days 276.51 nm.mol.mg<sup>-1</sup> compared to 5 days which gave an average of 191.65 nm.mol.mg<sup>-1</sup>. The results of this table showed that aspirin spraying increased the mean of glutathione concentration. The mean of glutathione increased from 291.49 nm.mol.mg<sup>-1</sup> when the aspirin was not sprayed to 299.23 nm.mol.mg<sup>-1</sup>, when spraying Brassinolide at a concentration with a higher concentration (1 mg.L<sup>-1</sup>) resulted in a significant increase in the mean concentration of glutathione at 297.86 nm.mol.mg<sup>-1</sup> compared with mean 293.18 nm.mol.mg<sup>-1</sup>. When not sprayed with Brassinolide, The results of the double interaction between irrigation periods and aspirin spraying showed significant differences at 15 days and concentration of 0mg.L<sup>-1</sup> of aspirin. The mean of Glutathione concentration was 270.44 nm.mol.mg<sup>-1</sup> but was significantly increased when spraying aspirin at a concentration 10 mg.L<sup>-1</sup> and

reached 281.01 nm.mol.mg<sup>-1</sup> under the same stress period of 15 days. The results of the table showed that when sprayed with 1 mg.L<sup>-1</sup> of brassinolide under 15 days of stress the mean of glutathione concentration was significantly increased to 272.75 nm.mol.mg<sup>-1</sup> compared to the same stress duration but when sprayed with brassinolide, the mean of glutathione concentration was 209.81 nm.mol.mg<sup>-1</sup>. The results of the triple interaction between the irrigation periods and concentrations of aspirin and brassinolide showed significant differences. It is believed that the formation of glutathione is one of the mechanisms used by the plant in the formation of types of short-chain proteins and resistance to oxidation and called this process by Glutathionylation of proteins [31]. The role of brassinolide and aspirin in increasing the concentration of glutathione is due to the role of the hormone and aspirin in increasing the effectiveness of the enzymatic antioxidants which works to remove the toxic effect of the free radicals and scavenging and then reduce the reactions of antioxidants and non-enzymatic antioxidants [32].

**Table 6: Effect of Aspirin and brassinolide hormone in Glutathione concentration (nm.mol.mg<sup>-1</sup>)**

water stress X aspirin concentrations	BL (mg/L <sup>-1</sup> ) Brassinolide Con.			Aspirin ASA(mg/L <sup>-1</sup> )	Water stress (days)
	1	0.5	0		
189.38	191.45	190.59	186.09	0	5
192.08	192.92	192.13	191.18	5	
193.48	194.65	193.44	192.36	10	
414.65	418.05	416.77	409.14	0	10
420.56	421.38	420.67	419.63	5	
423.20	424.48	423.20	421.92	10	
270.44	274.98	273.85	262.50	0	15
278.08	279.75	277.59	276.9	5	
281.01	283.02	281.15	278.85	10	
6.81	297.86	296.60	293.18	Brassinolide mean	

		4.548brassinolide effect			L.S.D(0.05)
		13.468 triple interaction			
brassinolide Concentrations X Effect of water periods					
Mean of water stress	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			(days) Water periods	
	1	0.5	0		
191.65	237.55	192.05	198.88	5	
419.47	421.30	420.21	416.90	10	
276.51	279.25	277.54	272.75	15	
4.55	7.676			L.S.D(0.05)	
brassinolide Concentrations X aspirin Concentrations					
Mean of aspirin	brassinolide Concentrations BL (mg/L <sup>-1</sup> )			aspirin Concentrations (mg/L-1)	
	1	0.5	0		
291.49	294.83	293.74	285.10	0	
296.91	298.02	296.80	295.91	5	
299.23	300.72	299.27	297.71	10	
4.55	23.88			L.S.D(0.05)	

## Conclusions and Recommendations

The conclusion from this study that the intensity of water stress, especially severe stress for a period of 15 days resulted in increase in oxidative stress by leakage of free radicals and inhibition of plant metabolism the spraying of aspirin and hormone and increased concentrations have

reduced the impact of water stress to the triple interaction between the factors of the experiment was the effect of factors (aspirin and brassinolide) in reducing the harmful effect of water stress is therefore recommended to spray higher concentrations and conduct studies on other environmental stresses and on other plants.

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