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SYNERGISTIC EFFECTS OF BIOCHAR AND GLUTATHIONE FOLIAR APPLICATION ON WHEAT PLANT GROWTH, PHOTOSYNTHETIC PIGMENTS, GRAIN YIELD, AND ITS COMPONENTS UNDER WATER DEFICIT CONDITIONS

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Abstract

Water stress, a severe abiotic stress, is currently posing a significant threat to wheat production globally. So, two experiments were conducted at the Experimental Farm of the College of Sciences and Humanities, Shaqra University, Al-Quwayiyah Governorate during the autumn seasons of 2021/2022 and 2022/2023 to explore the synergistic effects of biochar and glutathione foliar application on wheat plant growth, photosynthetic pigments, grain yield, and its components under water deficit conditions. These trials were performed in both seasons in a randomized complete block design using a split-plot arrangement, with three replications for each water regime treatment. Biochar application rates were applied in the main plots, whereas sub-plots used glutathione foliar application. Significant adverse consequences on plant growth, photosynthetic pigments, grain yield, and its components were observed as a result of a water deficit in both seasons. The foliar application of biochar and glutathione significantly mitigated the adverse consequence of water stress on plant growth, photosynthetic pigments, grain yield, and its components. Plant growth, photosynthetic pigments, grain yield, and components traits were significantly impacted by combination of water regimes with biochar application and glutathione application, with the most favorable triple interaction observed via increasing biochar application to 20 tons per hectare and glutathione application was up to 300 mg/l under various watered regimes, particularly under well-watered conditions. Accordingly, the application of biochar at a rate of 20 tons per hectare and glutathione at a rate of 300 mg/l was found to be a sustainable method for enhancing wheat production under water stress conditions.

Key words: Biochar, Glutathione, Grain yield, Water deficit, Wheat.

Introduction

Water scarcity is an important problem affecting the production of worldwide wheat production sustainability. Recently, scientists are exploring strategies to mitigate adverse effects of water scarcity on wheat production, such as foliar application of biochar and glutathione, which could result in a more sustainable and resilient approach to wheat production in water-limited environments (Ouda & Zohry, 2024).

This can lead to a decline in stomatal conductance, chlorophyll content, and leaf area because of the reduced activities of photosynthesis and nutrient uptake in wheat plants. This consequently results in a low grain yield with reduced grain quality. Environmental stress similarly affects the reproductive structures, floret fertility, and grain weight, therefore affecting the production of crops (Prasad *et al.*, 2015). Comprehending these impacts is crucial for farmers and researchers to create strategies to mitigate impact of water stress on wheat production. In this respect, Ali *et al.*, (2019) found that adversely affected by water stress on wheat plant growth, photosynthetic pigments, water relations, and seed yield. However, β-toc foliar fertigation increased tolerance to water stress and improved seed yield, hence mitigating the effects of water stress on wheat plants. Zhao *et al.*, (2020) indicated that a mild stress of 60-80% of field water capacity had optimum use of water resources with no significant reduction in yield of winter wheat in arid areas. Also, Habib *et al.*, (2020) found that the application of drought stress decreased the growth and yield in wheat plants, but the undesirable effects of the applied stress may be alleviated with sodium nitroprusside and hydrogen peroxide-treated seed priming. Cao *et al.*, (2021) observed that irrigation at the heading and grain-filling stages resulted in the highest grain yield, 6470 kg ha^{-1}, and improved the grain yield and photosynthetic traits of a flag leaf during wheat anthesis. Moreover, Al-Huqail *et al.*, (2023) showed that a benzothiazine derivative may increase wheat tolerance via improved growth and production of antioxidants despite drought stress by means of seed priming.

Biochar is a carbonaceous product produced from biomass pyrolysis, and it has been observed to enhance plant growth and productivity by improving soil fertility through improved water retention in soils, thus enhancing nutrient availability. This stimulates the beneficial microorganisms of the soil, improving soil structure, root growth, and nutrient uptake. Most recently, research has started to be conducted on its potential for mitigating water deficit stress on crop production. Improvement of plant growth and development might be achieved by biochar, as reported on by Aibdin *et al.*, (2023) and Zulfiqar *et al.*, (2022). Aibdin *et al.*, (2023) found that combining biochar with zinc-lysine significantly improved wheat plant growth, photosynthetic pigments, grain yield, and components performance. In a similar vein, Zulfiqar *et al.*, (2022) found that biochar application at a 5% rate reduced drought stress effects on plant growth, photosynthetic pigments, grain yield, and its components.

Glutathione is an antioxidant and thus may reduce the adverse impact of water deficit in wheat plants. It might behave as a promising tool for wheat production under water-stressed environments because it has the potential to enhance growth, improve photosynthetic pigments, and increase grain yield with foliar application. Nahar *et al.*, (2015) demonstrated that under drought stress, glutathione helped in improving the drought tolerance in mung bean seedlings by improving antioxidant defense, reducing methylglyoxal toxicity, and regulating proline and water content. Ascorbic acid and glutathione application as seed soaking and foliar spray have been reportedly enhancing the growth and productivity of faba bean plants under salt stress by Zaki *et al.*, (2018). Furthermore, Rehman *et al.*, (2021) found that the application of glutathione and moringa leaf extract improved wheat growth, physiology, and metabolic adaptation to salinity, thereby enhancing grain yield and reducing the negative effects of salinity on crop productivity. In addition, Mageed *et al.*, (2023) reported that exogenous glutathione can mitigate water deficits in bean plants grown in salty soil by enhancing antioxidant abilities and increasing glutathione and ascorbic acid content, thus improving productivity, photosynthetic efficiency, osmolytes, and antioxidant capacity.

Therefore, the main objective of this study was to investigate the potential effects of biochar and glutathione foliar application in the mitigation of the diverse effects of water stress on wheat plant growth, photosynthetic pigments, grain yield, and its components in the sandy soil conditions.

Material and Methods

Site description: Two experiments were carried out on Cv. Sakha 94 at the Experimental Farm of the College of Science and Humanities, Shaqra University, Al-Quwayiyah Governorate, 44º 46' N latitude and 24º 22' E longitude, and 819m above sea level, in central Saudi Arabia, during the autumn seasons of 2021/2022 and 2022/2023. The average daily temperature in the experimental area was 33.30°C during the day and 19.10°C at night, with an average precipitation of 18 mm/day and an average relative humidity of 46.75 - 47.50 %. Soil samples were prepared before planting by air-drying, grinding, sieving, and storing for further analysis after being finely sieved and sieved through a 2 mm sieve. The soil experiment, as described by Jackson (1973), involved a comprehensive analysis of physical and chemical parameters (Table 1). The crop planted earlier in both seasons was maize.

Experimental design: For each irrigation regimes, *i.e.,* mild (irrigation when 25% of available soil moisture was depleted (ASMD), T1), moderately (irrigation when 50% of ASMD, T2), and severe (irrigation when 75% of ASMD, T3), the experiments were performed in a randomized complete block design (RCBD) using a split-plot arrangement with three replications.

Table 1. Physicals and chemicals analyses of experimental sites at 030 cm depth of soil.

Seasons	Available			pH	EC mmh/v	Clay %	Silt %	Fine sand %	Texture
	N	P	K						
2021/2022	8.2	2.33	45	7.09	1.12	24.35	32.52	43.13	Loam
2022/2023	7.22	2.48	53	7.13	1.06	23.58	35.24	41.18	Loam

Table 2a. Mean squares of water regimes (W), biochar (B) and glutathione foliar application (G) and their interactions on wheat plant growth in both seasons.

S.O.V.	df	Shoot length		Leaf area per plant		No. of tillers per plant		No. of leaves per plant		Shoot DW per plant	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
W	2	1577.72**	2334.27**	13547.02**	26817.13**	4.03**	8.27**	41.68**	81.95**	15.15**	24.98**
Reps within W	6	70.61	97.82	621.34	1128.40	0.16	0.19	0.92	2.86	0.13	0.846
B	2	705.83**	1034.70**	6157.05**	11708.80**	1.86**	3.62**	18.72**	37.03**	6.84**	11.28**
W × B	4	125.77**	262.97**	1378.75**	2637.01**	0.40**	0.77**	4.27**	9.76**	1.55**	3.12**
Pooled Error a	12	20.78	33.54	232.53	340.81	0.05	0.06	0.33	1.02	0.05	0.36
G	2	934.94**	1163.05**	8264.45**	13419.45**	2.54**	4.28**	24.26**	41.13**	8.98**	12.55**
W × G	4	69.10**	98.73**	830.68**	921.30**	0.23**	0.23**	2.50**	3.34**	1.02**	1.16**
B × G	4	27.66*	42.86**	349.49*	413.04*	0.09*	0.13**	0.90**	1.57**	0.36**	0.38*
W × B × G	8	30.60**	32.46*	323.05*	384.18**	0.08**	0.13**	0.91**	1.24**	0.26**	0.28*
Pooled Error b	36	9.40	10.91	108.93	122.86	0.02	0.02	0.15	0.33	0.02	0.10

*, ** Significant at 0.05 and 0.01 probability level, respectively

Table 2b. Mean squares of water regimes (W), biochar (B) and glutathione foliar application (G) and their interactions on wheat photosynthetic pigments in both seasons.

S.O.V.	df	Chlorophyll a		Chlorophyll b		Carotenoid		Total photosynthetic pigments	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
W	2	1.8073**	2.0442**	0.3062**	0.4699**	0.0764**	0.1639**	4.7265**	6.3490**
Reps within W	6	0.0015	0.0063	0.0017	0.0006	0.0001	0.0025	0.0010	0.0024
B	2	0.8018**	0.8832**	0.1393**	0.2115**	0.0329**	0.0724**	2.1018**	2.7844**
W × B	4	0.1668**	0.1639**	0.0343**	0.0458**	0.0068**	0.0157**	0.4564**	0.5535**
Pooled Error a	12	0.0009	0.0020	0.0002	0.0004	0.0001	0.0007	0.0011	0.0012
G	2	1.0554**	1.2071**	0.1867**	0.2818**	0.0453**	0.0970**	2.7952**	3.7659**
W × G	4	0.0927**	0.0848**	0.0215**	0.0270**	0.0042**	0.0092**	0.2654**	0.3030**
B × G	4	0.0358**	0.0358**	0.0085**	0.0104**	0.0014**	0.0033**	0.1009**	0.1214**
W × B × G	8	0.0398**	0.0470**	0.0070**	0.0083**	0.0012**	0.0029**	0.1004**	0.1305**
Pooled Error b	36	0.0004	0.0012	0.0002	0.0002	0.0001	0.0003	0.0007	0.0009

*, ** Significant at 0.05 and 0.01 probability level, respectively

The main plots received biochar application rates of B0, which represents zero addition and serves as the control; B10, which corresponds to an application rate of 10 (ton ha^{-1}); and B20, which represents an application rate of 20 (ton ha^{-1}), while sub-plots were randomly distributed with glutathione foliar application at rates of 0.0, 150, and 300 mg/l. Each sub-plot had a size of $10.5m^2$ with 15 rows, 3.5m long, and 20 cm spacing between them. The normal agronomic practices of growing wheat were carried out from sowing to harvest, as recommended by Ministry of Environment, Water and Agriculture in the Kingdom of Saudi Arabia.

Recorded data

Plant growth measurements: Samples of ten guarded plants were taken at random from each plot of the three replications for measurement of growth traits. Various traits were recorded at harvest, such as shoot length (cm) and shoot dry weight, number of leaves and tillers per plant, as well as leaf area (cm2). The leaf area was measured using LI-COR (LI-3000; Lincoln NE, USA), and the dry weight (g) was recorded at 70◦C after oven drying.

Photosynthetic pigments: Photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, as well as total photosynthetic pigments were determined in fresh wheat leaves at 60 days after sowing using spectrophotometric analysis, following the recommended method of Moran (1982).

Yield measurement: Ten plants were randomly selected at harvest maturity to record spike length (cm), spikes number per plant, and grains number per spike from each replicate of each treatment. The final straw and grain yields were determined from 5 m^2 from experimental plots, and then converted them to tons per hectare.

Statistical analysis

The study investigated the impact of biochar and glutathione foliar application rates on all studied traits in three replications under different irrigation regimes in both seasons, in a randomized complete block design using a split-plot arrangement. The treatments were assessed by comparing their mean differences using the least significant difference (L.S.D. 5%), as outlined by Gomez & Gomez (1984).

Results and Discussion

Analysis of variances: The highest variations in plant growth traits were noted by applying of water regimes, followed by biochar application and glutathione foliar application, which were noted during both seasons (Table 2). It is clear that water availability significantly influenced plant growth, with plants showing variations under various water regimes. In addition, the foliar application of biochar followed by glutathione significantly impacted plant growth, in reverse order on photosynthetic pigments, grain yield, and its components, indicating their potential as growth-promoting treatments. Consequently, these results meant that combining water management practices with biochar and foliar applications was efficient in enhancing plant growth, and thereby wheat productivity in both seasons.

Moreover, the dual interactions of water regimes with biochar application had greater variations than with glutathione foliar application on plant growth, photosynthetic pigments, grain yield, and its components traits in both seasons. This suggested that the application of biochar, considered as a plant growth enhancer, had been found to have a more significant impact on plant growth in varying water regimes compared to the glutathione foliar application. In same regard, the dual interactions of biochar application with glutathione foliar application showed slightly greater variations on most plant growth and photosynthetic pigments, grain yield, and its components than the triple interaction of water regimes with two previous applications in both seasons. This showed that biochar addition to soil significantly improved the growth traits of plants, regardless of whether glutathione was applied or not, thus proving it as an important factor in plant health. These results are consistent with those reported by Rehman *et al.*, (2021), who showed that under salinity stress, exogenous application of glutathione and moringa leaf extract at seedling stage significantly improved wheat grain yield and thereby alleviated the negative effect of salinity on crop production. Further, Dawood *et al.*, (2020) confirm these results when they noticed a glutathione application at 300 mg/l was the most efficient to increase photosynthetic pigments in three wheat cultivars. Moreover, Saeed *et al.*, (2024) showed that exogenous application of glutathione alone or along with zinc significantly mitigated the adverse effects of water stress on maize growth, as a result of positive effects on antioxidant biosynthesis, total soluble proteins, total soluble, reducing, and non-reducing sugars.

Main effects

Effect of water regimes: Water scarcity had been found to have a negative impact on plant growth, photosynthetic pigments, grain yield, and its components, as shown in (Table 3). Water stress treatment in both seasons led to significant reductions in plant growth, photosynthetic pigments, grain yield, and components when compared to well-watered and moderate water stress.

The reductions in both seasons were 14.00 and 8.89 & 16.03 and 10.80% in shoot length, 12.27 and 8.01 & 16.56 and 10.78% in leaf area per plant, 13.38 and 8.62 & 18.38 and 11.92% in number of tillers per plant, 14.35 and 9.41 & 17.42 and 11.89% in number of leaves per plant, and 15.44 and 10.24 & 19.35 and 13.41% in shoot dry weight per plant compared to well-watered and moderate water stress, respectively.

The reductions in both seasons were 27.53 and 18.85 & 34.16 and 23.46% in chlorophyll a, 24.75 and 17.06 & 33.80 and 23.98% in chlorophyll b, 24.99 and 16.82 & 33.59 and 23.94% in carotenoid, and 26.43 and 18.08 & 33.97 and 23.68% in total photosynthetic pigments, compared to well-watered and moderate water stress, respectively.

Table 2c. Mean squares of water regimes (W), biochar (B) and glutathione foliar application (G) and their interactions on wheat grain yield, and its components in both seasons.

S.O.V.	df	Spike length		No. of spikes per plant		No. of grains per spike		Straw yield per hectare		Grain yield per hectare	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
W	2	77.06**	168.22**	102.73**	213.54**	714.69**	1988.59**	91.56**	150.09**	26.60**	63.70**
Reps within W	6	1.54	4.79	3.65	5.73	18.49	72.50	1.65	4.80	0.76	1.288
B	2	31.74**	68.90**	44.07**	90.79**	320.22**	798.43**	31.47**	63.26**	11.84**	26.58**
W × B	4	7.22**	17.79**	7.17**	25.49**	86.01**	199.71**	6.74**	16.29**	2.67**	6.73**
Pooled Error a	12	0.49	1.50	0.84	1.88	8.81	19.71	0.46	1.52	0.23	0.43
G	2	40.68**	75.17**	59.93**	97.14**	426.26**	898.65**	43.01**	67.00**	15.64**	28.86**
W × G	4	4.60**	5.26**	3.60**	7.17**	53.73**	61.67**	4.26**	4.30**	1.59**	1.86**
B × G	4	1.44**	2.36**	1.35*	4.22**	17.82**	28.60**	1.28**	2.54**	0.48**	0.96**
W × B × G	8	1.32**	2.00**	1.45**	4.03**	15.10**	22.86**	1.42**	2.54**	0.35**	0.82**
Pooled Error b	36	0.23	0.52	0.41	0.67	3.93	6.87	0.24	0.44	0.09	0.17

*, ** Significant at 0.05 and 0.01 probability level, respectively

Table 3a. The main effects of water regimes, biochar and glutathione foliar application on wheat plant growth in both seasons.

Traits / Main effects	Shoot length		Leaf area per plant		No. of tillers per plant		No. of leaves per plant		Shoot DW per plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water regimes										
25% of ASMD	108.48	114.65	361.42	377.59	5.73	5.98	17.15	19.75	9.61	9.81
50% of ASMD	102.40	107.93	344.67	353.13	5.43	5.54	16.22	18.51	9.05	9.13
75% of ASMD	93.29	96.27	317.06	315.05	4.96	4.88	14.69	16.31	8.12	7.91
LSD 0.05	2.70	3.43	9.04	10.95	0.13	0.15	0.34	0.60	0.13	0.35
Biochar application (ton ha^{-1})										
B 0	95.61	99.32	323.91	325.26	5.08	5.06	15.08	16.87	8.36	8.23
B10	103.24	108.37	346.81	355.22	5.47	5.58	16.32	18.57	9.12	9.16
B20	105.33	111.17	352.42	365.29	5.57	5.77	16.66	19.12	9.31	9.47
LSD 0.05	2.70	3.43	9.04	10.95	0.13	0.15	0.34	0.60	0.13	0.35
Glutathione foliar application (mg/l)										
0	94.94	99.22	321.64	324.63	5.04	5.04	14.98	16.86	8.29	8.21
150	102.79	107.44	345.89	352.43	5.46	5.54	16.26	18.41	9.10	9.08
300	106.46	112.20	355.61	368.72	5.63	5.83	16.83	19.30	9.40	9.56
LSD 0.05	1.69	1.82	5.76	6.12	0.08	0.08	0.21	0.32	0.08	0.18

ASMD refers to available soil moisture depletion

Table 3b. The main effects of water regimes, biochar and glutathione foliar application on wheat photosynthetic pigments in both seasons.

Traits / Main effects	Chlorophyll a		Chlorophyll b		Carotenoid		Total photosynthetic pigments	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water regimes								
25% of ASMD	1.8644	1.6022	0.8517	0.7735	0.4225	0.4593	3.1386	2.8351
50% of ASMD	1.6650	1.3783	0.7727	0.6737	0.3810	0.4010	2.8187	2.4530
75% of ASMD	1.3511	1.0549	0.6409	0.5121	0.3169	0.3050	2.3090	1.8720
LSD 0.05	0.0178	0.0265	0.0092	0.0111	0.0058	0.0162	0.0196	0.0201
Biochar application (ton ha^{-1})								
B 0	1.4327	1.1411	0.6734	0.5529	0.3339	0.3298	2.4400	2.0238
B10	1.6862	1.4088	0.7836	0.6859	0.3868	0.4075	2.8566	2.5021
B20	1.7616	1.4856	0.8083	0.7205	0.3998	0.4280	2.9698	2.6341
LSD 0.05	0.0178	0.0265	0.0092	0.0111	0.0058	0.0162	0.0196	0.0201
Glutathione foliar application (mg/l)								
0	1.4098	1.1131	0.6624	0.5394	0.3282	0.3220	2.4004	1.9745
150	1.6740	1.3954	0.7798	0.6827	0.3844	0.4048	2.8382	2.4829
300	1.7967	1.5269	0.8231	0.7372	0.4079	0.4385	3.0277	2.7026
LSD 0.05	0.0109	0.0189	0.0069	0.0082	0.0059	0.0102	0.0145	0.0163

ASMD refers to available soil moisture depletion

Table 3c. The main Effects of water regimes, biochar and glutathione foliar application on wheat grain yield, and its components in both seasons

Traits / Main effects	Spike length		No. of spikes per plant		No. of grains per spike		Straw yield per hectare		Grain yield per hectare	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water regimes										
25% of ASMD	17.07	18.28	19.36	22.29	61.28	64.36	17.04	17.96	10.42	11.13
50% of ASMD	15.75	16.46	17.74	20.22	57.62	58.05	15.37	16.26	9.70	9.99
75% of ASMD	13.71	13.35	15.47	16.73	51.12	47.38	13.36	13.30	8.46	8.09
LSD 0.05	0.42	0.73	0.54	0.81	1.76	2.63	0.40	0.73	0.28	0.39
Biochar application (ton ha^{-1})										
B 0	14.28	14.22	16.08	17.67	52.78	50.44	14.03	14.10	8.78	8.61
B10	15.90	16.63	17.98	20.45	57.90	58.61	15.66	16.42	9.77	10.10
B20	16.34	17.24	18.52	21.12	59.34	60.75	16.07	16.99	10.04	10.49
LSD 0.05	0.42	0.73	0.54	0.81	1.76	2.63	0.40	0.73	0.28	0.39
Glutathione foliar application (mg/l)										
0	14.15	14.36	15.88	17.87	52.26	50.81	13.86	14.27	8.68	8.70
150	15.83	16.03	17.89	19.71	57.80	56.64	15.58	15.82	9.74	9.74
300	16.55	17.70	18.80	21.66	59.96	62.35	16.32	17.42	10.16	10.77
LSD 0.05	0.27	0.40	0.35	0.45	1.09	1.45	0.27	0.37	0.17	0.23

ASMD refers to available soil moisture depletion

Table 4a. Interaction of water regimes (W) with biochar (B) and glutathione (G) foliar application on wheat plant growth in both seasons.

W	B ton ha1	G mg/l	Shoot length		Leaf area per plant		No. of tillers per plant		No. of leaves per plant		Shoot DW per plant	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
25% of ASMD	B 0	0	100.13	106.19	338.75	345.20	5.32	5.41	15.94	18.14	8.88	8.93
		150	105.17	112.01	353.27	369.33	5.61	5.79	16.69	19.23	9.40	9.58
		300	110.87	116.73	366.99	385.46	5.82	6.16	17.49	20.17	9.78	10.01
	B10	0	106.02	112.01	355.18	369.33	5.63	5.79	16.83	19.23	9.46	9.58
		150	109.82	115.73	364.46	382.33	5.78	6.07	17.27	20.08	9.71	9.92
		300	113.15	119.17	374.08	392.84	5.95	6.27	17.82	20.54	9.94	10.25
	B20	0	107.24	113.52	358.69	373.79	5.68	5.87	17.00	19.50	9.54	9.65
		150	110.59	116.87	366.55	385.79	5.82	6.17	17.47	20.21	9.77	10.03
		300	113.37	119.61	374.78	394.23	5.96	6.30	17.89	20.61	9.97	10.29
50% of ASMD	B 0	0	94.49	98.32	321.17	322.56	5.00	5.00	14.98	16.72	8.20	8.15
		150	99.24	105.05	336.74	341.43	5.28	5.33	15.76	17.95	8.78	8.81
		300	102.49	108.39	346.11	353.22	5.47	5.55	16.27	18.62	9.09	9.22
	B10	0	99.18	105.05	335.71	341.43	5.26	5.33	15.72	17.95	8.77	8.81
		150	104.24	109.37	352.04	357.13	5.58	5.62	16.57	18.79	9.26	9.33
		300	107.46	113.70	359.18	374.22	5.69	5.88	17.01	19.60	9.57	9.67
	B20	0	97.64	102.83	329.58	334.00	5.15	5.22	15.44	17.52	8.56	8.62
		150	105.42	111.84	354.13	368.40	5.61	5.78	16.69	19.21	9.42	9.57
		300	111.46	116.87	367.36	385.79	5.84	6.17	17.53	20.21	9.81	10.03
75% of ASMD	B 0	0	66.01	64.27	229.11	211.43	3.50	3.07	9.95	10.27	5.28	4.66
		150	89.06	85.74	305.12	280.68	4.74	4.29	13.93	14.23	7.67	6.70
		300	93.06	97.21	317.97	318.02	4.97	4.94	14.72	16.51	8.12	7.96
	B10	0	89.06	92.02	305.12	299.57	4.74	4.66	13.93	15.46	7.67	7.29
		150	99.04	101.34	334.61	331.04	5.25	5.16	15.70	17.23	8.75	8.48
		300	101.20	106.91	340.94	349.10	5.38	5.45	16.04	18.29	8.96	9.07
	B20	0	94.66	98.80	321.46	324.35	5.03	5.03	15.01	16.92	8.21	8.22
		150	102.49	108.97	346.11	355.70	5.47	5.60	16.27	18.73	9.09	9.28
		300	105.06	111.20	353.11	365.59	5.60	5.74	16.69	19.12	9.37	9.52
LSD 0.05			5.08	5.47	17.28	18.35	0.25	0.24	0.64	0.95	0.23	0.54

ASMD refers to available soil moisture depletion

The reductions in both seasons were 19.65 and 12.92 & 27.00 and 18.91% in spike length; 20.06 and 12.77 & 24.96 and 17.29% in the number of spikes per plant; 16.58 and 11.27 & 26.38 and 18.37% in the number of grains per spike; 21.59 and 13.07 & 25.94 and 18.18% in straw yield per hectare; and 18.82 and 12.83 & 27.32 and 19.03% in grain yield per hectare compared to well-watered and moderate water stress, respectively.

Effect of biochar application: The application of biochar at various levels, from zero to 20 tons ha^{-1}, led to a gradual increase in plant growth, photosynthetic pigments, grain yield, and components, as illustrated in (Table 3). Furthermore, when comparing the application of biochar with the nonapplication in both seasons, the application of 10 and 20 ton ha^{-1} resulted in significant increases in plant growth, photosynthetic pigments, grain yield, and its components.

The application of biochar at 10 and 20 ton ha^{-1} significantly increased the growth traits of plants, wherein shoot length increased by 7.98 and 10.16 & 9.11 and 11.93%, leaf area per plant increased by 7.07 and 8.80 & 9.21 and 12.31%, the number of tillers per plant increased by 7.80 and 9.76 & 10.30 and 13.94%, the number of leaves per plant increased by 8.22 and 10.49 & 10.10 and 13.30%, and shoot dry weight per plant increased by 9.16 and 11.35 & 11.31 and 15.11%, respectively. Moreover, the biochar application of 10 and 20 ton ha^{-1} significantly increased photosynthetic pigments traits, with chlorophyll an increasing by 17.70 and 22.96 & 23.46 and 30.19 %, chlorophyll b by 16.36 and 20.04 & 24.06 and 30.32 %, carotenoid by 15.86 and 19.74 & 23.53 and 29.76 %, and total photosynthetic pigments by 17.07 and 21.71 & 23.64 and 30.16 %, respectively. Furthermore, the application of biochar at the rates of 10 and 20 ton ha^{-1} resulted in a significant increase in grain yield and its components traits, where spike length was increased by 11.33 and 14.42 & 16.92 and 21.26%, the number of spikes per plant increased by 11.77 and 15.13 & 15.77 and 19.57%, the number of grains per spike increased by 9.69 and 12.41 & 16.19 and 20.43%, straw yield per hectare increased by 11.64 and 14.54 & 16.44 and 20.49%, and grain yield per hectare also increased by 11.24 and 14.33 & 17.26 and 21.85%, respectively.

This indicated that biochar application had been found to improve wheat plant growth, photosynthetic pigments, grain yield, and its components by enhancing water stress tolerance, soil fertility, and nutrient availability. It also enhanced water and nutrient retention, and promoted microbial activity, all of which contribute to the overall growth and health of wheat plants. In this respect, Aibdin *et al*., (2023) have found a significantly higher growth of wheat plants, photosynthetic pigments, grain yield, and its components with the application of biochar in combination with zinclysine. Moreover, Zulfiqar *et al.* (2022) reported that the application of 5% biochar had lowered the drought effects on plant growth, photosynthetic pigments, grain yield, and its components.

Effect of glutathione foliar application: The gradual increase in plant growth, photosynthetic pigments, grain yield, and components was observed with the application of glutathione foliar at different levels, from zero to 150 to 300 mg/l, as shown in Table 3. Furthermore, when comparing glutathione foliar application with the non-application in both seasons, the application of 150 and 300 mg/l of glutathione foliar resulted in significant increases in plant growth, photosynthetic pigments, grain yield, and its components.

Glutathione foliar application at 150 and 300 mg/l, compared to its non-application, led to a significant increase in plant growth traits during both seasons, where shoot length increased by 8.27 and 12.13 & 8.28 and 13.08%, leaf area per plant by 7.54 and 10.56 & 8.56 and 13.58 %, no. of tillers per plant by 8.42 and 11.82 & 9.77 and 15.62 %, no. of leaves per plant by 8.57 and 12.35 & 9.19 and 14.47 %, and shoot dry weight per plant by 9.78 and 13.47 & 10.51 and 16.39 %, respectively.

Compared to the non-application, the glutathione foliar application of 150 and 300 mg/l significantly increased photosynthetic pigments traits in both seasons: chlorophyll a by 18.74 and 27.44 % & 25.37 and 37.18 %, chlorophyll b by 17.72 and 24.26 & 26.56 and 36.67 %, carotenoid by 17.12 and 24.30 & 25.70 and 36.17%, and the total photosynthetic pigments by 18.24 and 26.14 & 25.75 and 36.87 %, respectively.

The glutathione foliar application of 150 and 300 mg/l compared to non-application led to a significant increase in grain yield, and its components in both seasons, with spike length increased by 11.80 and 16.91 & 11.57 and 23.23 %, no. of spikes per plant by 12.64 and 18.32 & 10.27 and 21.22 %, no. of grains per spike by 10.61 and 14.74 & 11.47 and 22.71 %, straw yield per hectare by 12.41 and 17.75 & 10.84 and 22.07 %, and grain yield per hectare by 12.22 and 16.99 & 11.89 and 23.77 %, respectively. This indicated that the application of glutathione foliar significantly improved wheat plant growth, photosynthetic pigments, grain yield, and its components, with the highest concentrations (300 mg/l) resulting in the most significant improvements, indicating its potential as a beneficial addition to enhance the quality and quantity of these traits. The findings of Dawood *et al.*, (2020) substantiated the importance of glutathione and selenium in enhancing plant growth, photosynthetic pigments, grain yield, and its components wheat plants, thereby highlighting their significant role in plant health.

Interaction effects: The combined effects of water regimes and biochar application exhibited a significant impact on the plant growth, photosynthetic pigments, grain yield, and its components, with the highest values at the application of biochar at 20 tons ha^{-1} under well-watered conditions (Table 4). This pointed to the importance of the application of biochar under water stress conditions in enhancing plant growth, photosynthetic pigments, grain yield, and its components. This result was in harmony with previously published findings of Abdelsatar *et al.*, (2024), who indicated that combining deficit irrigation with biochar application can improve seed yield and its components of three sesame varieties grown in sandy soil conditions. Therefore, biochar is considered one of the best applications to improve wheat yield under water stress conditions.

As it can be seen from Table 4, the interaction of water regimes and glutathione foliar application was highly significant for plant growth, photosynthetic pigments, grain yield, and its components. Application of glutathione foliar, especially at the rate of 300 mg/l under various water regimes, especially under well-watered conditions, led to the highest improvement of plant growth, photosynthetic pigments, grain yield, and its components. This indicated that under optimal watering conditions, the glutathione foliar application significantly enhanced plant growth, photosynthetic pigments, grain yield, and its components for healthier and more vigorous growth, hence acting as a valuable tool for plant performance, as proven by Nahar *et al.*, (2015), Rehman *et al.*, (2021), and Mageed *et al.*, (2023).

The plant growth, photosynthetic pigments, grain yield, and its components were significantly enhanced by the combination of biochar and glutathione foliar application (Table 4), with the highest values observed when biochar application was increased to 20 tons per hectare and glutathione application was up to 300 mg/l. This suggested that the combination of biochar and glutathione can significantly enhance plant growth, photosynthetic pigments, grain yield, and its components by enhancing nutrient uptake, water stress tolerance, and plant metabolism, thus enhancing overall plant health. This suggested that the synergistic effects of these factors can be beneficial.

The plant growth, photosynthetic pigments, grain yield, and its components were considerably affected by biochar application and glutathione application with the interaction of water regimes (Table 4). The best triple interacting effects were noticed with increasing levels of biochar application at a rate of 20 tons per hectare and increasing levels of glutathione up to 300 mg/L under variable water regimes, especially under well-watering. The results indicated that the interaction of biochar and glutathione enhanced growth, photosynthetic pigments, grain yield, and its components in the plants. Biochar improved the water retention capacity of soil, while glutathione reduced oxidative stress. This suitable combination of water regimes with biochar and glutathione has a high potential for enhancing the pigment traits, particularly under well-watered conditions. It further pointed out the synergetic interactions of these factors, interacting together under water-stress conditions.

Table 4b. Interaction of water regimes (W) with biochar (B) and glutathione (G) foliar application on wheat photosynthetic pigments in both seasons.

W	B ton ha^{1}	G mg/l	Chlorophyll a		Chlorophyll b		Carotenoid		Total photosynthetic pigments	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
25% of ASMD	B 0	0	1.6024	1.3150	0.7559	0.6379	0.3712	0.3815	2.7296	2.3344
		150	1.7608	1.4827	0.8143	0.7353	0.4031	0.4317	2.9782	2.6497
		300	1.9375	1.6917	0.8812	0.8080	0.4371	0.4799	3.2558	2.9796
	B10	0	1.7770	1.5025	0.8189	0.7440	0.4069	0.4387	3.0028	2.6852
		150	1.9117	1.6456	0.8707	0.7920	0.4320	0.4739	3.2144	2.9116
		300	2.0158	1.7664	0.9055	0.8400	0.4503	0.4999	3.3716	3.1062
	B20	0	1.8218	1.5466	0.8328	0.7541	0.4157	0.4481	3.0703	2.7489
		150	1.9316	1.6861	0.8787	0.8066	0.4352	0.4790	3.2455	2.9717
		300	2.0206	1.7834	0.9071	0.8439	0.4513	0.5010	3.3790	3.1283
50% of ASMD	B 0	0	1.4100	1.1027	0.6527	0.5278	0.3200	0.3170	2.3827	1.9474
		150	1.5594	1.2741	0.7397	0.6245	0.3629	0.3725	2.6620	2.2711
		300	1.6740	1.3709	0.7790	0.6788	0.3839	0.4060	2.8369	2.4557
	B10	0	1.5580	1.2655	0.7388	0.6203	0.3617	0.3704	2.6585	2.2561
		150	1.7289	1.4518	0.8014	0.7219	0.3955	0.4253	2.9258	2.5990
		300	1.8255	1.5511	0.8346	0.7546	0.4172	0.4500	3.0773	2.7557
	B20	0	1.5164	1.1966	0.7089	0.5866	0.3457	0.3508	2.5710	2.1339
		150	1.7639	1.4856	0.8152	0.7375	0.4040	0.4331	2.9831	2.6562
		300	1.9489	1.7061	0.8841	0.8113	0.4384	0.4843	3.2714	3.0016
75% of ASMD	B 0	0	0.3923	0.0649	0.2133	0.0201	0.1267	0.0183	0.7323	0.1033
		150	1.1844	0.9059	0.5823	0.4323	0.2827	0.2545	2.0493	1.5927
		300	1.3733	1.0616	0.6423	0.5114	0.3173	0.3073	2.3329	1.8803
	B10	0	1.1844	0.9059	0.5823	0.4323	0.2827	0.2545	2.0493	1.5927
		150	1.5517	1.2563	0.7365	0.6154	0.3602	0.3670	2.6485	2.2387
		300	1.6227	1.3338	0.7631	0.6529	0.3751	0.3874	2.7610	2.3741
	B20	0	1.4259	1.1180	0.6581	0.5319	0.3232	0.3191	2.4072	1.9690
		150	1.6740	1.3709	0.7790	0.6788	0.3839	0.4060	2.8369	2.4557
		300	1.7516	1.4770	0.8113	0.7341	0.4008	0.4309	2.9637	2.6420
LSD 0.05			0.0326	0.0568	0.0206	0.0245	0.0178	0.0307	0.0435	0.0490

ASMD refers to available soil moisture depletion

Table 4c. Interaction of water regimes (W) with biochar (B) and glutathione (G) foliar application on wheat grain yield, and its components in both seasons.

W	B ton ha^1	G mg/l	Spike length		No. of spikes per plant		No. of grains per spike		Straw yield per hectare		Grain yield per hectare	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
25% of ASMD	B 0	0	15.59	16.11	17.25	19.91	56.82	56.62	15.61	15.77	9.47	9.73
		150	16.50	17.48	18.55	21.29	59.83	61.84	16.44	17.13	10.10	10.62
		300	17.50	18.90	19.99	23.08	62.49	66.58	17.45	18.63	10.66	11.52
	B10	0	16.66	17.65	18.68	21.59	60.01	62.34	16.54	17.35	10.17	10.72
		150	17.27	18.59	19.77	22.57	61.82	65.50	17.21	18.28	10.58	11.33
		300	17.83	19.47	20.47	23.69	63.48	68.50	17.90	19.19	10.92	11.91
	B20	0	16.90	17.98	19.03	21.80	60.80	63.21	16.81	17.68	10.29	10.94
		150	17.49	18.76	19.96	22.85	62.36	66.05	17.45	18.39	10.65	11.44
		300	17.86	19.62	20.52	23.84	63.92	68.62	17.93	19.23	10.94	11.94
50% of ASMD	B 0	0	13.99	14.14	15.55	17.81	52.06	50.07	13.52	14.26	8.57	8.57
		150	15.16	15.44	16.89	19.07	55.96	54.11	14.78	15.31	9.35	9.35
		300	15.78	16.71	17.79	20.44	58.05	59.01	15.40	16.35	9.81	10.15
	B10	0	15.13	15.77	16.87	19.52	55.58	55.47	14.75	15.51	9.33	9.52
		150	16.14	16.92	18.28	20.60	59.23	59.94	15.87	16.54	10.02	10.23
		300	16.85	18.01	19.14	21.84	60.88	63.36	16.47	17.80	10.31	10.94
	B20	0	14.81	15.13	16.47	18.76	54.31	53.30	14.24	15.09	9.09	9.20
		150	16.37	17.05	18.57	20.79	59.95	60.42	15.98	16.77	10.13	10.37
		300	17.50	18.98	20.09	23.19	62.54	66.77	17.30	18.69	10.74	11.57
75% of ASMD	B 0	0	7.65	5.69	9.04	7.32	30.77	21.34	7.32	5.70	4.89	3.29
		150	12.60	9.67	14.37	12.54	47.50	35.18	12.41	9.92	7.74	5.91
		300	13.77	13.85	15.29	17.54	51.58	49.23	13.35	13.86	8.44	8.37
	B10	0	12.60	12.55	14.37	16.26	47.50	44.47	12.41	12.73	7.74	7.59
		150	15.11	14.38	16.83	17.94	55.53	50.67	14.70	14.47	9.31	8.77
		300	15.52	16.29	17.36	20.07	57.05	57.23	15.11	15.95	9.52	9.89
	B20	0	14.06	14.26	15.69	17.88	52.46	50.47	13.55	14.37	8.60	8.75
		150	15.78	15.94	17.79	19.72	58.05	56.03	15.40	15.59	9.81	9.62
		300	16.32	17.48	18.52	21.29	59.65	61.84	15.97	17.13	10.10	10.62
LSD 0.05			0.80	1.19	1.06	1.35	3.28	4.34	0.81	1.10	0.50	0.69

ASMD refers to available soil moisture depletion

Conclusion

We can, therefore, conclude that the use of biochar and glutathione foliar application markedly mitigated the adverse effects of water stress on plant growth, photosynthetic pigments, grain yield, and its components, proving that these sustainable methods enhance wheat production under water-stressed conditions with the application of biochar at 20 tons per hectare and glutathione at 300 mg/l.

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