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Biochar application affected biochemical properties, yield and nutrient content of safflower under water stress

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A two-year field trial was set up to investigate the effects of applying 3 tons ha^{-1} of wheat (3WB) and cotton biochar (3CB) alone or in combination with chemical nitrogen (N) and phosphorus (P) fertilizers on biochemical properties, yield and nutrient content of safflower under normal irrigation and water stress (irrigation cut-off at flowering stage) conditions. The total water applied in the chemical treatments [150 kg ha^{-1} N + 50 kg ha^{-1} P (100% of the recommended dose) and 112.5N + 37.5P (75% of the recommended dose)] under water stress, was significantly higher than other treatments. Application of 112.5N + 37.5P + 3CB increased RWC from 57.5 to 59.4% and the total chlorophyll content from 80.7 to 128.1%, compared to the control. The carotenoid content, catalase and peroxidase in 112.5N + 37.5P + 3CB were lower than chemical fertilizers. Under water stress, the seed yield of 112.5N + 37.5P + 3CB was 10.2–12.6% higher than 112.5N + 37.5P + 3WB. The higher chlorophyll content, RWC, remobilization efficiency and nutrient content in 112.5N + 37.5P + 3CB compared to other treatments was associated with seed yield enhancement. The findings indicate that the combination of CB with 75% recommended dosage of N and P, may be the optimal approach for enhancing safflower production under water stress conditions.

Keywords Chemical fertilizer, Cotton biochar, Chlorophyll content, Relative water content, Remobilization efficiency

In arid and semiarid regions of the world, water stress (drought stress) poses a significant threat to biomass production and seed yield[1]. The severity of water stress at the reproductive stages is difficult to predict and is influenced by various factors including time of the most recent precipitation, the soil's capacity to retain water and the rate of evaporation. Safflower (*Carthamus tinctorius* L.) is a widely cultivated crop in the southern regions of Iran. Recently, the cultivation of this crop has witnessed an increase among the farmers, due to its tolerance to water stress at the vegetative stages[2]. Although various cultivars of safflower typically exhibit tolerance to water deficit during the vegetative stages, it has been observed that flowering and seed-filling stages are sensitive to irrigation cut-off after flowering stage[3]. In the aforementioned stages, the occurrence of reduced rainfall and the onset of water stress prompt crops to respond to water stress by undergoing certain biochemical changes. These changes include a decrease in pigment content[4] and RWC[5,6], while some antioxidant enzymes such as catalase (CAT) and peroxidase (POX) may experience an increase[7].

To mitigate the adverse impact of water stress on the growth and yield of safflower, the application of biochar can be considered as a viable strategy in arid environments. This is primarily due to significant water retention capacity exhibited by biochar. Additionally, it has been found that biochar has the potential to alleviate the adverse impact of chemical fertilizers when applied at the recommended dosage[6,8,9]. Biochar is a carbonaceous substance produced via the process of pyrolysis and has various benefits. It has been found that biochar has a significant role to improve chemical and physical properties of soil, facilitate the gradual release of nutrients, and promote the accessibility and absorption of nutrients by plant roots[10].

Improvement in crop yield and biomass production is influenced by various factors including crop type, soil properties, water holding capacity of the soil, and biochar characteristics[11]. Liu et al.[12] conducted a comprehensive analysis of field and greenhouse experiments from 21 countries. Their findings revealed that the application of

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biochar resulted in an average increase of 11% in grain yield. On the contrary, the addition of biochar to the soil has been found to have a beneficial impact on nutrient availability and water retention capacity. This, in turn, leads to an increase in grain yield[9,11]. Biochar application has been shown to have an increase in water storage capacity and nutrient availability including phosphorus (P), calcium (Ca) and magnesium (Mg)[13]. Furthermore, the application of biochar has been found to have positive effects on plant growth, water stress tolerance and N in leaves[14]. Under conditions of limited P availability, the addition of biochar in conjunction with chemical fertilizers containing N and P has been found to increase the grain yield. However, when biochar is applied alone, it leads to a decrease in the chlorophyll content in leaves[15].

Water stress, especially at the reproductive stages of crops in southern Iran, is one of the main problems which limits crop productivity. Unfortunately, in some years, there is a lack of sufficient rainfall from April to June when the water requirement for the crop enhances to complete seed filling period and farmers have to irrigate the crops after the flowering. On the contrary, excessive utilization of chemical fertilizers, particularly urea, has been found to result in water and soil pollution, as well as elevated production costs[16]. Biochar application appears to be a viable approach for improving nutrient uptake and mitigate the adverse effects of water stress.

Little information has been published regarding the effect of different sources of biochar on the biochemical and physiological characteristics of safflower under late-season water stress. We hypothesized that the utilization of different source of biochar in conjunction with a reduced dosage of chemical fertilizer could potentially have a positive outcome in terms of photosynthetic pigments, RWC, assimilate remobilization, nutrient uptake and ultimately enhance safflower yield under water stress conditions. In fact, the assessment of plant's biochemical and physiological attributes plays a crucial role in understanding the biochar mechanism's ability to improve the safflower yield under water stress conditions. According to the biochemical and physiological characteristics of safflower, the identification of the optimal biochar type and determination of the appropriate dosage of chemical fertilizers under water stress conditions would greatly benefit farmers. Therefore, the objective of this study, is to investigate the impact of applying biochar derived from cotton and wheat either individually or in conjunction with 75% and 100% of the recommended nitrogen and phosphorous dosage on the changes of biochemical and physiological characteristics, as well as the yield of safflower subjected to water stress conditions.

Materials and methods

Field experiments and treatments

A two-year field experiment was conducted to study the application of biochar and chemical fertilizers on the biochemical and physiological characteristics, yield, and yield components of safflower under late-season water stress conditions. The experiment was conducted at the College of Agriculture and Natural Resources of Darab (28° 45.0′ N, 54° 26.8′ E), Fars province, Iran, during the 2019 and 2020 growing seasons. Before the experiment, composite soil samples were collected from five points of the field using an auger. The samples were taken from depths of 0–15 and 15–30 cm. Then, the soil samples were air-dried, sieved (< 2 mm) and analyzed for pH in the saturated paste[17], electrical conductivity (EC) in the saturated extract[18], soil texture using the hydrometer method[19] and organic carbon by wet oxidation through chromic acid and back-titrated with ferrous ammonium sulfate[20]. Also, total nitrogen (N) was measured using the Kjeldahl method[21]. Available phosphorus (P) was determined through bicarbonate extraction[22]. Available potassium (K) was extracted by shaking 5 g of soil with 25 ml of 1.0 M NH_4OAc (pH 7.0) for 10 min. Available iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were measured by adding 10 g of soil to a solution containing 20 mL of 0.005 M diethylentriamine pentaacetic acid, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ (pH 7.3), followed by 2 h of shaking[23].

The soil type of the experimental site is classified as fine-loamy, carbonatic, hyperthermic Typic Torriorthents[24]. The physical and chemical properties of the soil (depth of 0–30 cm) at the experimental site is shown in Table 1. Moreover, minimum and maximum air temperatures, monthly rainfall, and pan evaporation of the experimental site during the 2019–2020 and 2020–2021 growing seasons are given in Table 2.

	Soil	depth
Soil property	(0–15 cm)	(15–30 cm)
Sand (%)	39.0	41.0
Silt (%)	41.6	41.6
Clay (%)	19.4	17.4
pH	8.37	8.00
EC (dS m^{-1})	0.34	0.74
Organic carbon (%)	0.7	0.2
Total N (%)	0.03	0.01
Available P (mg kg^{-1})	22	26
Available K (mg kg^{-1})	170	130
Available Fe (mg kg^{-1})	0.68	1.24
Available Mn (mg kg^{-1})	7.49	0.64
Available Zn (mg kg^{-1})	1.01	0.54
Available Cu (mg kg^{-1})	3.05	0.47

Table 1. Physical and chemical properties of the soil (depth of 0–30 cm) in the experimental site.

Month	Temperature (°C)						Rainfall (mm)		Pan evaporation (mm)	
	2019–2020			2020–2021						
	Min	Max	Mean	Min	Max	Mean	2019	2020	2019	2020
December	6.3	19.8	13.0	6.4	23.1	14.7	117.3	0	70.0	92.6
January	3.9	17.6	10.75	5.9	16.8	11.3	122.8	169.3	71.1	61.2
February	3.8	18.3	11.0	4.7	19.1	11.9	24.6	7.1	108.2	84.5
March	7.9	23.1	15.5	9.5	25.4	17.4	9.4	3.0	147.3	142.3
April	10.8	23.6	17.2	13.6	31.5	22.5	58.7	0.9	125.3	234.1
May	18.1	31.9	25.0	18.3	34.8	26.5	0.0	1.9	244.2	286.1
June	21.1	40.5	30.8	23.3	41.9	32.6	2.6	0	356.2	403.9
Total							335.4	182.2	1122.3	1304.7

Table 2. Minimum and maximum air temperatures, monthly rainfall, and pan evaporation of the experimental site, during 2019–2020 and 2020–2021 growing seasons.

Each year, the experiment was carried out using a split plot design based on a randomized complete block design with three replicates. Treatments consisted of an irrigation regime as the main plot at two levels: normal irrigation, and water stress (irrigation cut-off at flowering stage; stage 61, based on the BBCH scale illustrated by Flemmer et al.[25]. Additionally, fertilizer type was included as subplot with the following treatments: control (C; without fertilizer), application of 3 ton ha^{-1} wheat biochar (3WB), application of 3 ton ha^{-1} cotton biochar (3CB), application of 150 kg ha^{-1} nitrogen (N) + 50 kg ha^{-1} phosphorus (P) (150N + 50P), application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P (112.5N + 37.5P), application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3WB (112.5N + 37.5P + 3WB), and application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3CB (112.5N + 37.5P + 3CB). While irrigated cotton and wheat cultivation is widespread in arid and semi-arid regions of Iran, their residues may be suitable for feedstock or biochar production. Addition of 3 ton ha^{-1} of biochar has been obtained according to the average yield of wheat and cotton residues in the agricultural lands of the region (6–8 ton ha^{-1}) and the average yield of biochar production (40–50%). The amount of biochar in this experiment was calculated based on the fact that when wheat and cotton residues are added to the soil (without converting it into biochar), typically 150 kg ha^{-1} urea (69 kg ha^{-1} N) and 50 kg of triple superphosphate (9.5 kg ha^{-1} P) is added to balance the C/N ratio. On the other hand, a preliminary experiment in the greenhouse showed that a 25% decrease in N and P doses did not significantly affect the yield.

The plot size was 3 m × 2 m, and it was surrounded by a 40 cm high earth-band with an 80 cm wide buffer space between the plots. The seedbed was prepared by moldboard plowing and disking. Seeds of safflower (cv. Goldasht) were hand-sown at a depth of 2 cm, with a row width of 50 cm, and a planting density of 40 plants per square meter on December 15th, 2019 and December 16th, 2020, respectively. Goldasht is a thornless and dwarf cultivar adapted to semi-arid regions. It is noteworthy that the use of plant in the present study complies with international, national and/or institutional guidelines.

Based on the soil test (Table 1), nitrogen (N) was applied as urea source at a rate of 150 kg ha^{-1} (100% of the recommended dose) and 11.2 kg ha^{-1} (75% of the recommended dose) and phosphorus (P) was applied as triple superphosphate source at a rate of 50 kg ha^{-1} (100% of the recommended dose) and 37.5 kg ha^{-1} (75% of the recommended dose) were used in the field experiment. In treatments with chemical fertilizers, total P as triple superphosphate (19% P) was incorporated into the soil before planting as the source of total P. Urea (46% N) was used as the source of nitrogen (N) in each plot, applied in three splits. One-third of the urea was used before sowing, another third at the branching stage [stage 20; Flemmer et al.[25]], and the remaining amount at the stem elongation stage (stage 39). Also, in each biochar treatment, before the safflower planting, wheat and cotton biochar were added into the soil as 3 ton ha^{-1} and each plot was plowed in order to completely mix the biochar with the soil.

The gravimetric method was used in each plot to monitor the soil water status at the root zone[26]. The soil profile was sampled at 30 cm depth down to 90 cm, using an auger. Then, the volume of water applied in normal irrigation was adjusted to restore root zone moisture deficit in the root zone. This was done when 50% of the available water was depleted to a depth of 90 cm reaching near-field capacity. A surface drip irrigation system was used for irrigation. A 20 mm diameter polyethylene pipe with in-line drippers at 40 cm intervals was placed on one side of each planting row. Overall, the plots were irrigated four times for normal irrigation and two times for irrigation cut-off at the flowering stage (water stress treatment). Climate monitoring of the region (Table 2) showed that safflower may experience water stress due to the limited precipitation during the flowering stage of plant in typical years. The total water applied (m^3) (irrigation amount + rainfall) in each irrigation regime and cropping system during the 2019 and 2020 growing seasons is presented in Fig. 1.

Biochar preparation and analysis

The biochar of wheat and cotton was produced from abundant plant residues in Darab region, located in southern of Iran. The plant residues were dried and ground to pass through a 2-mm sieve. The dried and ground plant residues (at 60 °C for 24 h) were pyrolyzed in a muffle furnace (Shimifan, F47) under limited oxygen conditions at a temperature of 400 °C for 4 h after being dried and ground at 60 °C for 24 h. The temperature was

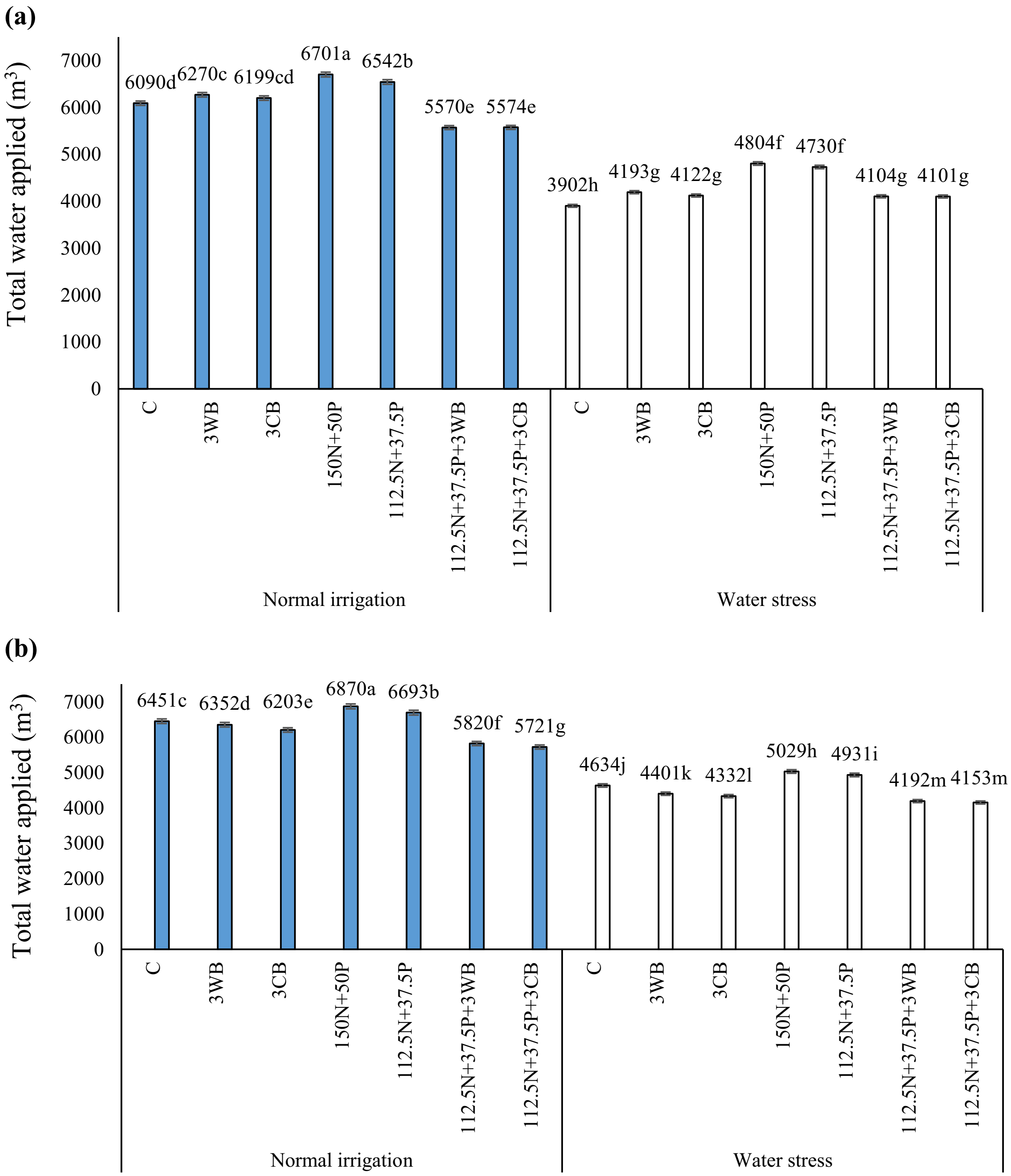

Fig. 1. Interaction effect of irrigation regime and fertilizer type on the total water applied of safflower during the 2019 (**a**) and 2020 (**b**) growing seasons. Means followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

increased at a rate of 5 °C min^{-1}. Biochar was ground and sieved (< 0.5 mm) before being applied to the soil[9]. Then, some characteristics of the biochar including the pH value[27], EC and the contents of carbon (C), hydrogen (H), nitrogen (N), phosphorus (P), potassium (K), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were determined[28] (Table 3).

To determine the photosynthetic pigments, antioxidant enzyme activity, and RWC, the upper five leaves of four plants were sampled and mixed together in each plot and considered as one replication. Mixed samples were then divided into 3 laboratory samples to check the reproducibility and accuracy of the measurements. The plant samples were taken when the capitula reached 50% of its final size (stage 75 based on the BBCH scale illustrated by[25]).

Biochar properties	Wheat residue	Wheat biochar	Cotton residue	Cotton biochar
pH	6.11	10.58	6.31	10.10
EC (dS m^{-1})	2.44	7.64	1.67	3.52
Total N (%)	2.5	1.1	3.1	3
Total P (%)	0.03	0.07	0.09	0.18
Total K (%)	0.36	0.63	0.30	0.74
Total Fe (mg kg^{-1})	85	183	201	361
Total Mn (mg kg^{-1})	27	50	151	241
Total Zn (mg kg^{-1})	10	18	15	27
Total Cu (mg kg^{-1})	3	5	6	10

Table 3. Some characteristics of the wheat and cotton biochar.

Chlorophyll and carotenoid content assessment

The chlorophyll content was measured by fresh tissue of the top leaf in each plot. Ten ml of 80% acetone was gradually added to 200 mg of leaf tissue and ground by a mortar and pestle. The created slurry was centrifuged for 10 min at 4000 rpm. The supernatant was then filtered using Whatman No. 2 filter paper, which was placed in a funnel during the transfer of the solution. Absorbance was measured by a double-beam UV–VIS spectrophotometer (UV-1900 spectrophotometer, Shimadzu, Japan) at wavelengths of 645 nm, 663 nm, and 470 nm. Chlorophyll *a*, *b*, total chlorophyll, and carotenoid levels were determined according to the method described by Lichtenthaler and Buschmann[29].

Antioxidant enzymes assay

The catalase enzyme activity (CAT) was determined using a spectrophotometer (UV-160A) according to the method described by Aebi[30]. CAT activity was expressed as units (µmol H_2O_2 consumed per minute) per milligram of protein. The peroxidase enzyme activity (POD), was evaluated using the method of Chance and Maehly[31]. POD was expressed as units (µmol guaiacol oxidized per minute) per milligram of protein.

Leaf relative water content

The leaf discs (8 mm in diameter) were taken from the top five leaves of safflower. Then, the relative water content (RWC) of the leaves was measured using the method of Machado and Paulsen[32].

Dry matter remobilization and remobilization efficiency

To determine the dry matter remobilization and remobilization efficiency of safflower, five plants in each treatment were harvested at the flowering and maturity stages**.** These traits were calculated according to Ercoli et al.[33] and Dordas[34] as follows:

$$\text{Dry matter remobilization}\ \left(\text{g m}^{-2}\right) = \text{dry matter at flowering} \\ - \text{dry matter of vegetative plant parts, including leaf, culm, and capitula, at maturity.}$$

$$\text{Remobilization efficiency (\%)} = \left(\text{dry matter remobilization/dry matter of the whole plant at flowering}\right) \times 100$$

Seed nutrient analysis

The macro and micronutrients of the harvested safflower seeds were determined using the following procedure. The dry grain obtained from each experimental plot was powdered using an electric mill, then ashed at 550 °C, and digested with 2 M HCl. The total nitrogen content of the grain was determined by the Kjeldahl method[21]. The total P concentration was determined calorimetrically, and the total K concentration was determined using a flame photometer (Corning 510, UK). Also, the total concentration of Fe, Cu, Zn, and Mn in the acid extract was determined using atomic absorption spectroscopy (PG 990, PG Instruments Ltd., UK).

Plant harvesting and analysis

Plants within an area of 1 m^2 from the center of the plots were hand-harvested at the physiological maturity (June 20, 2020 and June 19, 2021). The dry weight of the harvested plants was calculated after oven-drying at 70 °C for 48 h. Subsequently, yield components including number of capitula per plant, the number of seeds per capitula, the 1000-seed weight and the seed yield were determined.

Statistical analysis

Data were analyzed using SAS software 2012 (version 9.4), and the means were compared by Duncan's multiple range test (DMRT) at a 0.05 probability level ($p \le 0.05$). To check the normal distribution of data, Kolmogorov–Smirnov and Shapiro–Wilk tests were used and the skewness and kurtosis indices of data confirmed that the distribution of data was normal. A combined analysis of variance was used to analyze the data from the two years of the experiment. To reveal the relationships between variables and plant characteristics, correlation coefficients and stepwise linear regression analysis were utilized. Moreover, the principal component analysis (PCA) was conducted to condense a large set of traits into smaller sets and more interpretable sets of variables. One of the main purposes of the PCA is to determine variance using the minimum number of components.

Results

Analysis of variance

Results of the combined analysis of variance over two years showed that the year significantly affected the seed yield ($p \leq 0.05$) (Supplementary file S1) due to the variations of mean temperature, rainfall, and evaporation between the 2 years (Table 2). In addition, the interaction effects of year × irrigation regime, year × fertilizer type and irrigation regime × fertilizer type were significant at a 0.05 probability level (Supplementary file S1).

Weather conditions and total water applied

The study area had a semi-arid climate with cold and rainy winters as well as, warm and dry summers with little to no rainfall. According to Table 2, the average monthly temperature, and evaporation amounts in the second year were higher than those in the first year of the experiment. In both years, the minimum monthly temperatures were recorded from December to February, while the maximum temperatures were recorded from May to June. Total rainfall during the safflower growth stages in the 2019 and 2020 growing seasons was 335.4 and 182.2 mm, respectively. This means that in the first year, there was 153.2 mm more rainfall compared to the second year, which represents an 84% increase. The temporal distribution of rainfall in the first year was better than in the second year. In the first year, there was a considerable amount of rainfall in December (117.3 mm), January (122.8 mm) and April (58.7 mm). However, in the second year, the only month with substantial rainfall was January (169.3 mm). Unfortunately, for two consecutive years, there was no effective rainfall from May to June, a critical period when the water requirement for seed filling of safflower increases significantly. On the other hand, due to the higher average temperature in the second year, the amount of evaporation in 2020 (1304.7 mm) was higher than 2019 (1122.3 mm).

The total water applied (Fig. 1) included the irrigation amount and rainfall, which were determined in each treatment. Overall, the total amount of water applied in all treatments in 2020 (Fig. 1b) was greater than in 2019 (Fig. 1a). In both years, the total amount of water applied in 150N + 50P and 112.5N + 37.5P treatments, under both normal irrigation and water stress conditions, was significantly higher than in the other treatments ($p \leq 0.05$). In fact, in the second year, there was an increase in the average temperature and evaporation demand, particularly from April to June (Table 2). This resulted in an increase in the total water applied compared to the first year. The application of wheat and cotton biochar, either alone or in combination with chemical fertilizers, significantly reduced the total water applied for safflower compared to the recommended dose of chemical fertilizers ($p \leq 0.05$) (Fig. 1).

Chlorophyll and carotenoid contents

In both years, the interaction effect of the irrigation regime and fertilizer type influenced the pigment content of safflower (Table 4). Chlorophyll *a* content in all treatments under normal irrigation was higher than treatments under water stress. Additionally, the treatments that included chemical fertilizers, either alone or in combination with biochar had the highest chlorophyll *a* content compared to treatments with biochar application alone (3WB or 3CB) and control (without fertilizer). Also, in all treatments, the chlorophyll *a* content in the first year was higher compared to the second year. Water stress, negatively affected the chlorophyll *b* content of safflower in the late-season (Table 4). In 2019 and 2020, the highest amount of chlorophyll *b* content in both irrigation regimes was observed in the 112.5N + 37.5P + 3WB and 112.5N + 37.5P + 3CB treatments. Overall, in both years and irrigation regimes, combined application of biochar with chemical fertilizers improved the chlorophyll *b* content compared to 3WB and 3CB alone. The range of chlorophyll *b* content was 0.79 ± 0.01 to 0.98 ± 0.01 mg/g FW under normal irrigation and 0.45 ± 0.01 to 0.53 ± 0.04 mg/g FW under water stress. Total chlorophyll was found to be susceptible to water shortage. Under water stress in all fertilizer treatments, the amount of total chlorophyll decreased sharply compared to normal irrigation (Fig. 2a,b). At both irrigation levels, the application of cotton or wheat biochar in combination with chemical fertilizer had a significant effect on total chlorophyll compared to the application of biochar alone. Under water stress in the control treatment, the carotenoid content in 2019 (0.28 ± 0.01 mg/g FW) and 2020 (0.33 ± 0.05 mg/g FW) significantly increased compared to the other treatments ($p \leq 0.05$) (Table 4). In contrast, the lowest carotenoid content in both of the irrigation regimes was observed in 112.5N + 37.5P + 3WB and 112.5N + 37.5P + 3CB treatments. In both of the irrigation regimes, application of biochar alone and or combined with chemical fertilizers decreased the carotenoid content compared to control, significantly ($p \leq 0.05$). Similar to the total chlorophyll, the carotenoid content in the first year was higher than the second year for all treatments (Table 4).

Catalase and peroxides activity

Results of two years showed that catalase activity (CAT) increased significantly in the control treatment for both irrigation regimes (Table 4). Under water stress, increasing the CAT in control plants can alleviate the negative impacts of water deficit. In contrast, the combination of biochar with chemical fertilizers resulted in the lowest CAT activity ranging from 1.02 ± 0.17 to 1.23 ± 0.02 Unit mg^{-1} protein in normal irrigation and ranging from 1.22 ± 0.09 to 1.74 ± 0.03 Unit mg^{-1} protein when safflower plants were exposed to water stress. In two irrigation regimes, the combined application of biochar with chemical fertilizers (112.5N + 37.5P + 3WB and 112.5N + 37.5P + 3CB) decreased the CAT activity compared to 3WB and 3CB application alone. Peroxidase (POX) is another antioxidant enzyme that is enhanced by water stress and without fertilizer application (Table 4).

Irrigation regime	Fertilizer type	Chlorophyll *a* content (mg g^{-1} FW)		Chlorophyll *b* content (mg g^{-1} FW)		Carotenoid content (mg g^{-1} FW)		Catalase (units mg^{-1} protein)		Peroxidase (units mg^{-1} protein)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Normal irrigation	C	0.82 ± 0.03^{h}	0.74 ± 0.03^{i}	0.39 ± 0.02^{cd}	0.29 ± 0.01^{ij}	0.19 ± 0.01^{bc}	0.25 ± 0.01^{c}	1.94 ± 0.09^{e}	2.14 ± 0.02^{f}	1.98 ± 0.09^{de}	2.02 ± 0.14^{d}
	3WB	1.18 ± 0.16^{def}	1.10 ± 0.01^{f}	0.44 ± 0.11^{bcd}	0.36 ± 0.01^{g}	0.14 ± 0.01^{efg}	0.22 ± 0.01^{ef}	1.87 ± 0.14^{e}	2.01 ± 0.01^{g}	1.78 ± 0.14^{e}	1.88 ± 0.17^{de}
	3CB	1.25 ± 0.08^{de}	1.21 ± 0.01^{e}	0.43 ± 0.12^{bcd}	0.40 ± 0.01^{f}	0.13 ± 0.02^{efg}	0.21 ± 0.02^{f}	1.92 ± 0.08^{e}	1.97 ± 0.03^{h}	1.77 ± 0.19^{e}	1.83 ± 0.15^{e}
	150N + 50P	1.65 ± 0.04^{ab}	1.42 ± 0.03^{c}	0.58 ± 0.07^{b}	0.55 ± 0.02^{c}	0.12 ± 0.04^{efg}	0.18 ± 0.01^{f}	1.14 ± 0.06^{gh}	1.43 ± 0.01^{k}	1.40 ± 0.10^{f}	1.43 ± 0.06^{f}
	112.5N + 37.5P	1.30 ± 0.20^{cd}	1.33 ± 0.01^{d}	0.52 ± 0.08^{bc}	0.50 ± 0.02^{d}	0.12 ± 0.02^{efg}	0.19 ± 0.02^{g}	1.26 ± 0.06^{g}	1.50 ± 0.01^{j}	1.48 ± 0.07^{f}	1.58 ± 0.12^{f}
	112.5N + 37.5P + 3WB	1.64 ± 0.15^{a}	1.54 ± 0.02^{b}	0.91 ± 0.02^{a}	0.79 ± 0.01^{b}	0.11 ± 0.01^{g}	0.15 ± 0.02^{h}	1.09 ± 0.02^{gh}	1.23 ± 0.02^{m}	1.11 ± 0.09^{g}	1.15 ± 0.04^{g}
	112.5N + 37.5P + 3CB	1.69 ± 0.14^{a}	1.63 ± 0.03^{a}	0.98 ± 0.01^{a}	0.83 ± 001^{a}	0.11 ± 0.03^{g}	0.13 ± 0.02^{i}	1.02 ± 0.17^{gh}	1.12 ± 0.02^{n}	0.99 ± 0.01^{g}	1.02 ± 0.03^{g}
Water stress	C	0.78 ± 0.03^{h}	0.59 ± 0.01^{j}	0.31 ± 0.02^{d}	0.22 ± 0.02^{k}	0.28 ± 0.01^{a}	0.33 ± 0.01^{a}	2.88 ± 0.09^{a}	2.96 ± 003^{a}	2.75 ± 0.15^{a}	2.78 ± 0.11^{a}
	3WB	0.86 ± 0.06^{gh}	0.76 ± 0.02^{i}	0.41 ± 0.14^{cd}	0.31 ± 0.02^{hi}	0.16 ± 0.03^{cde}	0.24 ± 0.01^{cd}	2.11 ± 0.10^{d}	2.45 ± 01^{d}	2.22 ± 0.10^{c}	2.53 ± 0.16^{b}
	3CB	0.90 ± 0.11^{gh}	0.79 ± 0.02^{h}	0.40 ± 0.13^{cd}	0.32 ± 0.01^{h}	0.15 ± 0.01^{def}	0.23 ± 0.01^{de}	1.88 ± 0.07^{e}	2.22 ± 0.01^{e}	2.44 ± 0.07^{b}	2.31 ± 0.17^{c}
	150N + 50P	1.07 ± 0.07^{efg}	0.87 ± 0.01^{g}	0.35 ± 0.05^{d}	0.28 ± 0.01^{j}	0.18 ± 0.01^{bcd}	0.24 ± 0.02^{cd}	2.65 ± 0.14^{b}	2.77 ± 0.02^{b}	2.55 ± 0.31^{ab}	2.70 ± 0.12^{ab}
	112.5N + 37.5P	0.99 ± 0.14^{fgh}	0.76 ± 0.02^{i}	0.36 ± 0.07^{d}	0.25 ± 0.02^{k}	0.21 ± 0.02^{b}	0.27 ± 0.01^{b}	2.41 ± 0.08^{c}	2.57 ± 0.03^{c}	2.57 ± 0.14^{ab}	2.55 ± 0.13^{b}
	112.5N + 37.5P + 3WB	1.51 ± 0.10^{ab}	1.34 ± 0.02^{d}	0.53 ± 0.04^{bc}	0.45 ± 0.01^{e}	0.14 ± 0.01^{efg}	0.19 ± 0.02^{g}	1.58 ± 0.06^{f}	1.74 ± 0.03^{i}	2.05 ± 0.10^{cd}	1.80 ± 0.08^{e}
	112.5N + 37.5P + 3CB	1.47 ± 0.15^{bc}	1.39 ± 0.03^{c}	0.52 ± 0.05^{bc}	0.48 ± 0.01^{d}	0.12 ± 0.01^{efg}	0.18 ± 0.02^{f}	1.22 ± 0.09^{g}	1.40 ± 0.04^{l}	1.80 ± 0.06^{e}	2.22 ± 0.11^{c}

Table 4. Interaction effect of irrigation regime and fertilizer type on biochemical properties of safflower in 2019 and 2020 growing seasons. Means in each column followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

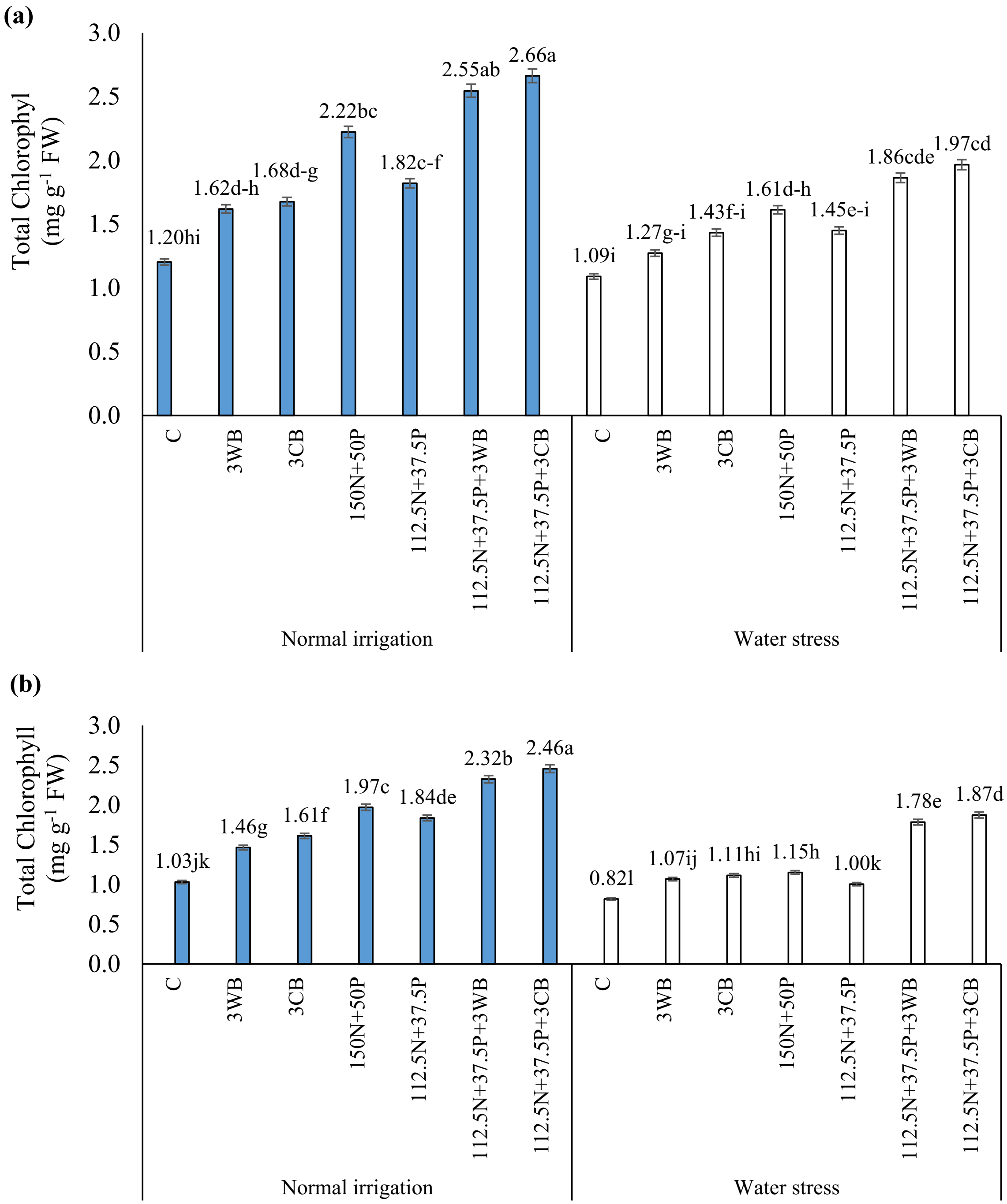

Fig. 2. Interaction effect of irrigation regime and fertilizer type on total chlorophyll during the 2019 (**a**) and 2020 (**b**) growing seasons. Means followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

Similarly, when safflower plants were subjected to water stress after the flowering stage, POX was positively enhanced in each fertilizer treatment compared to normal irrigation. In the control treatment under water stress, the activity of POX increased by 38.8% and 37.6% compared to normal irrigation in 2019 and 2020, respectively. On the other hand, the POX activity in combined treatments of biochar with chemical treatment was lower than biochar application alone. In addition, in the second year, there was an increase in mean temperatures and evaporation demand as well as, a decrease in rainfall at reproductive stages (Table 2). As a result, the levels of CAT and POX activity were generally higher compared to the first year.

Leaf relative water content (RWC)

Relative water content (RWC) was affected by the irrigation regime and type of fertilizer (Fig. 3). In both years, under normal irrigation, the treatment with 112.5N + 37.5P + 3CB resulted in the highest RWC with an increase of 82.4% and 63.9% compared to the control in 2019 (Fig. 3a) and 2020 (Fig. 3b), respectively. Under water stress, the application of cotton or wheat biochar combined with the chemical fertilizer, created the highest RWC, significantly ($p \leq 0.05$). After the combined treatment, the application of cotton and wheat biochar alone showed better performance in maintaining the RWC under water shortage. It appears that the application of biochar alone or in combination with chemical fertilizers, is more efficient in improving RWC under water stress, compared to using chemical fertilizers alone. Overall, in all irrigation regimes and fertilizer types, RWC in the second year (Fig. 3b) was lower than in the first year (Fig. 3a).

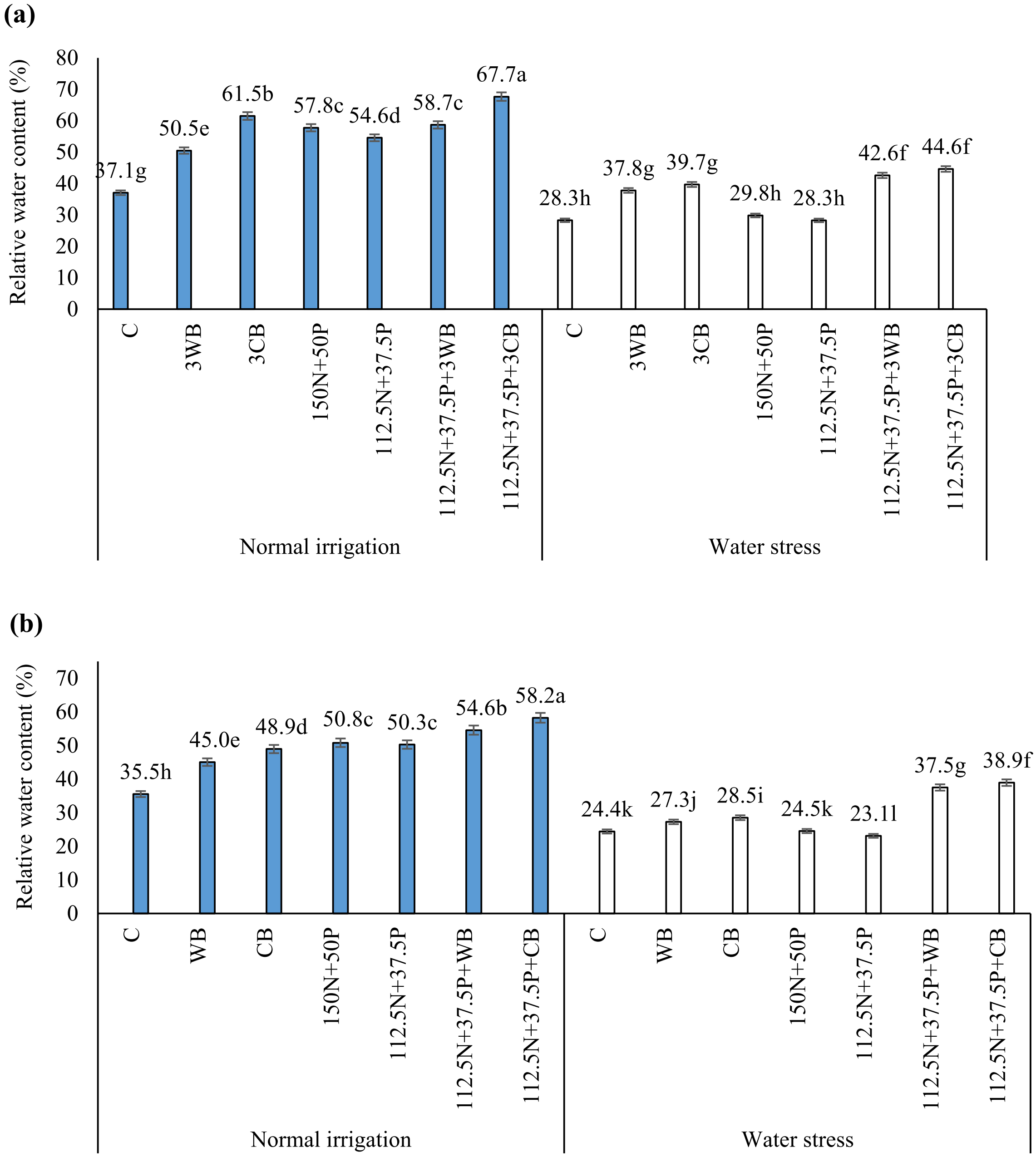

Fig. 3. Interaction effect of irrigation regime and fertilizer type on relative water content (RWC) during the 2019 (**a**) and 2020 (**b**) growing seasons. Means followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

Yield and yield components of safflower

The interaction effect of irrigation regime and fertilizer type was significant on the number of capitula per plant (Table 5). In both years, the number of capitula per plant was one of the main yield components which were affected by water stress. In each irrigation regime, the highest number of capitula per plant was obtained in the 112.5N + 37.5P + 3CB and 112.5N + 37.5P + 3WB treatments. Also, under water stress in each fertilizer treatment, the number of capitula per plant significantly decreased ($p \leq 0.05$) compared to normal irrigation. In both years, the number of seeds per capitula in each fertilizer treatment decreased due to water stress (Table 5). In 112.5N + 37.5P + 3CB and 112.5N + 37.5P + 3WB, the number of seeds per capitula was increased by 48.5–60.2% under normal irrigation and 40.1–47.1% under water stress conditions. In all of the fertilizer treatments, water stress after the flowering stage had a greater impact on the 1000-seed weight compared to normal irrigation (Table 5). Under water stress, the 1000-seed weight in the treatment with 112.5N + 37.5P + 3CB increased by 41.1% and 43.3% compared to the control in 2019 and 2020, respectively. In both years, under normal irrigation conditions, 112.5N + 37.5P + 3CB, 112.5N + 37.5P + 3WB, and 150N + 50P treatments had the highest seed yield, showing significant differences when compared together (Fig. 4). Under water stress, 112.5N + 37.5P + 3CB and 112.5N + 37.5P + 3WB treatments had the highest seed yield, demonstrating significant differences compared to the other treatments. However, it was observed that biochar application alone was not effective in alleviating the detrimental effects of drought stress as compared to the combined treatments. Overall, the number of capitula per plant, the number of seeds per capitula, 1000-seed weight (Table 5) and seed yield (Fig. 4) in the first year were higher than in the second year.

Dry matter remobilization and remobilization efficiency

In both years and irrigation regimes, the 112.5N + 37.5P + 3CB treatment significantly enhanced dry matter remobilization ($p \leq 0.05$). Remobilization increased by 66.8–79.5% under normal irrigation and 61.5–77.7% under water stress conditions compared to the control (Table 5). The control treatment of two irrigation regimes resulted in the lowest dry matter remobilization ranging from 10.3.0 ± 1.5 to 139.7 ± 2.0 g m^{-2}. Under water stress, the application of biochar with chemical fertilizer had a more noticeable effect on increasing the remobilization of the dry matter compared to the use of chemical fertilizers alone. In all treatments, the remobilization efficiency in the first year was higher than in the second year, particularly under water stress (Table 5). Under water stress, the remobilization efficiency increased from 19.60 ± 0.5% and 15.9 ± 0.04% in the control to 39.5% and 33.6% in the 112.5N + 37.5P + 3CB treatment in 2019 and 2020, respectively. Overall, application of biochar alone or combined with chemical fertilizers improved the dry matter remobilization and remobilization efficiency higher than control and or chemical fertilizer treatments.

Seed nutrient content

In both years, there were significant differences ($p \leq 0.05$) among fertilizer treatments in terms of seed N content in each irrigation regime (Table 6). Additionally, the N content was higher in normal irrigation compared to water stress conditions. The treatment 112.5N + 37.5P + 3CB had the highest N contents in both years and irrigation regimes, with a significant difference compared to the other treatments. The second highest seed N content was observed in the 112.5N + 37.5P + 3WB treatment in both irrigation regimes. Similarly, to N content, the P content in the seeds of the 112.5N + 37.5P + 3CB treatment reached to the highest level, ranging from 0.24 ± 0.01 to 29.0 ± 0.01% under normal irrigation conditions and from 0.22 ± 0.02 to 0.27 ± 0.02 under water stress conditions (Table 6). In both irrigation regimes, the application of 3CB and 3WB alone or in combination with chemical fertilizers showed better performance in increasing seed P content compared to chemical fertilizers. In 2019 and 2020, in each fertilizer treatment seed K content in normal irrigation was higher than in the water stress conditions. Therefore, the highest K content was observed in the 112.5N + 37.5P + 3CB and 112.5N + 37.5P + 3WB with no significant difference together (Table 6). When safflower is exposed to water stress after flowering, the seed K content in the control increased from 1.06 ± 0.05 to 2.48 ± 0.05 (37.7% increase) in 2019 and from 0.92 ± 0.09 to 2.35 ± 0.03 (45.9% increase) in 2020, when treated with 112.5N + 37.5P + 3CB. Generally, the macronutrient contents including N, P and K in normal irrigation was higher than the water stress conditions. In addition, biochar combined with chemical fertilizers treatments was more efficient in macronutrients improvement compared to biochar application alone or chemical fertilizers.

The seed Fe content was affected by fertilizer treatments under both irrigation regimes. Among the treatments, 112.5N + 37.5P + 3CB, 112.5N + 37.5P + 3WB, and 3CB had the highest Fe content (Table 7). Similarly, the lowest Cu, Zn and Mn content was observed in the control under normal and water stress conditions. In both irrigation regimes and years, application of 3CB alone or in combination with chemical fertilizers significantly improved the Cu, Zn and Mn content of safflower seed compared to the other treatments ($p \leq 0.05$) (Table 7). In each fertilizer type and irrigation regime, the Fe, Cu, Zn and Mn content was higher in the first year compared to the second year. Furthermore, the content of these micronutrients in the safflower seed was significantly lower in the 3WB application compared to 3CB (Table 7).

Correlation and stepwise linear regression between safflower yield and other traits

The correlation results between all of the traits are presented in Table 8. Total water applied had a positive correlation with other traits at a 0.01% probability levels, while it had a significant negative correlation with CAT and POX activity, dry matter remobilization, and remobilization efficiency. A positive correlation was also observed between chlorophyll *a*, *b* and total with yield and yield components of safflower, dry matter remobilization, remobilization efficiency and macro and micronutrients, significantly. A negative correlation was observed between carotenoid content and all of the traits except CAT ($r = 0.802^{**}$) and POX ($r = 0.708^{**}$). Seed yield is one of the main traits related to dry matter remobilization, remobilization efficiency, number of capitula per plant, number

Irrigation regime	Fertilizer type	Number of capitula per plant		Number of seeds per capitula		1000-seed weight (g)		Dry matter remobilization (g m^{-2})		Remobilization efficiency (%)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Normal irrigation	C	8.2 ± 0.36^{g}	8.0 ± 0.30^{f}	26.8 ± 0.36^{ef}	22.9 ± 0.47^{f}	24.6 ± 0.08^{de}	23.1 ± 0.11^{h}	117.8 ± 5.9^{i}	121.1 ± 1.9^{g}	17.6 ± 0.40^{e}	16.5 ± 0.31^{f}
	3WB	10.1 ± 0.15^{cd}	10.2 ± 0.11^{c}	30.3 ± 0.85^{d}	27.2 ± 0.35^{d}	26.8 ± 0.51^{bcd}	26.2 ± 0.13^{f}	125.9 ± 1.0^{i}	130.2 ± 1.1^{f}	19.0 ± 0.15^{de}	18.6 ± 0.22^{e}
	3CB	10.4 ± 0.34^{c}	10.4 ± 0.41^{c}	31.4 ± 1.23^{cd}	27.4 ± 0.41^{d}	27.1 ± 0.80^{bc}	27.3 ± 0.09^{d}	142.5 ± 3.3^{h}	144.9 ± 0.5^{de}	23.9 ± 0.17^{cde}	23.0 ± 0.10^{d}
	150N + 50P	10.3 ± 0.25^{c}	10.8 ± 0.26^{bc}	34.2 ± 0.41^{b}	31.4 ± 1.94^{b}	28.9 ± 0.20^{ab}	27.3 ± 0.05^{d}	157.1 ± 0.8^{f}	159.2 ± 0.3^{c}	28.0 ± 1.85^{b-e}	27.2 ± 0.09^{c}
	112.5N + 37.5P	9.5 ± 0.79^{de}	10.4 ± 0.12^{c}	33.1 ± 2.27^{bc}	30.1 ± 1.65^{c}	28.6 ± 0.55^{ab}	27.0 ± 0.15^{e}	148.6 ± 2.4^{g}	140.6 ± 0.6^{e}	24.2 ± 0.36^{cde}	23.1 ± 0.14^{d}
	112.5N + 37.5P + 3WB	11.3 ± 0.15^{b}	11.3 ± 0.25^{ab}	38.4 ± 0.70^{a}	35.9 ± 0.75^{a}	29.8 ± 0.57^{a}	27.6 ± 0.04^{c}	170.5 ± 3.7^{d}	178.7 ± 1.2^{b}	34.2 ± 4.33^{bc}	33.0 ± 0.16^{b}
	112.5N + 37.5P + 3CB	12.4 ± 0.28^{a}	11.9 ± 0.32^{a}	39.8 ± 0.51^{a}	36.7 ± 0.52^{a}	30.1 ± 0.85^{a}	28.1 ± 0.05^{a}	211.5 ± 2.1^{b}	202.0 ± 1.5^{a}	49.8 ± 2.02^{a}	38.2 ± 2.01^{a}
Water stress	C	6.5 ± 0.25^{h}	6.1 ± 0.15^{g}	21.2 ± 1.01^{h}	18.9 ± 0.21^{g}	21.2 ± 0.55^{f}	19.6 ± 0.21^{k}	139.7 ± 2.0^{h}	103.0 ± 1.5^{h}	19.6 ± 0.50^{de}	15.9 ± 0.04^{f}
	3WB	8.7 ± 0.32^{fg}	8.9 ± 0.22^{de}	24.2 ± 1.92^{g}	22.5 ± 0.15^{f}	22.8 ± 1.15^{ef}	22.1 ± 0.06^{j}	153.2 ± 1.5^{fg}	147.6 ± 2.1^{d}	29.0 ± 6.51^{b-e}	22.5 ± 1.33^{d}
	3CB	9.0 ± 0.20^{ef}	9.1 ± 0.25^{de}	24.4 ± 2.07^{g}	23.1 ± 0.30^{f}	22.9 ± 1.15^{ef}	22.4 ± 0.05^{i}	162.8 ± 3.0^{e}	161.1 ± 2.7^{c}	30.3 ± 5.97^{bcd}	23.2 ± 2.21^{d}
	150N + 50P	8.6 ± 0.75^{fg}	8.9 ± 0.23^{de}	25.2 ± 0.36^{fg}	24.9 ± 0.21^{e}	25.8 ± 3.47^{cd}	26.2 ± 0.04^{f}	140.4 ± 1.4^{h}	130.5 ± 1.8^{f}	23.3 ± 0.17^{cde}	19.9 ± 1.18^{e}
	112.5N + 37.5P	8.2 ± 0.85^{g}	8.6 ± 0.16^{ef}	23.6 ± 0.50^{g}	23.6 ± 0.23^{f}	25.2 ± 2.8^{cd}	25.9 ± 0.06^{g}	150.0 ± 0.5^{g}	122.3 ± 1.9^{g}	23.6 ± 0.18^{cde}	19.8 ± 1.41^{e}
	112.5N + 37.5P + 3WB	10.2 ± 0.10^{c}	9.4 ± 0.14^{d}	27.8 ± 0.41^{e}	25.1 ± 0.19^{e}	29.3 ± 0.47^{a}	28.0 ± 0.11^{b}	196.7 ± 7.2^{c}	157.8 ± 2.1^{c}	37.4 ± 0.55^{b}	29.8 ± 2.01^{c}
	112.5N + 37.5P + 3CB	10.1 ± 0.11^{cd}	9.5 ± 0.12^{d}	29.7 ± 0.52^{d}	27.8 ± 0.21^{d}	29.9 ± 0.15^{a}	28.1 ± 0.07^{a}	225.9 ± 1.6^{a}	183.1 ± 2.2^{b}	39.5 ± 0.31^{ab}	33.6 ± 1.13^{b}

Table 5. Interaction effect of irrigation regime and fertilizer type on yield components, dry matter remobilization and remobilization efficiency of safflower in 2019 and 2020 growing seasons. Means in each column followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

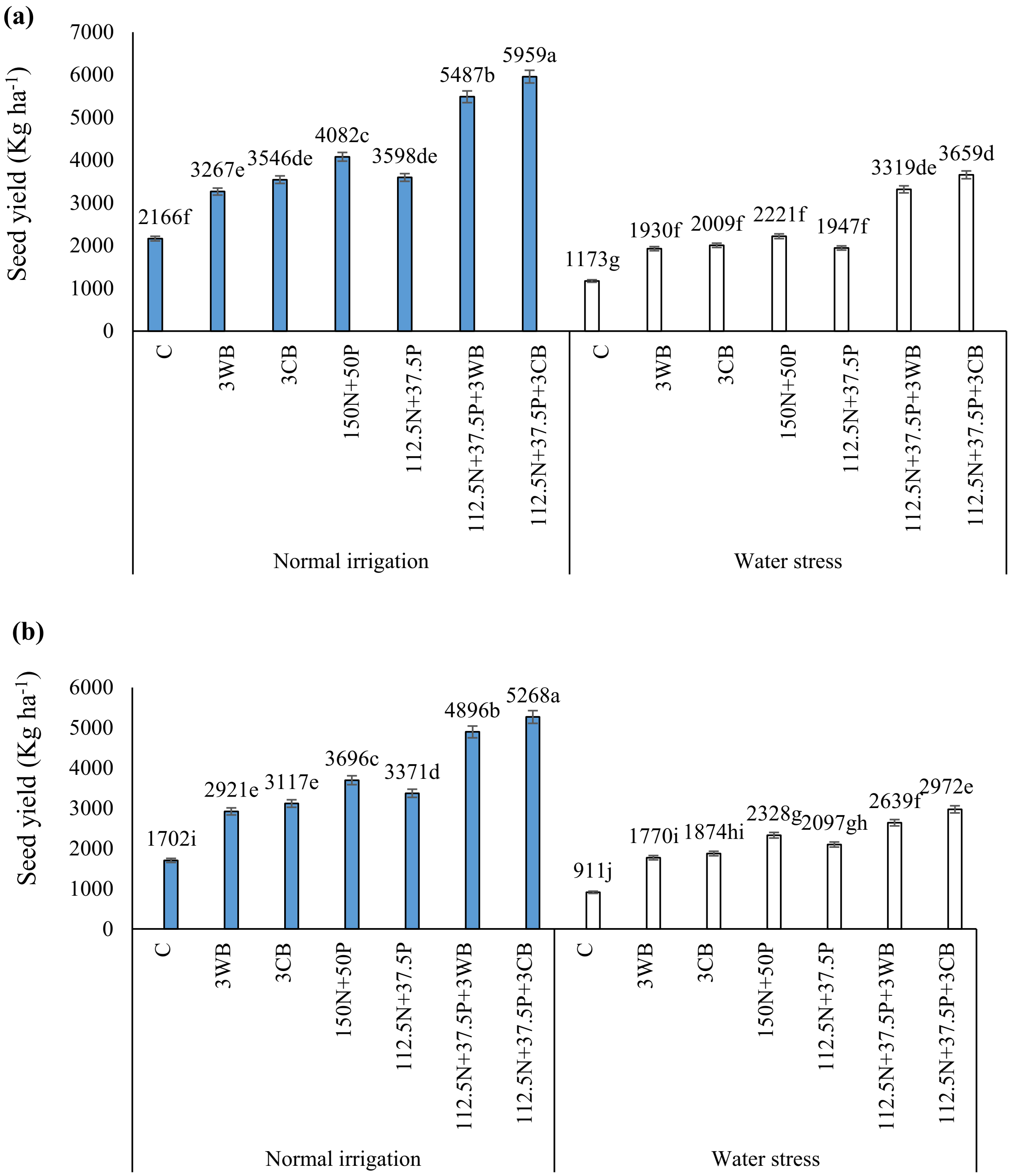

Fig. 4. Interaction effect of irrigation regime and fertilizer type on seed yield of safflower during the 2019 (**a**) and 2020 (**b**) growing seasons. Means followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

of seeds per capitula and 1000-seed weight, directly. Also, seed N content is positively related to P content at a 0.05 probability level, and with the other nutrients at a 0.01 probability level. On the other hand, seed P content correlated to K content (r = 0.287**) with no significant relationship with the other micronutrients. In addition, seed Fe content was highly related to Cu (r = 0.829**), Zn (r = 0.875**) and Mn (r = 0.852**) content. The regression results of safflower yield and other traits are presented in Table 9. Among the 20 traits measured, seed yield related to carotenoid content, number of seeds per capitula, 1000-seed weight, remobilization efficiency and seed Fe content, significantly. All of these traits except carotenoid content had a positive and significant correlation coefficient with grain yield.

Irrigation regime	Fertilizer type	Seed N content (%)		Seed P content (%)		Seed K content (%)	
		2019	2020	2019	2020	2019	2020
Normal irrigation	C	0.42 ± 0.01^{k}	0.51 ± 0.01^{j}	0.16 ± 0.01^{g}	0.19 ± 0.01^{d}	1.91 ± 0.05^{g}	1.67 ± 0.04^{ef}
	3WB	0.61 ± 0.01^{h}	0.68 ± 0.02^{h}	0.22 ± 0.01^{de}	0.24 ± 0.01^{b}	2.53 ± 0.09^{d}	2.29 ± 0.02^{bc}
	3CB	0.68 ± 0.02^{g}	0.71 ± 0.01^{g}	0.26 ± 0.02^{b}	0.27 ± 0.01^{a}	2.73 ± 0.05^{c}	2.49 ± 0.09^{ab}
	150N + 50P	1.15 ± 0.02^{c}	1.21 ± 0.01^{c}	0.18 ± 0.01^{f}	0.18 ± 0.01^{d}	2.82 ± 0.01^{b}	2.68 ± 0.04^{a}
	112.5N + 37.5P	1.11 ± 0.02^{d}	1.07 ± 0.01^{d}	0.17 ± 0.02^{fg}	0.18 ± 0.01^{d}	2.74 ± 0.05^{bc}	2.55 ± 0.05^{ab}
	112.5N + 37.5P + 3WB	1.28 ± 0.01^{b}	1.32 ± 0.03^{b}	0.24 ± 0.01^{c}	0.25 ± 0.01^{ab}	2.93 ± 0.03^{a}	2.70 ± 0.01^{a}
	112.5N + 37.5P + 3CB	1.35 ± 0.01^{a}	1.42 ± 0.03^{a}	0.29 ± 0.01^{a}	0.26 ± 0.01^{ab}	2.94 ± 0.06^{a}	2.76 ± 0.01^{a}
Water stress	C	0.30 ± 0.02^{m}	0.32 ± 0.01^{l}	0.08 ± 0.02^{h}	0.08 ± 0.01^{g}	1.06 ± 0.05^{j}	0.92 ± 0.09^{g}
	3WB	0.38 ± 0.01^{l}	0.43 ± 0.02^{k}	0.20 ± 0.01^{e}	0.20 ± 0.01^{cd}	1.80 ± 0.05^{h}	1.61 ± 0.07^{ef}
	3CB	0.40 ± 0.01^{l}	0.43 ± 0.02^{k}	0.22 ± 0.01^{de}	0.21 ± 0.01^{c}	2.20 ± 0.02^{f}	2.04 ± 0.02^{cd}
	150N + 50P	0.58 ± 0.03^{i}	0.67 ± 0.02^{h}	0.14 ± 0.01^{h}	0.13 ± 0.01^{e}	1.72 ± 0.02^{hi}	1.50 ± 0.03^{f}
	112.5N + 37.5P	0.54 ± 0.01^{j}	0.63 ± 0.01^{i}	0.12 ± 0.02^{h}	0.11 ± 0.01^{f}	1.68 ± 0.01^{i}	1.43 ± 0.02^{f}
	112.5N + 37.5P + 3WB	0.71 ± 0.01^{f}	0.82 ± 0.01^{f}	0.23 ± 0.02^{cd}	0.22 ± 0.02^{c}	2.37 ± 0.05^{e}	1.82 ± 0.09^{de}
	112.5N + 37.5P + 3CB	0.79 ± 0.02^{e}	0.88 ± 0.02^{e}	0.27 ± 0.02^{b}	0.26 ± 0.01^{ab}	2.48 ± 0.05^{d}	2.35 ± 0.03^{b}

Table 6. Interaction effect of irrigation regime and fertilizer type on macronutrient contents of safflower seed in 2019 and 2020 growing seasons. Means in each column followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

Irrigation regime	Fertilizer type	Seed Fe content (mg kg^{-1} DW)		Seed Cu content (mg kg^{-1} DW)		Seed Zn content (mg kg^{-1} DW)		Seed Mn content (mg kg^{-1} DW)	
		2019	2020	2019	2020	2019	2020	2019	2020
Normal irrigation	C	181.35 ± 1.07^{g}	179.60 ± 0.43^{g}	6.33 ± 0.15^{k}	5.90 ± 0.16^{h}	11.23 ± 0.15^{l}	9.57 ± 0.25^{j}	19.63 ± 0.51^{j}	18.07 ± 0.55^{fg}
	3WB	199.33 ± 1.85^{e}	196.73 ± 0.49^{e}	10.57 ± 0.05^{e}	9.73 ± 0.21^{de}	20.40 ± 0.36^{f}	18.10 ± 0.28^{f}	29.50 ± 0.98^{f}	27.30 ± 0.35^{d}
	3CB	209.23 ± 0.95^{c}	203.97 ± 4.9^{c}	15.33 ± 0.16^{b}	13.93 ± 0.32^{b}	30.17 ± 0.35^{b}	27.53 ± 0.31^{b}	44.93 ± 0.54^{b}	41.50 ± 1.01^{a}
	150N + 50P	193.43 ± 0.90^{f}	190.80 ± 1.17^{f}	9.27 ± 0.17^{h}	8.47 ± 0.25^{fg}	19.80 ± 0.43^{g}	17.72 ± 0.34^{f}	20.63 ± 0.51^{i}	19.07 ± 0.45^{ef}
	112.5N + 37.5P	191.20 ± 0.92^{f}	188.83 ± 0.85^{f}	8.67 ± 0.14^{i}	7.90 ± 0.17^{g}	18.37 ± 0.37^{h}	16.60 ± 0.32^{g}	19.80 ± 0.46^{ij}	18.13 ± 0.95^{fg}
	112.5N + 37.5P + 3WB	214.53 ± 1.41^{b}	211.60 ± 5.31^{b}	10.07 ± 0.13^{f}	9.83 ± 0.33^{d}	22.60 ± 0.26^{e}	20.23 ± 0.21^{e}	32.10 ± 0.31^{e}	29.57 ± 0.23^{c}
	112.5N + 37.5P + 3CB	218.63 ± 1.62^{a}	216.17 ± 1.50^{a}	16.70 ± 0.26^{a}	14.87 ± 0.29^{a}	33.43 ± 0.34^{a}	28.83 ± 0.12^{a}	46.53 ± 0.52^{a}	43.50 ± 1.22^{a}
Water stress	C	171.15 ± 0.98^{h}	169.40 ± 0.90^{h}	6.17 ± 0.06^{k}	5.93 ± 0.05^{h}	9.30 ± 0.21^{m}	8.50 ± 0.36^{k}	15.70 ± 0.12^{l}	14.83 ± 0.37^{h}
	3WB	192.67 ± 2.69^{f}	190.70 ± 1.71^{f}	9.20 ± 0.10^{h}	8.43 ± 0.11^{fg}	17.43 ± 0.32^{i}	15.27 ± 0.14^{h}	21.73 ± 0.63^{h}	19.80 ± 0.33^{ef}
	3CB	198.40 ± 1.31^{e}	197.53 ± 0.85^{e}	13.20 ± 0.11^{d}	12.37 ± 0.09^{c}	28.53 ± 0.30^{c}	25.23 ± 0.51^{c}	36.67 ± 0.52^{c}	33.80 ± 0.86^{b}
	150N + 50P	182.63 ± 0.40^{g}	181.00 ± 1.01^{g}	8.23 ± 0.14^{i}	7.80 ± 0.15^{g}	15.23 ± 0.15^{j}	13.73 ± 0.56^{i}	17.50 ± 0.26^{k}	16.43 ± 0.59^{gh}
	112.5N + 37.5P	180.40 ± 0.43^{g}	179.37 ± 1.19^{g}	7.30 ± 0.12^{j}	6.70 ± 0.19^{h}	14.07 ± 0.55^{k}	13.07 ± 0.68^{i}	17.77 ± 0.53^{k}	16.63 ± 0.24^{gh}
	112.5N + 37.5P + 3WB	200.67 ± 0.46^{e}	199.10 ± 0.96^{de}	9.67 ± 0.07^{g}	8.90 ± 0.18^{ef}	25.20 ± 0.46^{d}	22.10 ± 0.58^{d}	23.40 ± 0.60^{g}	21.07 ± 0.98^{e}
	112.5N + 37.5P + 3CB	203.83 ± 1.07^{d}	202.07 ± 1.06^{cd}	14.70 ± 0.14^{c}	13.10 ± 0.04^{bc}	30.07 ± 0.14^{b}	28.72 ± 0.21^{a}	33.73 ± 0.64^{d}	31.13 ± 0.95^{c}

Table 7. Interaction effect of irrigation regime and fertilizer type on micronutrient contents of safflower seed in 2019 and 2020 growing seasons. Means in each column followed by the same letters are not significantly different at 5% probability level using Duncan's multiple range test (DMRT). Bars represent mean ± SE. C: Control (without fertilizer); 3WB: application of 3 ton ha^{-1} wheat biochar; 3CB: application of 3 ton ha^{-1} cotton biochar; 150N + 50P: application of 150 kg ha^{-1} N + 50 kg ha^{-1} P, 112.5N + 37.5P: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P, 112.5N + 37.5P + 3WB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} wheat biochar; 112.5N + 37.5P + 3CB: application of 112.5 kg ha^{-1} N + 37.5 kg ha^{-1} P + 3 ton ha^{-1} cotton biochar.

Principal component analysis (PCA)

The PCA revealed two major principal components (with eigenvalues greater than one), which accounted for 78.657% of the total variance among the traits in this study (Table 10). The first Principal component (PC1) explained the highest variability in the data. It was positively influenced by the number of seeds per capitula, 1000-seed weight, RWC, chlorophyll *a* and *b* contents, total chlorophyll content, and N seed content had the

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Total water applied	1														
2. Chlorophyll *a* content	0.309**	1													
3. Chlorophyll *b* content	0.256*	0.838**	1												
4. Total chlorophyll content	0.298**	0.957**	0.918**	1											
5. Carotenoid content	0.242**	− 0.780**	− 0.737**	− 0.781**	1										
6. Relative water content	0.594**	0.806**	0.788**	0.813**	− 0.777**	1									
7. Catalase	− 0.381*	− 0.864**	− 0.815**	− 0.855**	0.802**	− 0.852**	1								
8. Peroxidase	− 0.599**	− 0.825**	− 0.840**	− 0.833**	0.708**	− 0.901**	0.301*	1							
9. No.of capitula per plant	0.410**	0.828**	0.779**	0.811**	− 0.730**	0.813**	0.804**	− 0.823**	1						
10. No. of seeds per capitula	0.511**	0.863**	0.904**	0.903**	− 0.786**	0.901**	− 0.856**	− 0.901**	0.865**	1					
11. 1000-seed weight	0.0.320**	0.841**	0.665**	0.802**	− 0.709**	0.681**	− 0.799**	− 0.672**	0.735**	0.779**	1				
12. Seed yield	0.429**	0.889**	0.916**	0.917**	− 0.752**	− 0.855**	− 0.889**	− 0.889**	0.913**	0.567**	0.811**	1			
13. Dry matter remobilization	− 0.251*	0.709**	0.684**	0.706**	− 0.645**	0486**	− 0.696**	− 0.506**	0.641**	0.591**	0.590**	0.647**	1		
14. Remobilization efficiency	− 0.166	0.667**	0.705**	0.687**	− 0.582**	0.517**	− 0.674**	− 0.513**	0.618**	0.676**	0.576**	0.669**	0.853**	1	
15. Seed N content	0.394**	0.724**	0.669**	0.717**	− 0.520**	0.578**	− 0.631**	− 0.687**	0.660**	0.177^{ns}	0.587**	0.694**	0.469**	0.354**	1
16. Seed P content	0.199	0.257*	0.2	0.238*	− 0.174	0.250*	− 0.267*	− 0.282**	0.313**	0.881**	0.171^{ns}	0.211^{ns}	0.189^{ns}	0.154^{ns}	0.266*
17. Seed K content	0.530**	0.831**	0.751**	0.819**	− 0.833**	0.910**	− 0.893**	− 0.857**	0.878**	0.770**	0.752**	0.866**	0.567**	0.541**	0.602**
18. Seed Fe content	0.139^{ns}	0.756**	0.772**	0.774**	− 0.748**	0.773**	− 0.771**	− 0.746**	0.859**	0.529**	0.646**	0.821**	0.725**	0.686**	0.547**
19. Seed Cu content	− 0.029^{ns}	0.537**	0.508**	0.546**	− 0.582**	0.596**	− 0.545**	− 0.460**	0.656**	0.560**	0.466**	0.581**	0.692**	0.623**	0.315**
20. Seed Zn content	− 0.067^{ns}	0.640**	0.557**	0.621**	− 0.670**	0.619**	− 0.647**	− 0.505**	0.714**	0.554**	0.573**	0.623**	0.763**	0.690**	0.368**
21. Seed Mn content	0.080^{ns}	0.178**	0.539**	0.519**	− 0.531**	0.637**	− 0.516**	− 0.511**	0.653**	0.511**	0.392**	0.599**	0.572**	0.537**	0.305**

Table 8. Correlation coefficients (Pearson's) between all measured plant parameters in this study.

Model	Unstandardized coefficient		Standardized coefficient	t	Sig	Collinearity statistics	
	B	Std. error	Beta			Tolerance	VIF
Constant	− 8077.701	578.486					
Chlorophyll *b* content	472.341	251.146	0.073	1.881	0.064	0.111	9.032
Carotenoid content	3189.617	526.422	0.145	6.059	0.000	0.287	3.486
Catalase	128.745	67.357	0.059	1.911	0.060	0.176	5.689
No. of seeds per capitula	185.765	9.981	0.782	18.612	0.000	0.094	10.692
1000-seed weight	57.839	10.497	0.131	5.510	0.000	0.292	3.422
Remobilization efficiency	12.541	3.278	0.086	3.826	0.000	0.325	3.072
Seed N content	130.857	72.996	0.035	1.793	0.077	0.438	2.282
Seed P content	139.626	77.779	0.024	1.795	0.077	0.887	1.127
Seed Fe content	14.544	2.340	0.149	6.215	0.000	0.288	3.470

Table 9. Regression results of safflower yield and other traits. Dependent variable: Yield.

	Components	
	1	2
No. of seeds per capitula	0.959	− 0.021
1000-seed weight	0.829	− 0.171
Relative water content	0.916	0.140
Chlorophyll *a* content	0.940	− 0.151
Chlorophyll *b* content	0.902	− 0.182
Total chlorophyll content	0.949	− 0.175
Carotenoid content	− 0.836	0.225
Peroxidase	− 0.934	− 0.165
Catalase	− 0.928	0.052
Seed N content	0.755	0.100
Seed P content	0.291	0.597
Total water applied	0.484	0.702
Eigenvalue	8.363	1.076
Proportional variance (%)	69.694	8.963
Cumulative variance (%)	69.694	78.657

Table10. Results of principal component analysis of different traits in safflower.

most positive contribution. However, carotenoid content, POX and CAT had a negative impact on PC1. In PC2, seed P content and total water applied had the most positive contribution. Interestingly, several biochemical traits including chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid content, POX and CAT activity played a significant role in this study. PC1 was dominated by chlorophyll *a*, *b* and total chlorophyll which had a positive effect. On the other hand, carotenoid content, POX and CAT activity were also important traits but they had a negative influence. These traits were strongly associated with other important traits such as the number of seeds in capitula, and 1000-seed weight, which are commonly known to play crucial roles in yield.

Discussion

In the current study, we examined the effect of wheat or cotton biochar application either alone or in combination with urea (N source) and triple superphosphate (P source) on biochemical properties, yield and yield components and nutrient uptake of safflower under water stress conditions. The type of biochar and its characteristics can affect the absorption of nutrients and subsequently can modify the amount of photosynthetic pigments. Asai et al.[15] stated that the application of biochar made from wood residues (8 and 16 ton ha^{-1}) can lead to a decrease in rice N uptake. This decrease can be attributed to N immobilization. Consequently, the chlorophyll content of rice decreased significantly. Similar to our results, they found that application of biochar can counteract the effect of N fertilizer on the enhancement of photosynthetic pigments. In another study, Carter et al.[35] reported that biochar of rice husk at 25 to 50 ton ha^{-1} with compost increased the chlorophyll content of lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*) by 7–10%. In fact, biochar improves the water holding of the soil, which in turn increases RWC and chlorophyll content. In the current study, the combination of biochar with 75% recommended dosage of N and P increased the total chlorophyll content of leaves by 112.5–138% at normal irrigation and 70.6 to 128.1% under water stress. Improving chlorophyll content in these combined treatments is related to the increase of RWC and N content which is absorbed from the soil. Carotenoids have a protective role in preventing the photo degradation of chlorophyll, especially under stressful environments[36]. As water shortage increases in the rhizosphere, it leads to the reduction of water and nutrient absorption, and consequently, the concentration of carotenoids increases in the leaves[1]. Younis et al.[37] stated that the photosynthetic rate, chlorophyll and carotenoid contents of spinach (*Spinacia oleracea* L.) were enhanced with the CB in a pot experiment. In the present study, CB had a higher N% compared to WB (3% vs. 1.1%). As a result, total chlorophyll levels were higher in CB alone compared to WB regardless of the irrigation regime. Carotenoid content was enhanced in control treatment without fertilizer, due to its protective effect on chlorophyll, under both normal and water stress conditions. On the other hand, the treatments containing WB or CB alone or in combination with chemical fertilizers resulted in less carotenoid content than other treatments, which may be attributed to the role of biochar in water retention in the soil[9], and consequently crop will be less subjected to water stress.

Charkhab et al.[38] revealed that water deficit in corn (irrigation at 50% of field capacity) increased POX activity by 45.7% compared to normal irrigation. The highest POX activity was observed in the control without the application of sugarcane biochar. It has been demonstrated that, biochar with its porous structure, is known to increase water retention capacity of the soil and can decrease POX activity when plants are exposed to water stress. Under water deficit, the plant's maintenance respiration is enhanced owing to the increase of CAT activity. Using biochar in order to increase water retention in the soil, creates a suitable condition for water uptake and consequently, the cost of plant for antioxidant enzyme production can be decreased[6,13,37,39]. Teodoro et al.[40] stated that the presence of wood compost with wood biochar reduced the production of POX in *Eruca sativa*. However, when wood compost applied alone, it actually increased the POX by reducing the soil's ability to retain water. The type of biochar used and the level of stress had a significant effect on the activity of antioxidant enzyme. It has been revealed that, as the water stress level increased and water retention decreased due to the absence of biochar, the CAT and POX activity increased[6,41]. In the current study, the control and chemical fertilizer treatments exhibited higher CAT and POX activity compared to co-application of biochar under water stress. This can be attributed to the fact that these treatments experience greater water stress due to the absence of biochar. Moreover, the chemical treatments (150N + 50P and 112.5N + 37.5P) consumed more water ranging from 4730 to 5029 m^3 before reaching the flowering stage under water stress. This consumption was significantly higher than that observed in the combined treatments.

Biochar application can enhance the availability of water during the crop growth stages, which in turn increases RWC and dry matter production by improving the electron transport rate in photosystem II[5,42]. When safflower was subjected to water stress, the rate of decrease in RWC in biochar application alone or in combination with chemical fertilizers was lower than the chemical fertilizer treatments due to the potential of biochar in water retention[6,8]. Although the chemical fertilizer treatments (150N + 50P and 112.5N + 37.5P) consumed more water under water stress in both years, their RWC was lower than that of cotton biochar application. It is demonstrated that, CB has the ability to save water, and creates favorable conditions for root uptake of nutrients and water[37]. Practically, the use of chemical fertilizers before flowering, leads to more water consumption, thereby increasing the transpiration surface area of the plant[6,8], which subsequently reduces the RWC of the leaves after flowering.

Some researchers have reported an increase in plant production through the application of biochar. This can be attributed to the improvement of water supply and essential nutrients in the soil[8,43,44]. However, some studies have shown a decrease in yield components and seed yield, which may be related to an increase in soil salinity by biochar application[45,46]. Despite this, the overall seed yield of safflower was improved when biochar was applied in combination with chemical fertilizers. In this study, the EC of WB was 117% higher than CB. Furthermore, the levels of macro and micronutrients in cotton biochar were higher than in wheat biochar. It appears that the superior characteristics of CB compared to WB, contribute to higher yield components of safflower, including number of capitula per plant, number of seeds per capitula, 1000-seed weight and ultimately, seed yield. Regarding the type of biochar under water stress, the seed yield of safflower in 112.5N + 37.5P + 3CB was 10.2 and 12.6% higher than 112.5N + 37.5P + 3WB treatment in 2019 and 2020, respectively. This indicates that, biochar with a porous structure has oxygen-containing functional groups on its surface area and that can act as a soil conditioner. This enhances dry matter production by increasing water availability and nutrient uptake[6,47]. In general, the advantages and disadvantages of biochar on seed yield and yield components depends on various factors. These include the source of biochar, the temperature of the pyrolysis process, the amount of biochar incorporated into the soil, physical and chemical properties of the soil, crop type and weather conditions[4,6,13,48].

The climatic conditions of the experimental site affected the yield components of safflower. In the second year, as a consequence of higher mean monthly temperatures and lower rainfall, total evaporation increased by 16.2% compared to the first year of the experiment. Under water stress conditions, the treatment 112.5N + 37.5P + 3CB had the highest yield components. Despite this, in the second year, the number of capitula per plant, number of seeds per capitula, 1000-seed weight and seed yield decreased by 6.3, 6.8, 6.4 and 23.1% compared to the first year, respectively. It is demonstrated that the seed filling period is highly sensitive to water shortage in the soil and increasing air temperature[2,49,50] therefore, cotton biochar can mitigate the adverse effect of water deficit on yield components by increasing water retention mainly at the late-season[8].

Some studies have shown that dry matter remobilization is enhanced when a crop is subjected to water stress[51,52]. As a result of a decrease in current photosynthesis after flowering, the contribution of pre-flowering of assimilate to grain filling increases significantly, depending on the severity of water stress[53]. In contrast, under normal irrigation, usually due to adequate water in the rhizosphere after flowering, the balance between sink and source is maintained at a high level, and the assimilate produced by the source is remobilized slowly. However, during the post-flowering stage, water and nutrient uptake from the roots decrease leading to deterioration in the sink-source relationship under water stress conditions. In this case, the sink size exceeds than the source size and the source organs must remobilize more assimilate from leaves and stems to seeds[46]. Biochar, on the other hand, enhances the contribution of pre-flowering of assimilate because of supplying adequate water and nutrients for the crop. In this condition, dry matter was more remobilized and consequently grain yield improved[38]. Despite this, in some cultivars of crops, water stress during the seed filling period can accelerate senescence, which may reduce the efficiency of assimilate remobilization to seeds and ultimately lead to a decrease in grain yield[51]. In the present study, biochar under water stress improved dry matter remobilization compared to the control. In 112.5N + 37.5P + 3CB treatment under water stress, the shoot had more time to transfer the assimilate before flowering, resulting in a 101.5 and 111.3% increase in remobilization efficiency compared to the control in 2019 and 2020, respectively. Overall, number of seeds per capitula which is a significant yield component, is higher in 112.5N + 37.5P + 3CB compared to 112.5N + 37.5P + 3WB. Also, dry matter remobilization and remobilization efficiencies are higher in 112.5N + 37.5P + 3CB treatment compared to 112.5N + 37.5P + 3WB (Table 5).

In addition to maintaining moisture, biochar plays an important role in providing micro and macro nutrients during the plant's growth season[9,13,43]. Lehmann[54] stated that biochar incorporated to the soil can slow down the turnover of organic carbon in the soil and reduce N demand for plants, indirectly via enhancing the N use efficiency. Moreover, certain types of biochar can serve as a source of available P for plants[55]. When biochar is present in the soil, it binds phosphate with free cations (such as Fe^{3+}, Mg^{2+} and Ca^{2+}) and released P is then utilized for crop growth again[56]. Furthermore, biochar can enhance the activity of K solubilizing bacteria and fungi, thereby, increasing P availability[57]. The increase in plant K concentration with biochar, may be owing to the mineral K content of CB and WB which in turn raises the soil CEC and can release K to the available form[9]. Jia et al.[58] also reported that regardless of the usefulness of biochar application which improved P uptake and increased corn growth parameters including plant height, stem diameter and biomass besides the immobilization of lead (Pb), the use of biochar resulted in less colonization of arbuscular mycorrhizal fungi. In the current study, the treatment of 112.5N + 37.5P + 3CB using CB with N and P fertilizers, significantly enhanced the seed content of N, P and K compared to the application of CB alone. Therefore, the stimulating effects of biochar on nutrient availability and crop yield may depend on various factors, including the source of biochar, the process of biochar preparation, the type of crop, soil type and environmental conditions[8,10,54]. In agreement to our results, Rafique et al.[59] stated that adding biochar to the soil promotes the absorption of P, K and Ca which accelerates the rate of photosynthesis and production of dry matter in corn. In the current study, the primary characteristics of CB and WB (Table 3) showed that CB has 117.0% less EC than wheat biochar. On the other hand, the content of N, P and K measured in cotton biochar was 172.7, 157.1 and 17.5% higher than wheat, respectively. Generally, the increase of N has a direct effect on the chlorophyll increment[9] and the increase of K and P in CB which is related to RWC[16] and nutrient uptake improvement[8,45], compared to WB.

Biochar can potentially influence the availability of micronutrients by absorbing metals on its surface, and increasing the soil CEC. Additionally, biochar can enhance the availability of Fe and Mn due to its high exchange capacity of these elements and release of certain complex agents[60]. Woldetsadik et al.[61] observed an increase in Mn content on sandy loam soil with biochar amendment, but this effect was reversed when higher amounts of biochar were used. The antagonistic effects of some micronutrients in terms of root absorption may also influence on their concentrations in plant tissues specially when different sources of biochar are used. Liu et al.[62] stated that low amount of nano-Tio2 doped biochar increased cation exchange capacity, P uptake, humic acid, microbial cooperation and mitigated cadmium mobility in the soil. In another study, Evangelou et al.[63] reported that biochar had a positive effect on increasing the K and Zn content in plant shoots, but there were no differences between biochar and control in P, Fe, Cu and Mn content. Biochar and fertilizer applications had positive effects on N, P, K and S uptake, although the extent of these effects varied depending on the soil type[64]. In the current study, the combination of biochar and chemical fertilizers, resulted in improved nutrient availability which consequently increased safflower yield.

De Almeida Silva et al.[65] revealed the significance of RWC, chlorophyll *a*, *b*, total chlorophyll and carotenoid contents on seed yield improvement. To model the seed yield of safflower, Abdipour et al.[66] used artificial neural network and PCA analysis. They identified five morphological and phenological traits, including plant height, number of branches per plant, number of capitula per plant, 1000-seed weight, and number of seeds per capitula. These traits were identified based on high values of Eigen vectors. In a similar study, La Bella et al.[67] evaluated new genotypes of safflower under Mediterranean conditions. They utilized the PCA method and identified 6 main components which described about 90% of the total variations. In the current study, various statistical approaches including correlation, stepwise regression and PCA, were integrated to analyze the data. Our results showed that, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid were the most important biochemical indices associated with safflower yield. Also, the results highlighted the importance of RWC, 1000-seed weight, number of seeds per capitula and seed N content which related to yield.

Conclusions

The application of biochar with chemical fertilizer affected chlorophyll content, assimilate remobilization, nutrient uptake and grain yield of triticale under water stress. When compared to wheat biochar, cotton biochar, due to its higher nutrient content, was found to improve safflower nutrition and relative water content to a greater extent. Additionally, the total water consumed in chemical fertilizer treatments was higher than the other fertilizer treatments. Application of cotton biochar with a 75% of the recommended dosage of N and P chemical fertilizers under water stress can increase grain yield of safflower. This will probably results in enhancing the relative water content of leaves, total chlorophyll, remobilization efficiency and nutrient uptake. Findings of this research associated to agronomic improvement in cotton biochar highlighted the significance of the biochar quality. We suggest that combining cotton biochar application with reduced amounts of chemical fertilizers is appropriate to decrease production costs and mitigate environmental hazards in sustainable agriculture. Different types of biochar from various crops are possible choices to increase our knowledge about the effects of biochar application under water stress conditions. Furthermore, the optimization of biochar preparation for example the temperature of biochar preparation (400–900 °C) and the combination of different sources of soil additives such as municipal compost or bentonite are possible interesting research factors.

Data availability

The datasets used and/or analyzed during the current research are available from the corresponding author on request.

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References

1. Assadi, N. M. & Bijanzadeh, E. Influence of relay intercropping of barley with chickpea on biochemical characteristics and yield under water stress. *PLoS ONE* **18**, e0273272 (2023).
2. Bijanzadeh, E., Moosavi, S. M. & Bahadori, F. Quantifying water stress of safflower (Carthamus tinctorius L.) cultivars by crop water stress index under different irrigation regimes. *Heliyon* **8**, e09010 (2022).
3. Bijanzadeh, E., Barati, V., Emam, Y. & Pessarakli, M. Assessment of the crop water stress index and color quality of bur clover (Medicago polymorpha L.) under different irrigation regimes. *Commun. Soil Sci. Plant Anal.* **50**, 2825–2835 (2019).
4. Zulfiqar, B. *et al.* Biochar enhances wheat crop productivity by mitigating the effects of drought: Insights into physiological and antioxidant defense mechanisms. *PLoS ONE* **17**, e0267819 (2022).
5. Haider, G. *et al.* Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. *Plant Soil* **395**, 141–157 (2015).
6. Semida, W. M. *et al.* Biochar implications for sustainable agriculture and environment: A review. *S. Afr. J. Bot.* **127**, 333–347 (2019).
7. Rizwan, M. *et al.* Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: A critical review. *Environ. Sci. Pollut. Res.* **23**, 2230–2248 (2016).
8. Najafi-Ghiri, M., Bijanzadeh, E. & Ghaedi, M. Soil potassium relations in an arid soil treated with biochar and chemical fertilizers after cultivation with safflower (*Carthamus tinctorius* L.) under water stress. *Arid Land Res. Manag0* **37**, 427–446 (2023).
9. Najafi-Ghiri, M., Bijanzadeh, E. & Bahadori, F. Effect of wheat-derived biochar on soil nutrients availability and nutrients uptake by two safflower (*Carthamus tinctorius* L.) cultivars under water stress. *Commun. Soil Sci. Plant Anal.* **53**, 1592–1606 (2022).
10. Atkinson, C. J., Fitzgerald, J. D. & Hipps, N. A. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant Soil* **337**, 1–18 (2010).
11. Yadav, N. K. *et al.* Biochar and their impacts on soil properties and crop productivity: A review. *J. Pharmacogn. Phytochem.* **7**, 49–54 (2018).
12. Liu, X. *et al.* Biochar's effect on crop productivity and the dependence on experimental conditions—a meta-analysis of literature data. *Plant Soil* **373**, 583–594 (2013).
13. Sohi, S. P., Krull, E., Lopez-Capel, E. & Bol, R. A review of biochar and its use and function in soil. *Adv. Agron.* **105**, 47–82 (2010).
14. Aguilar-Chávez, Á., Díaz-Rojas, M., del Rosario Cárdenas-Aquino, M., Dendooven, L. & Luna-Guido, M. Greenhouse gas emissions from a wastewater sludge-amended soil cultivated with wheat (Triticum spp. L.) as affected by different application rates of charcoal. *Soil Biol. Biochem.* **52**, 90–95 (2012).
15. Asai, H. *et al.* Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Res.* **111**, 81–84 (2009).
16. Dolkhani, F., Bijanzadeh, E., Boostani, H. R. & Hardie, A. G. Effect of nitrogen-fixing bacteria application on biochemical properties, yield, and nutrients of barley. *J. Soil Sci. Plant Nutr.* 1–15 (2022).
17. Thomas, G. W. Soil pH and soil acidity. *Methods Soil Anal. Part 3 Chem. Methods* **5**, 475–490 (1996).
18. Rhoades, J. Salinity: Electrical conductivity and total dissolved solids. *Methods Soil Ana. Part 3 Chem. Methods* **5**, 417–435 (1996).
19. Gee, G. W. & Or, D. 2.4 Particle-size analysis. *Methods Soil Anal. Part 4 Phys. Methods* **5**, 255–293 (2002).
20. Nelson, D. W. & Sommers, L. E. Total carbon, organic carbon, and organic matter. *Methods Soil Ana. Part 3 Chem. Methods* **5**, 961–1010 (1996).
21. Bremner, J. M. Nitrogen-total. *Methods of soil analysis: Part 3 Chemical Methods* **5**, 1085–1121 (1996).
22. Olsen, S. R. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (US Department of Agriculture, 1954).
23. Lindsay, W. L. & Norvell, W. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **42**, 421–428 (1978).
24. Soil, S. S. *Keys to soil taxonomy* (Natural Resources Conservation Service and Agriculture Department, 2014).
25. Flemmer, A., Franchini, M. & Lindström, L. Description of safflower (*Carthamus tinctorius*) phenological growth stages according to the extended BBCH scale. *Ann. Appl. Biol.* **166**, 331–339 (2015).
26. Voroney, P. Soils for horse pasture management. *Horse Pasture Manag*, 65–79 (2019).
27. Sun, J., Lian, F., Liu, Z., Zhu, L. & Song, Z. Biochars derived from various crop straws: Characterization and Cd (II) removal potential. *Ecotoxicol. Environ. Saf.* **106**, 226–231 (2014).
28. Yang, G. *et al.* Effects of pyrolysis temperature on the physicochemical properties of biochar derived from vermicompost and its potential use as an environmental amendment. *RSC Adv.* **5**, 40117–40125 (2015).
29. Lichtenthaler, H. K. & Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protocols Food Anal. Chem.* **1**, F4. 3.1–F4. 3.8 (2001).
30. Aebi, H. [13] Catalase in vitro. *Methods Enzymol.* **105**, 121–126 (1984).
31. Chance, B. & Maehly, A. [136] Assay of catalases and peroxidases (1955).
32. Machado, S. & Paulsen, G. M. Combined effects of drought and high temperature on water relations of wheat and sorghum. *Plant Soil* **233**, 179–187 (2001).
33. Ercoli, L., Lulli, L., Mariotti, M., Masoni, A. & Arduini, I. Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability. *Eur. J. Agron.* **28**, 138–147 (2008).
34. Dordas, C. Variation in dry matter and nitrogen accumulation and remobilization in barley as affected by fertilization, cultivar, and source–sink relations. *Eur. J. Agron.* **37**, 31–42 (2012).
35. Carter, S., Shackley, S., Sohi, S., Suy, T. B. & Haefele, S. The impact of biochar application on soil properties and plant growth of pot grown lettuce (Lactuca sativa) and cabbage (Brassica chinensis). *Agronomy* **3**, 404–418 (2013).
36. Bijanzadeh, E., Naderi, R. & Egan, T. P. Exogenous application of humic acid and salicylic acid to alleviate seedling drought stress in two corn (Zea mays L.) hybrids. *J. Plant Nutr.* **42**, 1483–1495 (2019).
37. Younis, U., Athar, M., Malik, S., Raza Shah, M. & Mahmood, S. Biochar impact on physiological and biochemical attributes of Spinach (Spinacia oleracea L.) in nickel contaminated soil. *Glob. J. Environ. Sci. Manag.* **1**, 245–254 (2015).
38. Charkhab, A., Mojaddam, M., Shahram, L., Sakinejad, T. & Dadnia, M. R. Evaluation of remobilization rate, grain yield and antioxidant content of maize in reaction to biochar and humic acid amounts under water deficiency stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **50**, 12603–12603 (2022).

39. Seyed Sharifi, R., Khalilzadeh, R., Pirzad, A. & Anwar, S. Effects of biofertilizers and nano zinc-iron oxide on yield and physicochemical properties of wheat under water deficit conditions. *Commu. Soil Sci. Plant Anal.* **51**, 2511–2524 (2020).
40. Teodoro, M. *et al.* Application of co-composted biochar significantly improved plant-growth relevant physical/chemical properties of a metal contaminated soil. *Chemosphere* **242**, 125255 (2020).
41. Jia, W. *et al.* Mineral elements uptake and physiological response of Amaranthus mangostanus (L.) as affected by biochar. *Ecotoxicol. Environ. Saf.* **175**, 58–65 (2019).
42. Liu, C. *et al.* Biochar increased water holding capacity but accelerated organic carbon leaching from a sloping farmland soil in China. *Environ. Sci. Pollut. Res.* **23**, 995–1006 (2016).
43. Boostani, H. R., Najafi-Ghiri, M. & Mirsoleimani, A. The effect of biochars application on reducing the toxic effects of nickel and growth indices of spinach (Spinacia oleracea L.) in a calcareous soil. *Environ. Sci. Pollut. Res.* **26**, 1751–1760 (2019).
44. Pokharel, P. & Chang, S. X. Manure pellet, woodchip and their biochars differently affect wheat yield and carbon dioxide emission from bulk and rhizosphere soils. *Sci. Total Environ.* **659**, 463–472 (2019).
45. Boostani, H. R., Hardie, A. G., Najafi-Ghiri, M. & Bijanzadeh, E. Investigation of interaction effects of biochars and silicon on growth and chemical composition of Zea mays L. in a Ni-polluted calcareous soil. *Sci. Rep.* **13** (2023).
46. Babaei, K., Seyed Sharifi, R., Pirzad, A. & Khalilzadeh, R. Effects of bio fertilizer and nano Zn-Fe oxide on physiological traits, antioxidant enzymes activity and yield of wheat (*Triticum aestivum* L.) under salinity stress. *J. Plant Interact.* **12**, 381–389 (2017).
47. Wang, Y. *et al.* Effects of biochar on growth, and heavy metals accumulation of moso bamboo (Phyllostachy pubescens), soil physical properties, and heavy metals solubility in soil. *Chemosphere* **219**, 510–516 (2019).
48. Boostani, H., Najafi-Ghiri, M. & Safizadeh, M. Can addition of biochar and zeolite to a contaminated calcareous soil mitigate the Pb-toxicity effects on Spinach (Spinacia oleracea L.) growth? *Commun. Soil Sci. Plant Anal.* **52**, 136–148 (2021).
49. Bijanzadeh, E., Emam, Y. & Pessarakli, M. Biochemical responses of water-stressed triticale (X *Triticosecale* wittmack) to humic acid and jasmonic acid. *J. Plant Nutr.* **44**, 252–269 (2021).
50. Barati, V. & Ghadiri, H. Assimilate and nitrogen remobilization of six-rowed and two-rowed winter barley under drought stress at different nitrogen fertilization. *Arch. Agron. Soil Sci.* **63**, 841–855 (2017).
51. Bijanzadeh, E. & Naderi, R. Remobilization efficiency and photosynthetic characteristics of five barley (*Hordeum vulgare* L.) cultivars under terminal drought stress. *Arch. Agron. Soil Sci.* **61**, 1199–1210 (2015).
52. Bijanzadeh, E. & Emam, Y. Evaluation of assimilate remobilization and yield of wheat cultivars under different irrigation regimes in an arid climate. *Arch. Agron. Soil Sci.* **58**, 1243–1259 (2012).
53. Bijanzadeh, E., Barati, V., Emam, Y. & Pessarakli, M. Sowing date effects on dry matter remobilization and yield of triticale (*Triticosecale* wittmack) under late season drought stress. *J. Plant Nutr.* **42**, 681–695 (2019).
54. Lehmann, J. A handful of carbon. *Nature* **447**, 143–144 (2007).
55. Agegnehu, G. *et al.* Biochar and biochar-compost as soil amendments: Effects on peanut yield, soil properties and greenhouse gas emissions in tropical North Queensland, Australia. *Agric. Ecosyst. Environ.* **213**, 72–85 (2015).
56. Zhang, H., Liu, Z. & Liu, S. HMGB1 induced inflammatory effect is blocked by CRISPLD2 via MiR155 in hepatic fibrogenesis. *Mol. Immunol.* **69**, 1–6 (2016).
57. Gao, S. & DeLuca, T. H. Biochar alters nitrogen and phosphorus dynamics in a western rangeland ecosystem. *Soil Biol. Biochem.* **148**, 107868 (2020).
58. Jia, Q., Sun, J., Gan, Q., Shi, N., & Fu, S. *Zea mays* cultivation, biochar, and arbuscular mycorrhizal fungal inoculation influenced lead immobilization. *Microbiol. Spectr.* 03427-23 (2024).
59. Rafique, M. *et al.* Residual effects of biochar and phosphorus on growth and nutrient accumulation by maize (Zea mays L.) amended with microbes in texturally different soils. *Chemosphere* **238**, 124710 (2020).
60. Najafi-Ghiri, M., Ghasemi-Fasaei, R. & Farrokhnejad, E. Factors affecting micronutrient availability in calcareous soils of Southern Iran. *Arid Land Res. Manag.* **27**, 203–215 (2013).
61. Woldetsadik, D. *et al.* Effects of biochar and alkaline amendments on cadmium immobilization, selected nutrient and cadmium concentrations of lettuce (*Lactuca sativa*) in two contrasting soils. *SpringerPlus* **5**, 1–16 (2016).
62. Liu, J., He, T., Yang, Z., Peng, S., Zhu, Y., Li, H., Lu, D., Li, Q., Feng, Y., Chen, K. and & Wei, Y. Insight into the mechanism of nano-TiO2- doped biochar in mitigating cadmium mobility in soil-pak choi system. *Sci. Total Environ.* **916**, 169996 (2024).
63. Evangelou, M. W., Brem, A., Ugolini, F., Abiven, S. & Schulin, R. Soil application of biochar produced from biomass grown on trace element contaminated land. *J. Environ. Manag.* **146**, 100–106 (2014).
64. Hossain, M. F., Piash, M. & Parveen, Z. Effect of biochar and fertilizer application on the growth and nutrient accumulation of rice and vegetable in two contrast soils. *Acta Sci. Agric.* **3**, 74–83 (2019).
65. De Almeida Silva, M. *et al.* Physiological changes and yield components of safflower (Carthamus tinctorius L.) lines as a function of water deficit and recovery in the flowering phase. *Agriculture* **13**, 558 (2023).
66. Abdipour, M., Younessi-Hmazekhanlu, M. & Ramazani, S. H. R. Artificial neural networks and multiple linear regression as potential methods for modeling seed yield of safflower (Carthamus tinctorius L.). *Ind. Crops Prod.* **127**, 185–194 (2019).
67. La Bella, S. *et al.* An agronomic evaluation of new safflower (Carthamus tinctorius L.) germplasm for seed and oil yields under Mediterranean climate conditions. *Agronomy* **9**, 468 (2019).

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Competing interests

The authors declare no competing interests.

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