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# Integrative Effects of Rice-Straw Biochar and Silicon on Oil and Seed Quality, Yield and Physiological Traits of *Helianthus annuus* L. Grown under Water Deficit Stress

Mahmoud F. Seleiman <sup>1,2,\*</sup> , Yahya Refay <sup>1</sup>, Nasser Al-Suhaibani <sup>1</sup>, Ibrahim Al-Ashkar <sup>1,3</sup>, Salah El-Hendawy <sup>1,4</sup> and Emad M. Hafez <sup>5</sup>

<sup>1</sup> Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; refay@ksu.edu.sa (Y.R.); nsuhaib@ksu.edu.sa (N.A.-S.); ialashkar@ksu.edu.sa (I.A.-A.); mosalah@ksu.edu.sa (S.E.-H.)

<sup>2</sup> Department of Crop Sciences, Faculty of Agriculture, Menoufia University, Shibin El-kom 32514, Egypt

<sup>3</sup> Agronomy Department, Faculty of Agriculture, Al-Azhar University, Cairo 11651, Egypt

<sup>4</sup> Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt

<sup>5</sup> Agronomy Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt; emadhafez2014@gmail.com

\* Correspondence: mahmoud.seleiman@agr.menofia.edu.eg; Tel.: +966-5531-53351

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**Abstract:** Water deficit stress can negatively affect oil quality, crop yields and soil infertility. Thus, we investigated the effects of rice-straw biochar, foliar silicon and their combination on quality, yield and physiological traits of sunflower grown under three water deficit stress treatments. Water stress treatments were 50% (WS<sub>0</sub>; no stress), 70% (WS<sub>1</sub>; moderate stress) and 90% (WS<sub>2</sub>; severe stress) depletion of the available soil moisture. The results showed that WS<sub>1</sub> and WS<sub>2</sub> negatively affected oil quality, mycorrhizal spores, yield and physiological traits of the sunflower; however, biochar, silicon and their combination significantly ( $p \leq 0.05$ ) improved most of those traits. Oil and oleic acid contents of sunflower grown under WS<sub>2</sub> were decreased by 18% and 25.8% compared to those grown under WS<sub>0</sub>, respectively. Nevertheless, the biochar and silicon combination resulted in higher oil (10.2%) and oleic acid (12.2%) in plants grown under WS<sub>2</sub> than those grown in untreated plots. Also, a significant increase (182% and 277%) in mycorrhizal spores was obtained in soil treated combination of biochar and silicon under WS<sub>1</sub> and WS<sub>2</sub> in comparison to untreated soil, respectively. On the other hand, plants grown under WS<sub>1</sub> and WS<sub>2</sub> exhibited reduced seed yield ha<sup>-1</sup> by 16.5% and 53.5% compared to those grown under WS<sub>0</sub>, respectively. However, seed yield ha<sup>-1</sup> were increased by 26.8% and 27.1% in plots treated with combined treatment compared to untreated plants, respectively. In addition, the biochar and silicon combination significantly increased stomatal conductance by 21.4% and 12.1%, reduced proline by 56.6% and 51.2% and reduced catalase activity by 13.4% and 17.3% under WS<sub>1</sub> and WS<sub>2</sub> compared to those grown in untreated plots, respectively. Therefore, the combined treatment of biochar and silicon can minimize and alleviate the negative effects of WS<sub>1</sub> and WS<sub>2</sub>, improve oil quality, physiological traits, microbial activity and seed yield ha<sup>-1</sup> in sunflower plants.

**Keywords:** rice-straw biochar; exogenous silicon; sunflower; productivity; oil quality; water deficit stress

## 1. Introduction

Biochar is a carbonaceous substance resulting from the thermochemical decomposition of agricultural biomass and wastes [1–5]. One promising management approach is to convert rice straw [1] or rice husk [4] into biochar, which can be suitable source of silicon. Biochar is used as a conditioner to improve soil quality and yield in agricultural systems [3,5,6], enhance soil carbon sequestration potential [1,7,8], enhance nutrient cycles in agricultural lands [3,9–11] and alleviate the harmful effects of drought stress for chickpea, *Cicer arietinum* L. [12] and soybean *Glycine max* L. [13]. It also promotes plant growth through improving nutrients uptake and assimilation, improving soil microbial activity [5] and phytohormone synthesis [14]. However, the role of biochar in alleviating water deficit stress and the resultant effect on the quality (unsaturated fatty acids, protein and oil), yield and physiological traits (proline, antioxidant enzyme and chlorophyll content, as well as stomatal conductance) of sunflower has not been fully elucidated in arid and semi-arid regions.

On the other hand, biochar is considered slow-release silicon source, thus it might be insufficient to meet the demand for silicon in agriculture and alleviating the negative effects of abiotic and biotic stresses. Therefore, exogenous/foliar applications of some elements such as silicon can act as a complement effect with the soil amendment of biochar for alleviating the negative effects of water deficit stress on plant growth, productivity and quality. Silicon is the second most abundant element in the Earth's crust [15]. Although not essential for higher plants, it can be beneficial for growth of plant species [16]. Silicon does not exist in free form but is present as silicates and oxides [17]. Silicon can be taken up by plant roots through passive and active uptake mechanisms as a water soluble silicic acid ( $H_4SiO_4$ ). There are three fractions for silicon in soils, that is, liquid, adsorbed and solid fractions [18,19]. Liquid and adsorbed phases of Si consist of  $H_4SiO_4$ , in addition to polysilicic acids [19]. The silicic acid occurring as monomeric ( $H_4SiO_4$ ) is the plant-available form of soil Si. On the other hand, polysilicic acid has not been shown to be plant-available form of soil Si as  $H_4SiO_4$ ; however, it can link soil particles via the formation of silica bonds, which improve soil aggregation and water-holding capacity [20]. Based on Si accumulation, plants are categorized into high accumulators ( $>15\text{ g Si kg}^{-1}\text{ DM}$ ), intermediate accumulators ( $5\text{--}10\text{ g Si kg}^{-1}\text{ DM}$ ) and low-accumulators ( $>5\text{ g Si kg}^{-1}\text{ DM}$ ) [21,22]. Rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), sugarcane (*Saccharum officinarum* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) are Si accumulator, while maize (*Zea mays* L.) and pearly everlasting (*Anaphalis margarita* L.) are considered intermediate and non-Si accumulators, respectively [22–24]. Rice, wheat, sugar beet, sugar cane and soybean can accumulate 41.6, 24.5, 23.4, 15.1 and 14.0  $\text{g Si kg}^{-1}\text{ DM}$ , respectively [24]. Silicon can enhance resistance of plants to diseases caused by fungi or bacteria [25] through structural reinforcement [26], inhibiting pathogen colonization, antimicrobial compound production [27] and increasing plant resistance through activating signaling pathways and defense-related gene expression [28]. In addition, silicon can ameliorate harmful effects of various environmental stresses on plant growth and productivity [15,17,29]. It can enhance plant growth under drought stress [29,30] by maintaining plant water balance [31], photosynthetic efficiency, erectness of leaves and the structure of xylem vessels under high transpiration rates caused by high temperature and moisture stress [29,32,33]. Silicate minerals accumulate in epidermal cells to form a barrier and reduce water loss using the cuticles [34]. These minerals not only strengthen the plant cell wall but also enhance cell wall elasticity through extension growth [16].

Various abiotic and biotic stresses affect agricultural productivity in arid and semi-arid regions [3,15,35]. The most critical abiotic factors in these regions are severe water deficit stress, salinity and heat stresses, which can significantly decrease the yield and quality of crops. Bray et al. [36] have reported that abiotic stress can reduce the yield by 82% for wheat (*Triticum aestivum* L.), 69% for soybean (*Glycine max* L.) and 66% for maize (*Zea mays* L.). Drought is a major environmental stress factor that affects the growth, yield and quality of various plant species [35,37–40]. Moradi-Ghahderijani et al. [41] reported that severe water stress lessened the sunflower oil yield by 58%. Thus, proper management of organic soil amendments and plant nutrients can play a crucial role in improving plant tolerance and adaptation to severe water deficit stress [35,42]. Sunflower (*Helianthus annuus*

L.) is highly sensitive to water deficit stress from the early flowering stage to the achene filling stage due to inefficient regulation of leaf expansion and transpiration levels under insufficient soil moisture availability [43]. A decline in soil moisture content causes leaf wilting, resulting in a substantial reduction in the yield in semi-arid areas [39].

Sunflower is one of the most widely cultivated oil crops worldwide, particularly in arid and semiarid regions [39,44,45]. It accounts for approximately 8% of the global oilseed production. In 2018, the global cultivation area of sunflower was 26.6 M ha with a total seed production of 47.8 Mt [46]. Egypt annually imports approximately 60,000 and 30,000 t of sunflower and soybean oils, respectively, due to the small cultivation area (6,000 ha) and low total seed production (20,000 t) of sunflower [46] resulting from various abiotic stresses. Sunflower provides raw materials that can be used in several food, fodder and bioenergy sectors [39]. Oils with a high percentage of oleic acid are beneficial, while those with higher linoleic acid content are used in the biofuel industry. Sunflower seeds contain up to 50.4% oil [45] and 17%–20% protein [39]. The oil quality of sunflower depends on the fatty acid composition, particularly the ratio of oleic, linoleic and linolenic acids [47]. In addition, the consumption of oils with a high content of unsaturated fatty acids has been shown to exert a positive effect on human health [45].

Therefore, this study was performed to investigate the effects of soil amendment (rice-straw biochar), exogenous application of silicon and the combined treatment of biochar and silicon on the quality, mycorrhizal spores, plant elemental analysis, yield and physiological traits of sunflower grown under water deficit stress conditions (control, moderate water stress and severe water stress) in arid and semi-arid regions. The hypothesis was that the combination of rice-straw biochar as soil amendment and silicon as exogenous application can improve microbial activity in soil, alleviate negative effects of water stress and improve quality and productivity of sunflower grown under water deficit stress.

## 2. Materials and Methods

### 2.1. Experimental Design and Field Practices

Two experiments were performed at the Experimental Farm, Faculty of Agriculture, University of Menoufia, Egypt (Latitude: 30°33'31" and Longitude: 31°00'36") during the summers of 2016 and 2017 to investigate the role of soil amendment (rice-straw biochar), exogenous application of silicon and their combination on oil and seed quality, mycorrhizal spores, yield and physiological traits of sunflower (*Helianthus annuus* L., cv Sakha 53) grown under different water deficit stress conditions [50% (WS<sub>0</sub>; no stress), 70% (WS<sub>1</sub>; moderate stress) and 90% (WS<sub>2</sub>; severe stress) depletion of the available soil moisture from the depth 0–60 cm].

Briefly, biochar was produced by fast pyrolysis (temperature 500–550 °C; time 20 min) from rice-straw feedstock. Biochar analysis was pH 8.3, electrical conductivity 0.14 dS m<sup>-1</sup>, water holding capacity 53.01%, silicon 6.4 g kg<sup>-1</sup>, Carbon 465.4 g kg<sup>-1</sup>, Nitrogen 9.8 g kg<sup>-1</sup>, Phosphorus 1.9 g kg<sup>-1</sup>, Potassium 20.5 g kg<sup>-1</sup>, Calcium 55.2 g kg<sup>-1</sup>, Magnesium 3.7 g kg<sup>-1</sup>, H 17.1 g kg<sup>-1</sup>, O 50.2 g kg<sup>-1</sup> and specific surface area 22.4 m<sup>2</sup> g<sup>-1</sup>. The biochar was homogenized and applied at the rate of 10.0 t ha<sup>-1</sup> before sowing. Subsequently, the biochar was mixed with the top layer of soil (0–10 cm depth). Silicon was exogenously applied as K<sub>2</sub>Si<sub>3</sub>O<sub>7</sub> at 30 and 55 days after sowing (DAS) at the rate of 150 g Si ha<sup>-1</sup>.

The experimental design was split plot-based on randomized complete block design with three replications. Irrigation treatments were placed in the main plots, while soil biochar amendment, exogenous silicon and their combination treatments were placed in subplots. The sub-plot size was 20 m<sup>2</sup> (5 m length × 4 m width). The plot was bordered with a 1.5-m alley to prevent mixing of various water treatments.

Sunflower seeds were purchased from the Oil Crops Institute, Agricultural Research Center, Egypt. Seeds were sown on 6th June 2016 and 8th June 2017 at the rate of 10 kg ha<sup>-1</sup>. They were sown in rows (0.6 m apart) and on hills (2–3 seeds per hill) with a distance of 20 cm between two hills. At 18 DAS, plants were thinned into one plant per hill. Calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) fertilizer was added

at the rate of 37 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> prior to sunflower sowing. Ammonium nitrate (33.5% N) fertilizer was added at the rate of 72 kg ha<sup>-1</sup> in two equal doses (i.e., before first and second irrigations). Potassium was added in the rate of 50 kg K<sub>2</sub>O ha<sup>-1</sup> before the first irrigation. The preceding cultivated crop was wheat (*Triticum aestivum* L.) in both seasons.

The investigated area is an arid region with no precipitation and remains hot and dry during summer (June–September). The monthly mean of air temperature, relative humidity and precipitation during the growth seasons of 2016 and 2017 are presented in Figure 1. The soil type was clay loam with pH 7.10 and EC 0.82 dS m<sup>-1</sup>; Si 0.34 g kg<sup>-1</sup>, available N 27.7 ppm, available P 9.60 ppm, available K 294.5 ppm and other physical properties are presented in Table 1.

## 2.2. Measurements

### 2.2.1. Physiological and Yield Traits

The free proline content in leaves was determined at the flowering stage (BBCH stage 61) [48] following the method of Bates et al. [49]. The plant leaf samples (1.0 g) were homogenized in 10 mL aqueous sulfosalicylic acid (3%) using a mortar and pestle and subsequently filtered. Thereafter, 2 mL of the filtrate was mixed with 2 mL glacial acetic acid and 2 mL ninhydrin reagent and the reaction mixture was incubated in a water bath for 1 h at 100 °C. After cooling the reaction mixture, 4.0 mL toluene was vortexed for 20 s and added to the reaction mixture, which was subsequently transferred to a separating funnel. The chromophore containing free proline was aspirated and absorbance was measured at 520 nm against toluene blank using a spectrophotometer (UV-160A, Shimadzu, Japan). Proline content was calculated from a calibration curve and expressed as μmol proline g<sup>-1</sup> fresh weight (FW).

Stomatal conductance was measured from the youngest fully expanded leaf at the flowering stage (BBCH stage 61) [48] between 10:00 to 12:00 am using the leaf porometer system (Model AP4, Delta-T Devices Ltd., Cambridge, UK), following the user manual instructions. Stomatal conductance measurement was recorded as the average from five different plants in each plot.

The activity of antioxidant enzymes (catalase and peroxidase) was analyzed from 500 mg sunflower leaves. Briefly, the sample was homogenized at 0–4 °C in 3.0 mL of 50.0 mM Tris buffer (pH 7.8), containing 1.0 mM EDTA-Na<sub>2</sub> and 7.5% polyvinylpyrrolidone. Thereafter, samples were centrifuged at 12,000 rpm for 20 min at 4.0 °C. Catalase activity (CAT) was determined from changes in the absorbance at 240 nm at each 30 s interval for 3 min using a UV-160A spectrophotometer. Guaiacol peroxidase (POX) activity was determined from changes in the absorbance at 470 nm at each 30 s interval for 3 min. CAT and POX activities were expressed as the increase in absorbance min<sup>-1</sup> g<sup>-1</sup> FW.

Chlorophyll content of the topmost fully expanded leaves on the main stem at the flowering stage (BBCH stage 61) [48] between 10:00 to 12:00 am was measured using the SPAD meter (Model: SPAD-502, Minolta Sensing Ltd., Osaka, Japan). It was expressed as SPAD units (Soil Plant Analysis Development). SPAD values were recorded from 10 plants within each plot and the readings were averaged to obtain a single value for each replication.

In addition, five plants were randomly collected from each plot at the flowering stage (BBCH stage 61) [48] for measuring the plant height (from soil surface to the plant top), stem diameter (at 30 cm from the soil surface), leaf area plant<sup>-1</sup> (LI-3000C, Portable Leaf Area Meter, LI-COR Inc., Lincoln, NE, USA) and relative water content (RWC). RWC was analyzed gravimetrically from the topmost fully expanded leaves at the flowering stage (BBCH stage 61) [48] between 10:00 to 12:00 am. The leaves were weighted immediately after detaching them from the plants to obtain FW and were subsequently rehydrated in distilled water for 24 h to obtain the turgid weight (TW). Finally, they were dried at 60 °C in an oven for 48 h to obtain the dry weight (DW). RWC was measured according to the following formula:

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100 \quad (1)$$

On 8th September 2016 and 10th September 2017, plants with an area of 2.0 m<sup>2</sup> were harvested for measuring head diameter (cm), number of seeds per head, head weight (g), seed index (100-seed weight, g) and seed yield (kg ha<sup>-1</sup>).

### 2.2.2. Mycorrhizal Spores and Plant Analysis at Harvest

Mycorrhizal spores density in soil (10 g) treated with different treatment was counted as described by Seleiman et al. [50] and according to the modified method of Allen et al. [51]. Soil samples were collected from each plot and were sieved through 1 and 63 µm. Specific weight from the 10 g was inserted in a centrifuge tube containing 15 mL of distilled water and was left for 15 min to hydrate. Then, samples at 2000 rpm for 10 min to remove the organic matter. Afterward, samples were re-suspended in 20 mL of 2 M sucrose solution and centrifuged at 2000 rpm for 10 min. The supernatant, including the spores, was poured into a separatory funnel. The liquid was afterwards slowly drained out of the separatory funnel (10 mL min<sup>-1</sup>). Mycorrhizal spores were washed gently from the funnel walls with 2 mL of distilled water into a Petri dish. Finally, number of mycorrhizal spores was counted directly using a stereo microscope (Leica MZ FL III, Fluorescent Stereo Microscope, Heerbrugg, Germany).

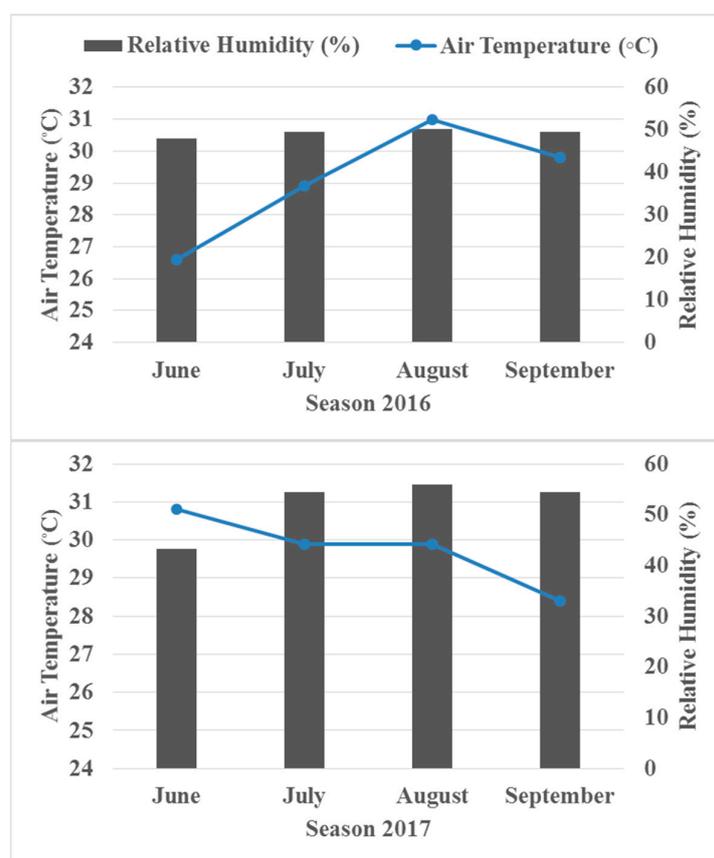
Total Phosphorus, Potassium and Silicon was analyzed as described by Seleiman et al. [52]. Grinded plant biomass samples (300 mg) were inserted in PTFE Teflon tubes with 6 mL nitric acid (67%–69%) and 1 mL hydrogen peroxide (30%) for digestion in a microwave heating (MARSXpress, MARS 240/50, CEM, Matthews, NC, USA). The digested samples were filtered using a Whatman paper (Grade No. 42, pore size 2.5 µm, GE Healthcare, UK) and then were diluted in purified water to a specific volume. Finally, diluted samples were analyzed using an ICP-Optical Emission Spectrometry (iCAP 6200, Thermo Fisher Scientific Inc., Cambridge, UK).

For analyzing total N content, grinded plant samples (0.2 g) were weighted and analyzed following the Dumas combustion method by using a Vario MAX CN (Elementar Analysensysteme GmbH, Hanau, Germany).

**Table 1.** Analysis of investigated experimental soil before sowing in 2016 and 2017 seasons.

Physical and chemical properties								
Seasons	Properties	EC (dS m <sup>-1</sup> )	pH	OM (%)	N	P	K	Total Si (g kg <sup>-1</sup> )
					Available (meq L <sup>-1</sup> )			
	2016	0.80	7.2	1.75	28.01	9.73	297.4	0.31
	2017	0.84	7.0	1.82	27.34	9.44	291.6	0.36
Mechanical properties								
Seasons	Properties	Sand (%)		Silt (%)	Clay (%)		Soil texture	
	2016	21.00		41.04	37.96		Clay loam	
	2017	21.03		42.02	36.95		Clay loam	

EC = Electrical conductivity, OM = Organic matter.



**Figure 1.** Monthly average of air temperature, relative humidity and precipitation level during 2016 and 2017 seasons. Source: Central Laboratory for Agricultural Climate, Agricultural Research Center, Ministry of Agriculture & Land Reclamation, Egypt.

### 2.2.3. Quality Traits

Seeds were dried at 40 °C for 4 h using a ventilated vacuum oven and were subsequently ground using a Waring Blendor (Waring Product Division, New Hartford, CT, USA). The percentage of oil seeds was analyzed using the petroleum ether extraction technique with the Soxhlet meter [53]. The oil extract was evaporated through distillation in a rotary evaporator at 35 °C until the solvent was removed completely. Finally, the crude extract was weighed to obtain the percentage of seed oil.

Oil yield ( $\text{kg ha}^{-1}$ ) was calculated as follows:

$$\text{Oil yield } (\text{kg ha}^{-1}) = \text{Oil seed\%} \times \text{seed yield } (\text{kg ha}^{-1}) \quad (2)$$

To analyze the fatty acids (oleic acid and linoleic acid), a 2-g aliquot of sunflower seed oil was transferred to a screw-capped vial containing 0.3 mL methanol:sodium methylate (28.5:1.5 w/w) and incubated for 2 h at 90 °C for the methylation of fatty acids. The fatty acid composition (oleic and linoleic acids) of sunflower oil was measured, as described by Rotunno et al. [54] using a Fisons model GC-8160 gas chromatograph (Italian Fisons, Milan, Italy). Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture (Larodan) of fatty acid methyl esters (FAME). Oleic/linoleic ratio was calculated as follows:

$$\text{Oleic/linoleic ratio} = \frac{\text{Oleic content}}{\text{Linoleic content}} \quad (3)$$

Protein content—the seeds were finely ground and the protein content was determined according to the method of Kjeldahl (ISO 20483:2006). Crude protein content was calculated by multiplying the N content by 6.25.

### 2.3. Statistical Analysis

Data obtained from the treatment of biochar, silicon and their combination, as well as water deficit stress treatments and their effect on oil and seed quality, mycorrhizal spores, plant elemental analysis, yield and physiological traits of sunflower were subjected to analysis of variance (ANOVA) with the general linear model using PASW statistics 21.0 (IBM Inc., Chicago, IL, USA). The means were compared using Tukey's multiple range test (at significant difference of  $p \leq 0.05$ ). The standard error of mean (S.E.M.) was calculated for each parameter.

## 3. Results

### 3.1. Physiological and Yield Traits

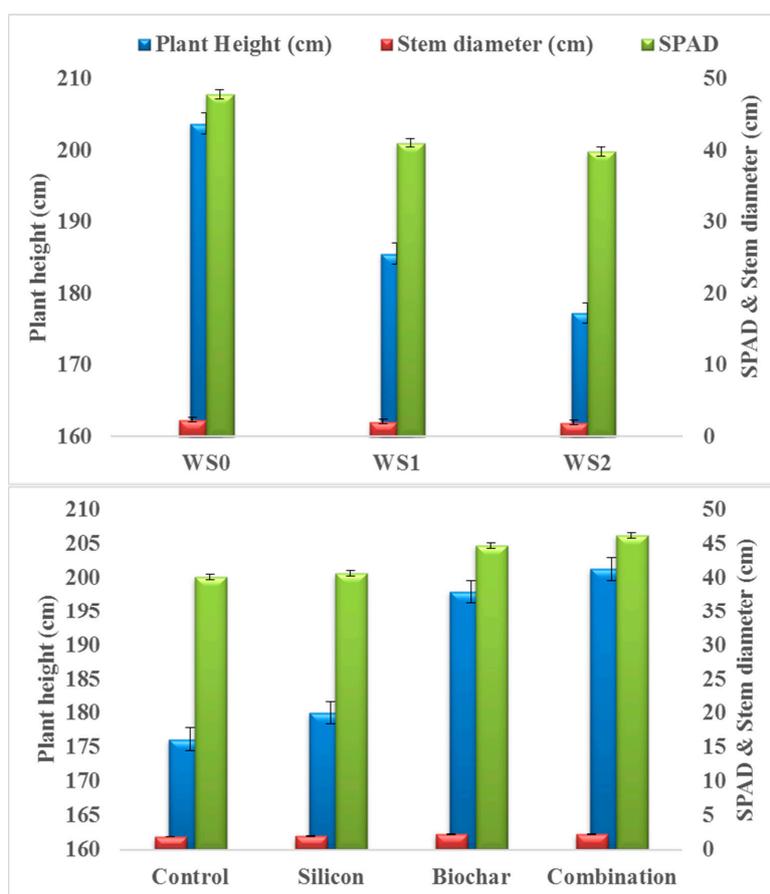
Physiological and yield traits of sunflower showed significant differences with respect to water deficit stress, soil and exogenous amendments during the two growing seasons (Tables 2–4 and Figure 1). The stomatal conductance of the sunflower plants grown under moderate (WS<sub>1</sub>) and severe (WS<sub>2</sub>) water deficit stress conditions was significantly ( $p \leq 0.01$ ) reduced by 16.13% and 23.96%, respectively, relative to that of the well-watered (WS<sub>0</sub>) plants (Table 2). However, treatment with biochar, silicon and their combination increased the stomatal conductance by 7.9%, 16.9% and 21.43% and by 2.8%, 6.1% and 12.1% for the plants grown under WS<sub>1</sub> and WS<sub>2</sub> conditions, respectively, compared to those grown in the untreated plots (Table 2). On the other hand, the proline content and POX and CAT activities were significantly increased ( $p \leq 0.01$ ) by 38.5%, 183.4% and 63.3% and by 7.2%, 95.2% and 45.5% for the plants grown under WS<sub>2</sub> and WS<sub>1</sub> conditions, respectively, compared to WS<sub>0</sub> plants (Table 2). However, treatment with silicon, biochar and their combination significantly ( $p \leq 0.01$ ) decreased the proline content and POX and CAT activities in the sunflower plant grown under different water deficit stress treatments compared to those grown in the untreated plots (Table 2). Moreover, the combined treatment of biochar and silicon reduced the proline content and CAT activity by 56.6% and 13.4% and by 51.2% and 17.3% for the plants grown under WS<sub>1</sub> and WS<sub>2</sub> conditions, respectively, compared to those grown in the untreated plots.

The leaf area plant<sup>-1</sup>, RWC, plant height and stem diameter of sunflower were significantly reduced with increasing water deficit stress (Table 3 and Figure 2). The leaf area plant<sup>-1</sup> and RWC obtained from WS<sub>0</sub> plants increased by 31.5% and 39.8% compared to that obtained from plants grown under severe water deficit stress treatment (Figure 2). However, these negative effects of water deficit stress were significantly minimized when sunflower plants were treated with biochar, silicon or their combination. Plants grown under WS<sub>1</sub> condition and receiving combined treatment (biochar + silicon) did not exhibit significant differences in the leaf area plant<sup>-1</sup> and RWC with those grown in WS<sub>0</sub> plots without any amendments (Figure 2). The leaf area plant<sup>-1</sup> and RWC of sunflower plants grown under WS<sub>2</sub> condition were reduced by 40.6% and 44.3%, respectively, in the absence of biochar or silicon (control treatment), 29.3% and 24.3%, respectively, with exogenous silicon application, 18.8% and 15.8%, respectively, with soil biochar application and 11.35% and 12.16%, respectively, with combined treatment compared to those of WS<sub>0</sub> plants without any amendments (Table 3).

**Table 2.** Interaction effects of amendments and water deficit stress treatments on physiological traits of sunflower during growing seasons 2016 and 2017.

Traits		Stomatal Conductance $g_s$ ( $\text{Mmol H}_2\text{O m}^{-2} \text{S}^{-1}$ )		Proline ( $\mu \text{mol g}^{-1} \text{FW}$ )		POX Activity ( $\text{Mmol H}_2\text{O}_2 \text{FW min}^{-1}$ )		CAT Activity ( $\text{Mmol H}_2\text{O}_2 \text{FW min}^{-1}$ )	
Water Stress	Amendments	S1	S2	S1	S2	S1	S2	S1	S2
WS <sub>0</sub>	Control	48.87	49.56	8.90	9.20	1.18	1.38	150.33	153.7
	Silicon	50.78	51.48	9.54	9.84	1.04	1.24	125.33	127.0
	Biochar	53.73	54.42	8.70	9.00	0.75	0.95	72.67	76.6
	Combination	55.06	55.76	8.14	8.44	0.60	0.80	44.33	48.3
WS <sub>1</sub>	Control	38.76	40.10	10.59	10.56	2.21	2.75	229.20	192.0
	Silicon	41.91	43.19	9.24	9.53	1.92	2.36	176.80	149.3
	Biochar	45.44	46.78	9.22	9.50	1.52	1.85	126.00	105.0
	Combination	47.19	48.57	9.02	9.30	1.34	1.55	97.60	85.3
WS <sub>2</sub>	Control	37.53	37.72	13.67	13.77	3.23	3.33	252.00	214.0
	Silicon	38.60	38.79	12.69	12.79	3.06	3.16	191.60	162.6
	Biochar	40.96	41.16	11.82	11.92	2.55	2.65	137.20	119.3
	Combination	42.07	42.27	11.30	11.40	2.21	2.31	121.20	106.0
S.E.M		0.56	0.59	0.042	0.113	0.049	0.051	3.78	3.4
Significant									
Water stress (WS)		*	**	**	**	**	**	**	*
Amendments (A)		**	**	**	**	**	**	**	**
WS × A		**	**	**	**	**	**	**	**

POX = Peroxidase, CAT = Catalase, WS<sub>0</sub> = well-watered plants (50% depletion of the available soil moisture; DAM), WS<sub>1</sub> = Moderate stress (70% DAM), WS<sub>2</sub> = Severe stress (90% DAM), S1= Season 1, S2= Season 2, S.E.M. = Standard error of means; Probability ( $p$ )  $\geq 0.05$  = ns (not significant); \* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$ .



**Figure 2.** Effect of biochar and silicon amendments on growth of sunflower under water deficit stress treatments. Bars = Standard error of means (S.E.M.), WS<sub>0</sub> = well-watered plants (50% depletion of the available soil moisture; DAM), WS<sub>1</sub> = Moderate stress (70% DAM), WS<sub>2</sub> = Severe stress (90% DAM).

**Table 3.** Interaction effect of amendments and water deficit stress treatments on leaf area per plant and relative water content of sunflower during two growing seasons 2016 and 2017.

Treatments		Traits		Relative Water Content (%)	
		Leaf Area Plant <sup>-1</sup> (cm <sup>2</sup> )		S1	S2
Water stress	Amendments	S1	S2	S1	S2
WS <sub>0</sub>	Control	5768.3	5868.3	80.43	83.93
	Biochar	6765.3	6865.3	89.50	93.00
	Silicon	5889.3	5989.3	84.20	87.70
	Combination	6873.6	6973.7	92.10	95.60
WS <sub>1</sub>	Control	5104.1	5224.5	61.88	65.60
	Biochar	5988.5	5506.9	76.51	80.54
	Silicon	5211.8	5333.4	69.29	73.17
	Combination	6102.7	5957.2	79.29	83.39
WS <sub>2</sub>	Control	3441.0	3471.0	45.03	46.53
	Biochar	4710.0	4740.1	68.47	69.97
	Silicon	4099.2	4129.2	61.47	62.97
	Combination	5142.7	5172.8	71.43	72.93
S.E.M		15.6	15.4	0.28	0.281
Significant					
Water stress (WS)		**	**	**	**
Amendments (A)		**	**	**	**
WS × A		**	**	**	**

WS<sub>0</sub> = well-watered plants (50% depletion of the available soil moisture; DAM), WS<sub>1</sub> = Moderate stress (70% DAM), WS<sub>2</sub> = Severe stress (90% DAM), S1 = Season 1, S2 = Season 2, S.E.M. = Standard error of means, Probability ( $p$ )  $\geq 0.05$  = ns; \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ .

On the other hand, chlorophyll content was significantly reduced by 14.2% and 16.7% in sunflower plants grown under  $WS_1$  and  $WS_2$  conditions, respectively, compared to that in  $WS_0$  plants (Figure 2). However, the combined treatment and the single application of biochar-soil amendment increased the chlorophyll content by 13.1% and 10.3%, respectively, in plants grown under water deficit stress treatments compared to those grown in untreated plots (Figure 2). These results indicated that soil amendment of biochar, exogenous application of silicon and their combined treatment alleviates water deficit stress-induced chlorophyll degradation in sunflower plants.

The interaction effects between the water stress treatments and amendment applications were significant on seed yield ( $\text{kg ha}^{-1}$ ) and related traits (Table 4). Plants grown under  $WS_1$  and  $WS_2$  conditions showed a significant reduction ( $p \leq 0.01$ ) in the head diameter by 11.2% and 24.9%, number of seeds  $\text{head}^{-1}$  by 20.9% and 35.6%, 100-seed weight by 10.5% and 30.7% and seed yield ( $\text{kg ha}^{-1}$ ) by 16.5% and 53.5%, respectively, compared to  $WS_0$  plants (Table 4). However, the application of soil biochar, exogenous silicon or their combination ameliorated the effect in those plants. The number of seeds  $\text{head}^{-1}$ , 100-seed weight and seed yield ( $\text{kg ha}^{-1}$ ) of the sunflower plants grown with exogenous silicon application under  $WS_1$  condition were only reduced by 5.6%, 9.2% and 3.4%, respectively, compared to that of the  $WS_0$  plants without any amendment (Table 4).

However, these traits were increased by 5.2%, 2.4% and 1.6%, respectively, with soil biochar treatment and by 11.2%, 5.8% and 9.7%, respectively, with combined treatment, compared to that in the  $WS_0$  plants in untreated plots (Table 4). Furthermore, the seed yield of the plants grown under  $WS_2$  condition was reduced by 55.6% ( $1624 \text{ kg ha}^{-1}$ ) in the untreated plots (control), 48.2% ( $1410 \text{ kg ha}^{-1}$ ) in the plots treated with exogenous silicon, 43.9% ( $1284 \text{ kg ha}^{-1}$ ) in the plots treated with biochar and 33.0% ( $965 \text{ kg ha}^{-1}$ ) in the plots treated with combined treatment over an average of two growing seasons, compared to that of the  $WS_0$  plants grown in the absence of soil biochar or exogenous treatments (Table 4). Overall, under all water stress conditions, the number of seeds  $\text{head}^{-1}$ , 100-seed weight and seed yield ( $\text{kg ha}^{-1}$ ) were increased by 26.80%, 18.06% and 27.09%, respectively, for plants receiving the combined treatment, by 22.53%, 14.06% and 19.06%, respectively, for those receiving soil biochar and by 11.20%, 18.06% and 11.12%, respectively, for those receiving exogenous silicon, compared to that for the  $WS_0$  plants (Table 4).

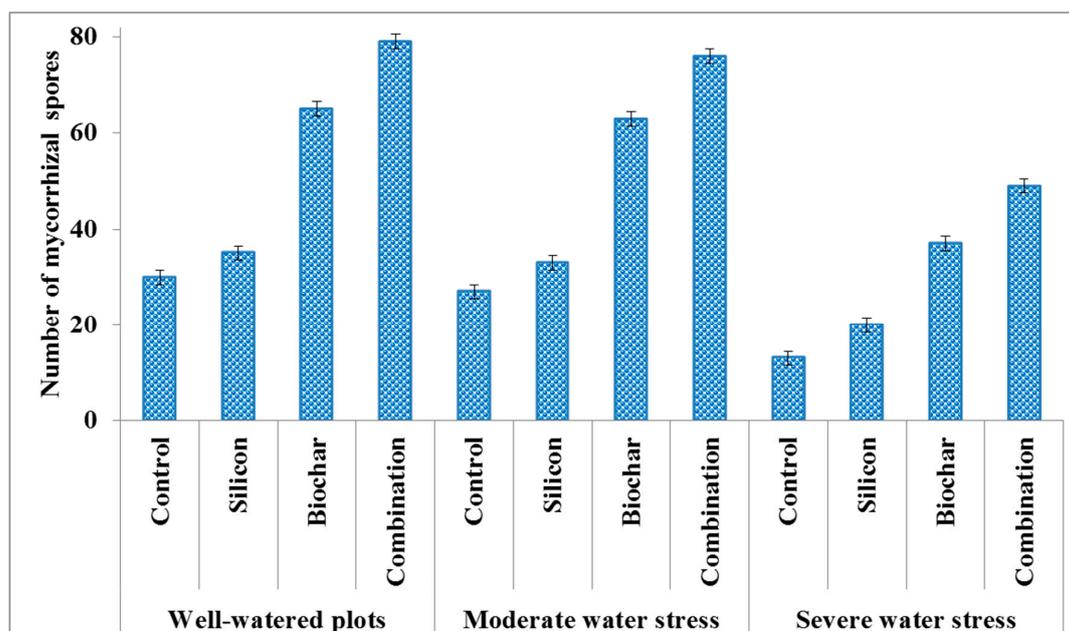
**Table 4.** Interaction effects of amendments and water deficit stress treatments on yield and related traits of sunflower during two growing seasons 2016 and 2017.

Treatments		Traits		Head Diameter (cm)		Number of Seeds Per Head		100-Seed Weight (g)		Seed Yield (kg ha <sup>-1</sup> )	
		Water Stress	Amendments	S1	S2	S1	S2	S1	S2	S1	S2
WS <sub>0</sub>	Control	18.2	19.1	1033	1053	6.27	6.57	2914.0	2933.9		
	Silicon	18.6	19.5	1231	1251	6.43	6.73	3155.0	3174.9		
	Biochar	22.0	22.9	1440	1460	7.13	7.43	3619.3	3639.3		
	Combination	22.6	23.5	1555	1575	7.23	7.53	3973.0	3993.0		
WS <sub>1</sub>	Control	16.7	17.3	942	956	5.57	5.58	2574.4	2316.1		
	Silicon	16.7	18.3	979	991	5.83	5.83	2661.0	2988.3		
	Biochar	19.5	19.6	1120	1075	6.59	6.56	2964.3	2977.0		
	Combination	19.8	19.8	1161	1158	6.81	6.78	3144.4	3268.6		
WS <sub>2</sub>	Control	12.0	12.3	702	707	4.10	4.20	1294.6	1304.5		
	Silicon	14.0	14.3	810	815	4.53	4.63	1509.0	1518.9		
	Biochar	17.6	17.9	932	937	4.87	4.97	1634.8	1644.8		
	Combination	18.3	18.6	959	964	5.47	5.57	1953.5	1963.4		
S.E.M		0.22	0.23	25	26	0.075	0.074	39.9	39.7		
Significant											
Water stress (WS)		**	**	**	**	**	*	**	**		
Amendments (A)		**	**	**	**	**	*	**	**		
WS × A		*	*	**	**	**	*	**	**		

WS<sub>0</sub> = well-watered plants (50% depletion of the available soil moisture; DAM), WS<sub>1</sub> = Moderate stress (70% DAM), WS<sub>2</sub> = Severe stress (90% DAM), S1= Season 1, S2= Season 2, S.E.M. = Standard error of means; Probability (*p*) ≥ 0.05 = ns (not significant); \* = *p* ≤ 0.05; \*\* = *p* ≤ 0.01.

### 3.2. Mycorrhizal Spores and Plant Elemental Analysis

A significant increase (182% and 277%) in mycorrhizal spores was noticed in soil treated with combination treatment (biochar + silicon) in comparison to untreated soil under moderate and severe water stress, respectively (Figure 3). While a significant increase of 133% and 184% was obtained in soil treated with biochar in comparison to untreated soil under moderate and severe water deficit stress, respectively.

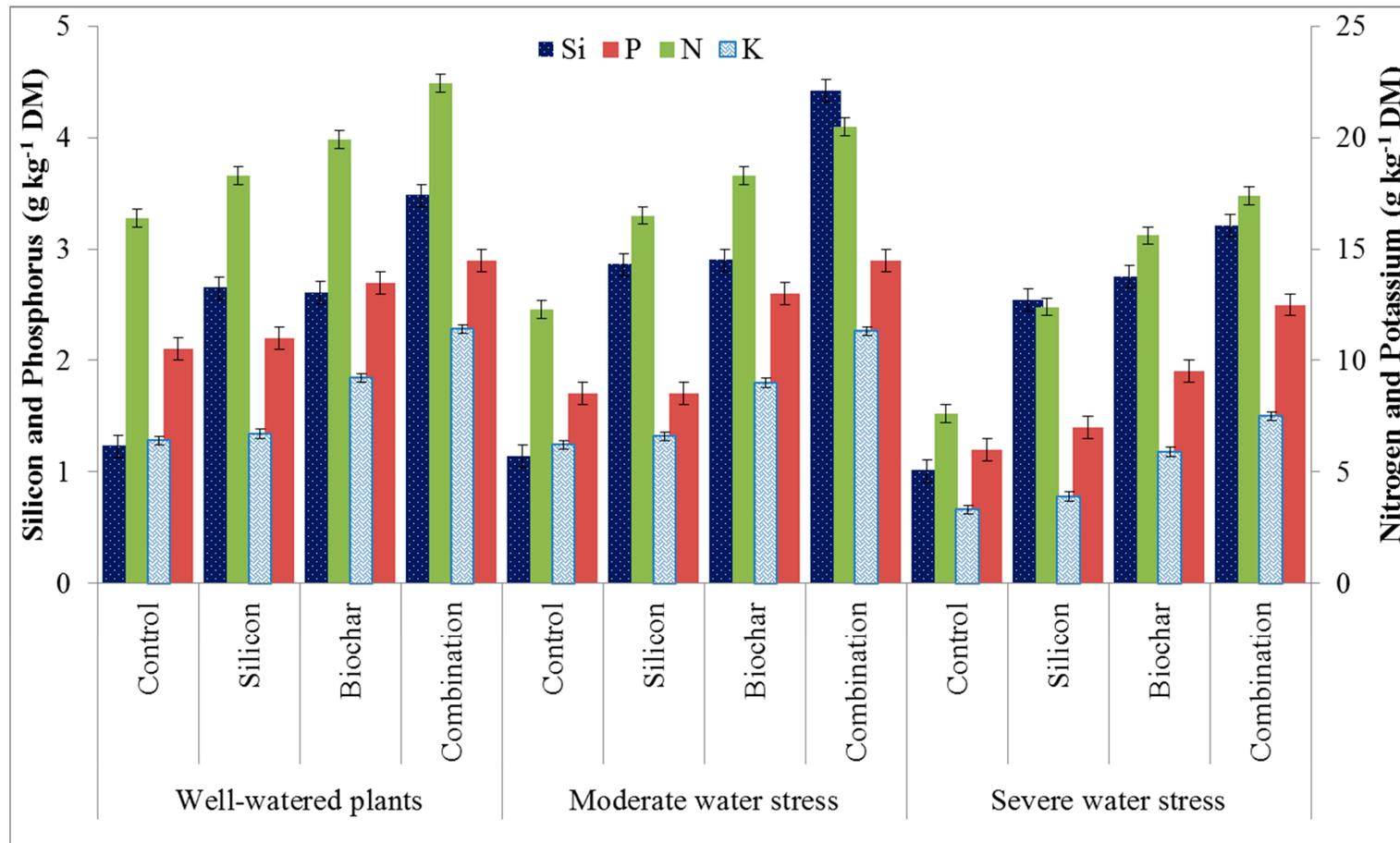


**Figure 3.** Number of mycorrhizal spores in soil treated with biochar, silicon and their combination under different water deficit stress treatments. Bars = Standard error of means (S.E.M.).

Besides that, the highest content of Si, N, P and K in sunflower biomass was obtained when plots were treated with combination of biochar and silicon in comparison to individual treatment of biochar or silicon and untreated soil under different water stress treatments (Figure 4). For example, the combination treatment of biochar and silicon resulted higher Si (288%), N (71%), P (67%) and K (82%) in sunflower biomass than those obtained from untreated soil (Figure 4).

### 3.3. Oil and Seed Quality Traits

As shown in Table 5, the oil percentage, seed oil yield and oleic acid content were significantly decreased ( $p \leq 0.05$ ) by 17.98%, 60.93% and 25.79% and by 6.52%, 21.84% and 9.09%, for plants grown under moderate  $WS_2$  and  $WS_1$  conditions, respectively, compared to that for  $WS_0$  plants. However, the combined treatment of biochar and silicon, as well as the application of soil biochar and exogenous silicon increased the oil percentage by 10.17%, 9.37% and 4.13% for the plants grown under all water stress conditions, compared to that for the  $WS_0$  untreated plants (control) (Table 5). Furthermore, the seed oil yield ( $\text{kg ha}^{-1}$ ) was increased by 33.85%, 26.30% and 14.39% for the plants subjected to the combined treatment, biochar and silicon application, respectively, compared to  $WS_0$  plants (Table 5). The combined treatment significantly ameliorated ( $p \leq 0.01$ ) the negative effects of  $WS_1$  and  $WS_2$  conditions for oil percentage by +10.86% and -39.85%, oil yield by -1.46% and -25.31% and oleic acid content by 2.52% and 14.44%, respectively, compared to  $WS_0$  treatment (Table 5). Linoleic acid and protein content were increased by 477% and 6% under  $WS_2$  condition and by 156% and 15% under  $WS_1$  condition, respectively, compared to that in  $WS_0$  plants (Table 5).



**Figure 4.** Effect of biochar and Silicon and their combination on Silicon (Si), Phosphorus (P), Nitrogen (N) and Potassium (K) content of sunflower biomass under water deficit stress treatments. Bars = Standard error of means (S.E.M).

**Table 5.** Interaction effects of soil and foliar amendments and water deficit stress treatments on oil traits and fatty acids of sunflower during two growing seasons 2016 and 2017.

Treatments		Oil (%)		Oil Yield (kg ha <sup>-1</sup> )		Protein (%)		Oleic Acid (%)		Linoleic Acid (%)		Oleic/Linoleic Ratio	
Water Stress	Amendments	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
WS <sub>0</sub>	Control	41.9	42.0	1221	1232	19.3	20.0	85.0	84.5	5.8	6.2	14.7	13.6
	Silicon	43.4	43.6	1369	1384	20.0	20.8	86.4	86.9	4.7	5.0	18.4	17.4
	Biochar	45.3	45.5	1639	1655	19.5	20.0	87.4	87.9	4.2	4.5	20.8	19.5
	Combination	45.4	45.5	1803	1816	20.3	21.3	88.4	88.7	3.7	4.1	23.9	21.6
WS <sub>1</sub>	Control	40.9	41.4	1052	958	20.5	21.1	72.8	73.2	17.6	17.9	4.2	4.1
	Silicon	42.4	42.4	1128	1267	21.2	21.7	74.6	75.6	16.5	16.8	4.5	4.6
	Biochar	45.1	45.2	1336	1345	20.7	21.6	82.7	83.0	7.3	7.5	11.3	11.1
	Combination	45.1	45.0	1418	1470	21.7	22.3	84.6	85.0	7.1	7.2	11.9	11.8
WS <sub>2</sub>	Control	33.6	33.7	435	439	22.5	22.7	60.5	60.7	31.5	31.2	1.8	1.9
	Silicon	35.8	35.8	540	543	22.9	23.2	62.7	62.3	29.1	29.4	2.1	2.2
	Biochar	38.1	38.3	622	630	23.2	23.4	66.8	67.4	25.5	25.7	2.6	2.6
	Combination	39.1	39.1	763	767	23.6	23.9	67.9	68.1	23.9	24.3	2.9	2.8
S.E.M		0.3	0.4	16.4	17.8	1.01	0.89	0.3	0.4	0.9	1.1	0.05	0.04
Significant													
Water stress (WS)		**	**	**	**	*	*	**	**	**	**	**	**
Amendments (A)		*	*	**	**	*	*	**	**	**	**	**	**
WS × A		*	*	**	**	*	*	**	**	**	**	**	**

WS<sub>0</sub> = well-watered plants (50% depletion of the available soil moisture; DAM), WS<sub>1</sub> = Moderate stress (70% DAM), WS<sub>2</sub> = Severe stress (90% DAM), S1= Season 1, S2= Season 2, S.E.M. = Standard error of means; Probability (*p*) ≥ 0.05 = ns (not significant); \* = *p* ≤ 0.05; \*\* = *p* ≤ 0.01.

## 4. Discussion

Water resource shortage is a serious problem worldwide and particularly in the arid and semi-arid regions [35,39,55,56]. In the present study, physiological, yield, oil and seed quality traits, mycorrhizal spores in soil and plant elemental analysis were negatively affected when the sunflower plants were grown under WS1 and WS2 conditions. However, this effect was reversed in plots treated with soil biochar, exogenous silicon and their combination (Tables 2–5 and Figures 2–4). This can be attributed to the beneficial effects of the soil nutrients, such as N, P, K, Ca and Mg; moisture availability induced by biochar [56] and Si-induced plant growth under water deficit stress. Biochar is condensed at high pyrolysis temperatures ( $\geq 500$  °C), owing to the aromatization of carbon and silicon crystallization.

### 4.1. Physiological and Yield Traits

The physiological and growth traits, such as stomatal conductance, POX and CAT activities, leaf area plant<sup>-1</sup>, RWC, chlorophyll content and plant height were negatively affected when plants were exposed to water deficit stress (Tables 2 and 3 and Figure 2). However, treatment with biochar, silicon or their combined treatment improved these traits. Total silicon content (6.4%) in the biochar or exogenous silicon is thought to cause this effect, in part, by reducing the negative impact of water deficit stress via increasing stomatal conductance and decreasing transpiration. The positive enhancements of biochar as soil amendment on plant-water relationship due to increased availability of water for the plants have been stated [57]. In addition, higher water availability results in better hydrated plants with higher capacity for stomatal opening, which is in agreement with our observations. Plant leaves generally transpire through the stomata and cuticle [58]. This process is important under water and environmental stresses. Kaya et al. [59] have reported that Na<sub>2</sub> SiO<sub>3</sub> (2 mM) can increase leaf RWC by 26.5% in maize grown under water stress (50% of field capacity). Silicon can also improve stomatal resistance to water deficit stress thereby increasing leaf chlorophyll content [60].

The increased chlorophyll content in the current study upon the application of silicon and biochar under water deficit stress conditions might be associated with improved antioxidant defense and therefore maintained physiological processes such as photosynthesis. Zhang et al. [29] have reported that the expression of some photosynthesis-related genes are down-regulated in plants grown under water deficit stress conditions; however, exogenous Si can partly up-regulate their expression. This result suggests that Si plays a vital role in alleviating the effect of water deficit stress by modulating photosynthesis-related genes, regulating photochemical process and promoting photosynthesis [29]. Under water stress, photosynthesis in the sunflower plant can be affected by two mechanisms—(a) reduced CO<sub>2</sub> diffusion within the leaf owing to closure of stomata and (b) metabolic inhibition of CO<sub>2</sub> [61]. On the other hand, biochar exerts a beneficial influence by increasing the availability of soil water and field capacity, resulting in improved growth [56]. Biochar can directly supply nutrients to soil [62] and increase plant growth and crop productivity [9]. The enhanced plant growth in the soil treated with biochar during water stress correlated with increased reduction in stomatal conductance, suggesting that the higher water-use efficiency is major factor for enhanced crop performance of biochar-amended plants [63].

The highly aromatic structure, porosity and surface area of the biochar were the main advantages for its stability in soil as well as for enhancing nutrient bioavailability and increasing crop productivity [3]. Furthermore, biochar exhibits high propensity to absorb water and increase its residence time in treated soils [6]. On the other hand, Si plays an important role in alleviating negative water deficit stress through different mechanisms, such as enhancing the antioxidant defense system of plants [17], preventing transpirational water loss [64], increasing water uptake via plant roots [31], improving photosynthetic enzyme activity [33] and regulating growth substance levels [65].

Seed yield and related traits, such as head diameter, number of seeds per head and 100-seed weight of sunflower plants grown under WS<sub>1</sub> and WS<sub>2</sub> conditions were negatively affected compared to that of WS<sub>0</sub> plants (Table 4). Flower initiation and anthesis are the most important growth stages affecting the seed yield. A high number of fertile flowers and florets results in high seed yield [39,43].

The exposure of sunflower plants to WS<sub>2</sub> conditions at this stage is critical; WS<sub>2</sub> coupled with high temperature can lead to pollen infertility, low head diameter and reduced seed yield [66]. For instance, the seed yield of sunflower plants was reduced by 83% upon exposure to water deficit stress at the flowering stage owing to pollen sterility, reduction in the number of seeds per head and 100-seed weight [67]. However, the reduction in the seed yield (kg ha<sup>-1</sup>) under WS<sub>1</sub> (16.4%) and WS<sub>2</sub> (52.3%) conditions in the present study was lower than that obtained by Jabari et al. [67] (83%) due to the enhancement of sunflower growth and physiological traits by the coupling effects of biochar and exogenous application of silicon.

Improving crop yield and its related traits as well as the increase in plant content of Si, N, P and K can be attributed to the great content of nutrients present in biochar and the very high specific surface area [63], when biochar was applied in a combination with silicon in the current investigation. The application of biochar as soil amendment with exogenous silicon to agricultural land can enhance the availability of Si, N, P and K in the soil, support sustainable agriculture via silicon and nutrient cycling in the soil and improve crop productivity [11]. Biochar application to the soil can significantly enhance nitrification by increasing the substrate area availability to nitrifying bacteria [68] and facilitate continuous nitrification by holding nitrogenous nutrients in the root zone soil for an extended time [69]. However, in the untreated soil, a fraction of nitrogen was volatilized and the remainder was leached beyond the rhizosphere, reducing the possibility of continuous nitrification.

#### 4.2. Mycorrhizal Spores and Plant Elemental Analysis

The significant increase in the number of mycorrhizal spores following the individual application of biochar or the combination treatment (biochar + silicon) indicates an enhancement of growth and sporulation of the fungus (Figure 3). Seleiman et al. [50] reported that organic amendment in soil can enhance mycorrhizal spores and root colonization. On the other hand, the lowest number of mycorrhizal spores was obtained from the control treatment (only synthetic fertilizer) in the current study (Figure 3), this can be due to the plentiful N supply which can cause deleterious to the mycorrhiza fungi [50,70]. In addition, the highest contents of Si, N, P and K in sunflower biomass were obtained from plants treated with combination treatment under different irrigation treatments in comparison to other treatments (Figure 4). This can be due to the high content of those elements in rice-straw biochar that applied into soil, particularly silicon. Increasing silicon in soil can increase the availability of soil phosphorus to plants as reported by Eneji et al. [71]. K uptake in soil is improved through the activation of H-ATPase, when silicon is applied even at low Si concentration [72]. Moreover, the increased mycorrhizal fungi and its spores in soil treated with organic amendments can enhance N and P uptake by plants [50].

The highest content of silicon in sunflower biomass at harvest was obtained from plants that received the combination treatment of biochar and foliar silicon application under moderate water stress in comparison to well-watered plants and severe water stress (Figure 4). This means that coupling biochar and foliar silicon application act very well under moderate condition of water stress compared with well-watered or severe stressed plants.

#### 4.3. Oil and Seed Quality Traits

Sunflower oil is a high quality edible oil with a high content (up to 90%) of unsaturated fatty acids, particularly oleic and linoleic acids and low content of saturated fatty acids (8%–10%), such as palmitic and stearic acids [39,73]. The high density of oleic acid in oil is important because the human body cannot synthesize this fatty acid [38]. High mono-unsaturated fatty acids, such as high-oleic acid, can significantly reduce the susceptibility of sunflower oil to oxidative degradation compared to high poly-unsaturated fatty acids [74]. Consequently, high-oleic oil is naturally stable and does not require hydrogenation.

Therefore, a process to increase the content of oleic acid instead of linoleic acid is a major task for scientists and plant breeders. In the current study, water deficit stress conditions negatively affected the

quality traits of the sunflower plant; however, the application of biochar, silicon and their combination improved seed and oil quality by increasing oleic acid content, oleic/linoleic ratio, oil percentage, oil yield ( $\text{kg ha}^{-1}$ ) and protein % and reducing linoleic acid content (Table 5). A higher oil percent is correlated with a reduction of seed weight, this was not in agreement of our results and results obtained from other studies. For instance, Flagella et al. [44] and Anastasi et al. [45] reported that oil seed content of sunflower was significantly reduced by decreasing water supply, although Göksoy et al. [75] found that there was no a significant response of seed oil content to full and limited irrigation. In our study, sunflower plants grown under well-watered and moderate water stress treatments had the heaviest seeds and the highest oil seed content compared to those grown under severe water stress. Our results also were in agreement with those of Baldini et al. [74] who reported that fatty acid composition of sunflower oil seeds is affected by water deficit stress and the ratio of oleic acid to linoleic acid can change the oxidative properties of the oil [44]. Changes in oleic acid content are mainly dependent on synthesis and oleate desaturase enzyme activation [74], which could be affected by water deficit stress. The increase in the oleic/linoleic acid ratio in sunflower plants treated with silicon, biochar or their combination under water deficit stress (Table 5) suggests a possible role of silicon and biochar in the activation of oleate desaturase in sunflower seeds. On the other hand, Flagella et al. [44] have reported a reduction in oleic acid content and an increase in linoleic acid content under full irrigation compared to that in the non-irrigated plants. In addition, Petcu et al. [37] have reported that water stress does not affect oil quality traits due to the low intensity of stress and cultivation of high oleic sunflower genotypes.

## 5. Conclusions

Severe water deficit stress flowed by moderate water stress significantly reduced most of sunflower studied traits. However, the combined treatment (rice-straw biochar as soil amendment and silicon as exogenous application) alleviates and minimized the negative impact of moderate and severe water deficit stress conditions on sunflower plants. Under different water deficit stress treatments, the combined treatment of biochar and silicon resulted in the highest oil quality (i.e., oil and oleic acid percentages), highest content of elements in plant biomass (i.e., Si, N, P, K) and highest seed yield ( $\text{kg ha}^{-1}$ ) of sunflower in comparison to individual application of biochar or silicon and/or control treatment. In addition, the highest number of mycorrhizal spores in soil and leaf stomatal conductance and the lowest antioxidant enzyme activities and proline content were obtained when the combined treatment was applied in comparison to the individual application of biochar or exogenous silicon under  $WS_1$  and  $WS_2$  conditions. In conclusion, the combined application of biochar and silicon is recommended due to positive influences on sunflower productivity and quality traits as well as alleviating and/or mitigating the adverse effects of water deficit stress.

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