Italian Journal of Agronomy 2022; volume 17:2086

Agronomic response of sunflower subjected to biochar and arbuscular mycorrhizal fungi application under drought conditions

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Highlights

- *The combined effects of biochar and arbuscular mycorrhizal fungi on sunflower are studied.*
- *Biochar application and mycorrhiza inoculation improved plant performance.*
- *Biochar and AMF positively affected the net photosynthesis rate of sunflower plants.*
- *The adoption of biochar and AMF may mitigate the effect of drought conditions.*
- *Biochar and AMF can support sunflower cultivation.*

Abstract

There is growing interest in developing environment-friendly farming practices that can limit the impact of drought stress in agriculture. The main objective of this study was to investigate the combined effects of biochar and arbuscular mycorrhizal fungi (AMF) on the agronomic responses of sunflower. Field experiments were conducted in the 2018 and 2019 growing seasons in semi-arid environments of Iran. The following treatments were adopted: i) three levels of biochar [0, 2.5 and 5 t ha^{-1} of biochar called B_l, B_m and B_h, respectively]; and ii) three irrigation levels (50, 30 and 10% of the maximum available water (MAW) called 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively)]; iii) two levels of AMF inoculation (with and without the addition of AMF called $^+$AMF and $^-$AMF, respectively). The experimental design was a randomized complete block design. At flowering, the leaf area index (LAI) was generally higher in the plants subjected to B_h-$^+$AMF (on average 4.95), even if the LAI values changed according to biochar application ($B_h > B_m > B_l$) and the level of irrigation ($50^{MAW} > 30^{MAW} > 10^{MAW}$). At harvesting, sunflower seed yield was highest in $^+$AMF and in Bh (on average 53.9 and 51.2 g plants^{-1}, respectively). Sunflower plants subjected to B_h-$^+$AMF showed the highest seed yield under all irrigation levels (79.4, 57.1 and 32.3 g plant^{-1} in 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively). The application of biochar combined with AMF resulted in an increase in agronomic responses compared to untreated plants (B_l-$^-$AMF) such as root biomass (+15%), stem diameter (+12%), plant height (+5%) and head diameter (+15%). Seed protein was higher in $^+$AMF than $^-$AMF (on average 20.7 *vs* 17.2 g m^{-2}, respectively) and in B_h and B_m compared with B_l (on average 19.4 *vs* 18.2 g m^{-2}, respectively). The oil content of seeds was affected by biochar application and AMF inoculation, especially under 50MAW and 30MAW irrigation levels; conversely, no differences were observed under the 10MAW irrigation level. Sunflower yield characteristics were positively correlated to the net photosynthesis rate and negatively affected by hydrogen peroxide and malondialdehyde content. The results showed that the adoption of biochar and AMF may represent as a successful strategy to balance crop productivity in a semi-arid environment. Although further research is required for a better understanding of the irrigation and fertilization schedule, these preliminary results could be extended to other crops which have similar requirements to sunflower.

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Key words: Soil amendment; physiological parameters; irrigation; drought; arbuscular mycorrhizal fungi symbiosis; sustainable agriculture.

Acknowledgements: the authors wish to thank the Agronomy Department, Payame Noor University, Gorgan, I. R. Iran for financial support. The authors would also like to thank the anonymous reviewers for their valuable comments on earlier drafts of this paper. Moreover, the authors also thank Ken Jacobsen for his evaluable assistance in checking and correcting the English text.

Received for publication: 3 April 2022.
Revision received: 18 August 2022.
Accepted for publication: 18 August 2022.

Italian Journal of Agronomy 2022; 17:2086
doi:10.4081/ija.2022.2086

Introduction

Food security is severely threatened by drought conditions that adversely affect crop growth and yield, especially in arid areas of the world (Niu *et al*., 2014; Chaves *et al*., 2003). Under drought conditions, yield is reduced by as much as 50% when compared to the average potential yield that could be observed in most major cultivated crops (Langeroodi *et al*., 2020; Gavili *et al*., 2019; Duc *et al*., 2018; Hussain *et al*., 2018; Bernardo *et al*., 2017; Shafiq *et al*., 2014). Accordingly, the constraints caused by drought stress may cause economic losses for farmers, and overall decreases in the year-on-year capability to meet the food demands of an ever-increasing global population (Nagarajan and Nagarajan, 2010). Additionally, it is expected that water availability will be reduced due to climate change, with the onset of early drought increasing competition for this resource amongst the domestic, industrial, and agricultural sectors (Niu *et al*., 2014). Consequently, all crops included those considered to be drought-tolerant, will be severely affected by drought stress, making the management of agroecosystems more challenging.

Sunflower (*Helianthus annuus* L.) is an annual crop that belongs to the family *Asteraceae*. Sunflower is cultivated worldwide due to its adaptability to different agro-environmental conditions and production of seeds that are appreciated for their high-quality oil and protein content. The root system of sunflower plants is characterized by the development of roots that may access water from deeper soil layers, which could be good option in semi-arid environments (Karam *et al*., 2007). Although the extensive root system allows sunflower plants to tolerate water stress to a greater extent than other cultivated plants, sunflower could be negatively influenced by water and heat stresses, especially during the reproductive stage until to achene filling (Hussain *et al*., 2018; García-López *et al*., 2016). Limited soil water availability caused by drought conditions can cause leaf wilting contributing to a significant reduction in achene yield, oil yield and its quality (García-López *et al*., 2016). In addition, drought stress negatively affects sunflower throughout the entire growing season by limiting stem elongation and leaf area (Hussain *et al*., 2018). Therefore, alternative agronomical approaches are needed to sustain the productivity of sunflower and meet the challenge of sustainable agricultural production systems, which can increase the efficiency of resource utilization, such as water under semi-arid environmental conditions. Biochar is the term used for a product obtained from different organic materials exposed to thermal degradation in an anaerobic environment (pyrolysis). Recently, biochar has received great attention for its beneficial effects as a soil amendment in modern agricultural practices (Lone *et al*., 2015). Indeed, biochar application in agricultural soils can enhance carbon storage and improve soil fertility compared with unamended soils (Egamberdieva *et al*., 2017; Rutigliano *et al*., 2014). Several studies reported that biochar-enriched soils are generally associated with improved physical and chemical characteristics, such as higher water holding capacity, higher exchange capacity (CEC), sorption of pesticides and nutrient ions and improvement of soil structure (Tanure *et al*., 2019; Egamberdieva *et al*., 2017; Shanta *et al*., 2016; Paneque *et al*., 2016; Lone *et al*., 2015). In addition, the application of biochar may affect microbial community structure and its role in the efficient use of nutrients and water (Rutigliano *et al*., 2014). The potential of biochar to sustain crop productivity under arid environments is associated with the enhancement of soil water-holding capacity through improved retention of rain-water and reductions in the required frequency and amount of irrigation water (Paneque *et al*., 2016). The association between the cultivated plant and arbuscular mycorrhizal fungi (AMF) represents a promising strategy for sustainable agriculture that can support crop tolerance to drought due to the ability of AMF to explore a greater amount of soil thus allowing the plants to access more water and, therefore, show signs of reduced leaf water potential later than plants grown in uninoculated plants (Bernardo *et al*., 2017; Rozpadek *et al*., 2016). Several studies have reported the role of AMF in improving plant water parameters in different host plants such as chicory, maize, chickpea, sesame, and tomato (Leventis *et al*., 2021; Gholinezhad and Darvishzadeh, 2021; Langeroodi *et al*., 2020; Hashem *et al*., 2019; Ren *et al*., 2019). AMF produce extra-radical hyphae that effectively extend the plant roots in the rhizosphere and improve water absorption (Safahani Langeroodi *et al*., 2021). In addition, endo-mycorrhization stimulates the synthesis pathway of secondary metabolites, such as phenolic compounds, phytoalexins and peroxidases, which sustain plant protection mechanisms against different stresses (Gholinezhad and Darvishzadeh, 2021; Kabir *et al*., 2020; Meddich *et al*., 2015). Recently, Wang *et al*. (2022) observed that AMF enhances nutrient uptake, especially phosphorous, by improving availability as well as translocation. The increased nutrient uptake may allow to increase photosynthate accumulation and biomass production, facilitating a potential reduction in the application of synthetic fertilizers by up to 50% (Wang *et al*. 2022). Based on these characteristics, the AMF association with cultivated plants should be considered by farmers because represent a key functional group of soil biota that could support sustainable crop productivity from both agricultural and ecological points of view, especially under severe environmental conditions.

The interaction effects of biochar and inoculation with AMF on sunflower crop under limited availability of irrigation water on agronomical performance, seed yield and oil composition have not been thoroughly investigated. This study aimed to pave the way to an in-situ application of biochar and AMF for sunflower cultivation, even in the areas where drought could threaten satisfactory seed production. Therefore, this study hypothesized that the adoption of biochar and AMF may mitigate the effect of drought conditions in a cultivated area characterized by limited water availability and, also be successfully adopted as water-saving approaches while maintaining seed yield and quality. The main objectives were to investigate the effect of different combinations of biochar and AMF under deficit irrigation conditions on: i) agronomic response of sunflower plants; ii) sunflower productivity in terms of seed yield and yield components; and iii) the connection between sunflower yield characteristics and plant physiological parameters.

Materials and methods

Research area and Experimental setup

Field trials were carried out on sunflower during the 2018 and 2019 growing seasons at the research farm of the College of Agriculture, Payame Noor University, Golesan province, Iran (lat. 36°50’ N; 54°22’ E; and 61 m a.s.l.). The climate of the experimental area is semi-arid with long dry summers and temperate winters. The historical data (30-year period) showed an average annual rainfall of 312 mm, distributed mainly from November to April (255 mm), and a mean annual temperature of 18.3°C, with the lowest mean monthly temperature in February (8.3°C) and the highest in August (28.8°C). The weather data in terms of the minimum and maximum air temperatures, precipitation and solar radiation were recorded in both sunflower growing seasons by a meteorological station placed about 500 m from the experimental fields. The soil of the research area was a silty loam with the following characteristics: pH (1:2.5 H_2O) 7.58, organic matter (1.04%), total nitrogen (0.79 g kg^{-1}) of dry soil, available phosphorous (5.6 mg kg^{-1}) of dry soil and available potassium (101 mg kg^{-1}) of dry soil.

The field experiments were conducted in two adjacent fields using a fully randomized complete block design of a factorial combination of the following treatments: i) three levels of biochar applications [0 (no application), 2.5 and 5 t ha^{-1} of biochar hereafter called B_l, B_m and B_h, respectively]; and ii) three irrigation levels (50, 30 and 10% of the maximum available water (MAW), hereafter called 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively, determined based on the maximum allowable depletion (MAD) of the threshold of available water)]; iii) two levels of AMF inoculation (with and without the addition of AMF, hereafter called $^{+}$AMF and $^{-}$AMF, respectively). The treatments were replicated three times for a total of 54 plots. Each plot was 21 m^2 (5×4.2 m) and included 7 sunflower rows placed 60 cm apart.

Farming operation description

Biochar was obtained by using paper sludge collected in a recycling paper placed in a brick kiln and exposed at the charring temperature of 400°C for 4 days. After the pyrolysis exposure (4 days) the obtained biochar was crushed and sieved (<1 cm) to homogenize the final products for field applications. The biochar had the following characteristics: pH of 8.9, electrical conductivity (EC) of 79 (H_2O) µS cm^{-1}, total N content of 0.3%, total C content of 51.4%, Cation-exchange capacity (CEC) of 12.5 Cmolc. kg^{-1}, P of 36 mg kg^{-1}, K of 1500 mg kg^{-1}, Mg 300 mg kg^{-1}, specific area of 39.8 m^2 g^{-1} (Brunauer-Emmett-Teller analysis).

In both growing seasons, biochar at the above-mentioned rates and phosphorous as triple superphosphate at a rate of 80 kg of P ha^{-1} were manually spread on the soil surface. Then the soil was ploughed by a moldboard plough to incorporate biochar and phosphorous to a depth of 35 cm and disked twice for seedbed preparation. Sunflower seeds of cultivar Farrow were sown by hand one week after biochar application on 1 June 2018 and 3 June 2019, respectively, at a density of 7 plants m^{-2}. Urea fertilizer at a rate of 120 kg ha^{-1} of N was applied at sunflower sowing (40 kg ha^{-1} of N), and at the beginning of the flowering stage (80 kg ha^{-1} of N), respectively. The application of N was performed according to common practices adopted by the farmers in the area.

Before sowing, the $^{+}$AMF plots received 150 g m^{-2} of inoculants, containing 125 spores g^{-1} of substrates, and distributed in the furrows that had been opened for sunflower sowing. After sowing, the furrows were carefully buried with soil (Gholamhoseini *et al*., 2013). The AMF inoculant adopted in the experiments was *Funneliformis mosseae* provided as pure isolates by Royan Co. (Karaj, Iran). The selected inoculant was chosen because it is commercially available in Iran. Each year, onion (*Allium cepa* 'Selmouni Red') was grown, under greenhouse conditions, in pots filled with autoclaved soil (3 times, 121°C, 30 min). Onion pots were used to prepare AMF in the previous period of sunflower growing season (March to May). The soil used in the pot cultures was collected from a depth of 0-30 cm of the soil layer in the same field where the field experiments were performed. The collected soil was dried in an oven and sieved (2 mm mesh) then mixed with sand at a ratio of 1:1 (V:V). The AMF spore count in the native field soil was minimal (~1 spores 100 g^{-1} air-dried soil). Root colonization by AM was measured at sunflower flowering by preparing root samples (1 g) following the method proposed by Philips and Hayman (1970). Roots were stained using the Gridline-Intersect Method (Giovannetti and Mosse, 1980). The mycorrhizal dependency of sunflower plants was expressed as the change in plant growth due to the mycorrhizal colonization and calculated using the formula suggested by Menge *et al*. (1978).

Three weeks before sunflower sowing, the pot contents were air-dried, and the onion roots were cut into pieces of 1 cm size and homogenized with the potting substrate. At that point, the inoculum was mainly composed of onion roots with 85% colonization, spores, and hyphae.

During the sunflower cultivation, irrigation water that was obtained from a deep-well was distributed using flat drip piping with 15 cm emitters placed alongside the crop row with a flow rate of 2 L h^{-1}. All plots were over-irrigated to the third week after sunflower sowing to ensure seedling establishment and avoid crop failure. Irrigation water was applied according to the specific irrigation schedule for each treatment. A water meter was used to measure the amount of water applied in each irrigation treatment. Soil moisture was measured gravimetrically, and the volumetric soil moisture content before irrigation was taken at different times using a weighing method. The amount of water needed for irrigation was calculated according to the following formula proposed by Safahani Langeroodi *et al*. (2021):

$$Vd=MAD\times ASW\times Rz\times A \tag{1}$$

In this formula, Vd is the amount of irrigation water (mm), ASW stands for the available soil water (equal to 130 mm per meter of soil depth), Rz is the effective root depth (0.80 m, Langeroodi *et al*., 2014) and A is the surface area of the plot. Available soil water is the amount of water present in the root area between the crop capacity and the permanent wilting point. Due to the application of drip-tape irrigation and proper irrigation management during the growing season based on soil moisture measurements, drainage from the root zone was assumed to be zero. All weeds were removed by hand for the whole sunflower growing season to avoid interference with the crop growth. Sunflower plants were harvested at seed maturity in the first week of October in both experimental years.

Data collection and analysis

Sunflower plants were assessed at the beginning of the flowering stage by harvesting the plants from one linear meter randomly chosen within each plot. Plant samples were dried in an oven at 80°C until a constant weight was achieved to determine the total dry matter (hereafter called TDM). At the same time, a potable area meter (LI-COR 2000) was placed below the sunflower canopy at soil level to measure the leaf area index (LAI). In each plot, the LAI measurements were performed five times at 12:00 p.m. on a sunny day, the data reported are the average value of all LAI measurements observed in each plot. Ten representative plants in the center of each plot were selected and marked to carry out observations from flowering (BBCH 65) to physiological maturity (BBCH 89). At physiological maturity, the same plants per plot were manually harvested to determine the following sunflower traits: biological yield, plant height, stem diameter, root dry weight, head diameter, seed per head and thousand seed weight (hereafter called TSW). The seed yield was determined by harvesting a 3 m^2 area placed in the middle of each plot. In the table, the seed yield is reported at 13% of moisture content. The sunflower harvest index (HI) was calculated as the ratio between seed weight and biological yield. Protein content was calculated using Kjeldahl nitrogen analysis (N×5.7). The petroleum ether (Soxhlet method) was used for oil extraction from sunflower seeds and expressed as a percentage. In each treatment, the oil sample was dissolved in 15 ml carbon tetrachloride, 25 mL Wijs' reagent and 10 mL of 5% KI solution, mixed well and kept for 30 min in the dark. The iodine extracted from the mixture was then titrated against starch (indicator) and 0.1 N standard sodium thiosulfate solutions to estimate the iodine value (IV) (AOAC, 1997). Free fatty acids (FFAs) were determined using the method of AOCS (1998). Physiological and biochemical assays performed in sunflower plants at the flowering stage reported by Safahani Langerrodi *et al*. (2021) were used for correlation analysis with the main agronomical attributes measured on sunflower plants. The parameters used for the correlation analysis were proline, determined using the method of Bates *et al*. (1973), superoxide dismutase (SOD) and peroxidase activity (POX) calculated following the method of Orman (1980), ascorbate peroxidase (APX) measured using the protocol of Cakmak (1994), catalase (CAT) analysis proposed by Ismail *et al*. (2004), abscisic acid (ABA) concentration calculated following Quarrie *et al*. (1988), hydrogen peroxide (H_2O_2) determined with the mothed of Velikova *et al*. (2000), and malondialdehyde (MDA) content following Janero (1990). In addition, chlorophyll (Chl) was estimated in sunflower leaves in accordance with the method of Lichtenhaler (1987) and the net photosynthesis rate (PN) was directly measured on sunflower plants by means of a Photosynthesis System LCpro+ (ADC BioScientific Ltd., Hertsfordshire, UK) under conditions of 1800 μmol m^{-2} s^{-1} photosynthetic active radiation supplied by a light unit mounted on the top of leaf chamber, and 365 μmol mol^{-1} ambient CO_2. The relative water content (RWC) was determined by applying the formula suggested by Smart and Bingham (1974).

Statistical analysis

All data and parameters measured in the study were subjected to the analysis of variance (ANOVA) carried out with the JMP statistical software v.4.0 by adopting a three-way factorial analysis where irrigation, biochar and AMF were the main factors, and the growing season was designated as a repeated measure (Gomez and Gomez, 1984). Averages were compared with Tukey's HSD test (α=0.05). Pearson's linear correlation test was conducted to detect significant correlations between selected sunflower leave parameters and yield characteristics.

Results

Leaf area index and total dry matter of sunflower at flowering stage

The leaf area index (LAI) of sunflower plants measured at the flowering stage varied according to the interaction irrigation × biochar × AMF (P<0.05, Table 1). The LAI was high in 50^{MAW} (on average 5.84), intermediate in 30^{MAW} (on average 4.32) and low in 10^{MAW} (on average 2.95). Similarly, LAI was higher in B_h followed by B_m and B_l (on average 4.57, 4.39 and 4.16, respectively). In addition, the LAI values were always higher in $^{+}$AMF compared with $^{-}$AMF (on average 4.74 *vs* 4.00), even if the differences tended to be higher in 50^{MAW} than 10^{MAW} (Table 1). Likewise, the TDM of sunflower plants at the flowering stage were decreased among the irrigation (on average 1562, 1140, and 981 g m^{-2} of DM in 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively) and biochar (on average 1279, 1234 and 1172 g m^{-2} of DM in B_h, B_m and B_l, respectively) levels and it was increased by the inoculum of AMF 1329 *vs* 1025 g m^{-2} of DM in $^{+}$AMF *vs* $^{-}$AMF respectively, Table 1).

Sunflower plant characteristics

Root's colonization of inoculated sunflower plants showed a significant reduction in intensity when irrigation water was reduced (data not shown). However, biochar treatment protected AM fungi from the deleterious effects of drought by improving the number of spores, mycelium, vesicles and arbuscules in drought-stressed plants (data not shown). The intensity of colonization declined by 33.7 and 73.1% in 30^{MAW} and 10^{MAW} treatments compared to 50^{MAW}, respectively. The application B_h and B_m enhanced an increase in the intensity of colonization by 40.9% and 50.6%, respectively, compared to B_l treatment.

Results regarding the interaction effect of irrigation × biochar × AMF on the root dry matter (RDM), stem diameter (SD), plant height (PH), head diameter (HD) and days to maturity (DM) of sunflower plants at harvesting are reported in Table 2. The RDM ranged from 87.8 g $plant^{-1}$ in 50^{MAW}-B_h-$^{+}$AMF to 41.1 g $plant^{-1}$ to 10^{MAW}-B_l-$^{-}$AMF and it tended to show high values in 50^{MAW}, intermediate in 30^{MAW} and low in 10^{MAW} (on average 76.7, 61.7 and 49.0 g $plant^{-1}$, respectively). A significant increase in RDM was also observed under B_h as compared to B_l (Table 2). Moreover, the RDM was higher in $^{+}$AMF than in $^{-}$AMF (on average 66.4 *vs* 58.5 g $plant^{-1}$, respectively). The SD showed a similar trend to that

Table 1. The interaction effect of Irrigation level × AM fungi × biochar rate on leaf area index and total dry matter (TDM) in leaves of sunflower at flowering stage.

Treatment		LAI		TDM (g m^{-2} of DM)	
		+AMF	–AMF	+AMF	–AMF
50^{MAW}	B_h	6.50^{Aa}	5.70^{Ba}	1760^{Aa}	1514^{Ba}
	B_m	6.30^{Aa}	5.40^{Bb}	1654^{Aab}	1461^{Bab}
	B_l	5.92^{Ab}	5.20^{Bb}	1602^{Ab}	1390^{Bb}
30^{MAW}	B_h	5.01^{Ac}	4.05^{Bd}	1320^{Ac}	1069^{Bc}
	B_m	4.75^{Ad}	3.93^{Bd}	1280^{Acd}	1030^{Bc}
	B_l	4.55^{Ad}	3.65^{Be}	1188^{Ad}	954^{Bc}
10^{MAW}	B_h	3.36^{Ae}	2.78^{Bf}	1070^{Ae}	941^{Bd}
	B_m	$3.25A^{ef}$	2.68^{Bf}	1063^{Ae}	916^{Bd}
	B_l	3.05^{Af}	2.58^{Bf}	1022^{Ae}	874^{Bd}
S.E.		0.30		65.16	

Data averaged over 2 years (2018 and 2019 growing seasons). Values belonging to the same parameter without common letters in a row for AM fungi inoculation (upper case letter) and in columns for irrigation level of each biochar rate (lower case letter) are statistically different according to least significant difference test (0.05). LAI, leaf area index; TDM, total dry matter; S.E., standard error.

observed in RDM, except in biochar where no differences were observed between B_h and B_m (Table 2). The PH varied according to the irrigation level (161.5, 143.7 and 131.3 cm in 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively), biochar level, (on average 147.7, 146.0 and 142.8 cm in B_h, B_m and B_l, respectively, and AMF (148.7 and 142.3 cm in $^{+}$AMF and $^{-}$AMF, respectively). The HD varied from 19.5 to 10.1 cm and was always the highest in 50^{MAW} (on average 17.4 cm) and $^{+}$AMF (on average 15.3 cm), while among biochar levels HD values were similar in B_h and B_m and higher than B_l (on average 14.8 *vs* 13.6 cm, respectively), even if in 50^{MAW}-$^{-}$AMF it was similar across all biochar levels (Table 2). The DM was higher in 50^{MAW} (on average 124.3 days), compared to 30^{MAW} (on average 114.0 days) and 10^{MAW} (on average 107.7 days). In addition, DM tended to be high in B_h even if the differences were greater in $^{+}$AMF than $^{-}$AMF (Table 2).

Sunflower yield and its characteristics

The interaction effect of irrigation × biochar × AMF on sunflower yield and yield characteristics was reported in Figure 1. The biological yield (BY) ranged from 16.0 t ha^{-1} in 50^{MAW}-B_h-$^{+}$AMF to 5.9 t ha^{-1} in 10^{MAW}-B_l-$^{-}$AMF and it was greater in $^{+}$AMF than in $^{-}$AMF (on average 11.1 *vs* 9.9 t ha^{-1}, respectively), even if under each irrigation level BY increased as biochar level increased. The seed yield was highest in 50^{MAW} followed by 30^{MAW} and 10^{MAW} (on average 68.1, 51.0 and 28.7 g plant^{-1}) and was higher in $^{+}$AMF than in $^{-}$AMF (on average 53.9 *vs*. 44.6 g plant^{-1}, respectively), while under each irrigation level it showed similar values between B_h and B_m (Table 3). The BY and the sunflower seed yield parameters were positively correlated with relative water content (RWC) (r=0.75** and 0.53*, respectively), chlorophyll content (r=0.66* and 0.53*, respectively) and net photosynthesis rate (r=0.67* and 0.60*, respectively), while they were negatively affected by the hydrogen peroxidase content (r= –0.53* and -0.54*, respectively, Table 3). As expected, the number of seeds per head varied according to the seed yield and it was the highest in 50^{MAW}-B_h-$^{+}$AMF (1260 n. head^{-1}) and the lowest in 10^{MAW}-B_l-$^{-}$AMF (671 n. head^{-1}), even under the 10^{MAW} irrigation level no differences were observed among the biochar treatments. The thousand weed weight (TSW) increased from the 10^{MAW} irrigation level (on average 40.3 g) to the 50^{MAW} irrigation levels (on average 71.5 g), and it was affected by the AMF inoculation (on average 57.7 *vs* 51.4 in $^{+}$AMF and $^{-}$AMF, respectively). Among the biochar treatments, the TSW was generally high and similar in B^h and B^m, even if the greatest differences were observed in the 50^{MAW} irrigation level (Figure 1). The TSW showed a positive relationship with the net photosynthesis rate (r=0.58*, Table 3).

The chemical characteristics of sunflower seeds, in terms of seed protein (SP), oil content, IV and FFAs as affected by the irrigation × biochar × AMF interaction are reported in Figure 2. The SP content in sunflower seeds was always greater in $^{+}$AMF than $^{-}$AMF (on average 20.7 *vs* 17.2 g m^{-2}, respectively), and it was generally higher in B_h and B_m compared to B_l (on average 21.4 *vs* 14.0 g m^{-2}, respectively), except in 10^{MAW}-$^{-}$AMF where the SP showed similar values among all biochar treatments (Figure 2). Moreover,

Table 2. The interaction effect of Irrigation level × AM fungi × biochar rate on sunflower plant characteristics at harvesting stage.

Treatment		RDM (g plant^{-1})		SD (cm)		PH (cm)		HD (cm)		DM (days)	
		$^{+}$AMF	$^{-}$AMF	$^{+}$AMF	$^{-}$AMF	$^{+}$AMF	$^{-}$AMF	$^{+}$AMF	$^{-}$AMF	$^{+}$AMF	$^{-}$AMF
50^{MAW}	B_h	87.8^{Aa}	75.6^{Ba}	2.25^{Aa}	1.99^{Ba}	168^{Aa}	160^{Ba}	19.5^{Aa}	16.9^{Ba}	126^{Aa}	124^{Aa}
	B_m	81.7^{Ab}	70.8^{Bbc}	2.18^{Aa}	1.94^{Ba}	165^{Aa}	159^{Bab}	18.8^{Aa}	16.4^{Ba}	126^{Aa}	124^{Aa}
	B_l	75.1^{Ac}	69.1^{Bc}	1.99^{Ab}	1.91^{Ba}	161^{Ab}	156^{Bb}	16.9^{Ab}	16.1^{Ba}	125^{Aa}	$121B^{b}$
30^{MAW}	B_h	67.8^{Ad}	60.6^{Bd}	1.88^{Ac}	1.68^{Bb}	151^{Ac}	143^{Bc}	15.8^{Ac}	13.8^{Bb}	120^{Ab}	111^{Bc}
	B_m	64.9^{Ade}	58.8^{Bd}	1.84^{Ac}	1.60^{Bbc}	148^{Acd}	140^{Bd}	15.4^{Ac}	13.0^{Bbc}	117^{Ac}	111^{Bc}
	B_l	61.1^{Ae}	56.9^{Bd}	1.71^{Ad}	1.58^{Bc}	145^{Ad}	135^{Be}	14.1^{Ad}	12.8^{Bc}	115^{Ad}	110^{Bc}
10^{MAW}	B_h	55.8^{Af}	$47.6B^{e}$	1.59^{Ae}	1.44^{Bd}	134^{Ae}	130^{Bf}	12.9^{Ae}	11.4^{Bd}	110^{Ae}	106^{Bd}
	B_m	53.7^{Afg}	45.8^{Bef}	1.55^{Aef}	1.37^{Bde}	134^{Ae}	130^{Bf}	12.5^{Aef}	10.7^{Bde}	110^{Ae}	106^{Bd}
	B_l	50.1^{Ag}	41.1^{Bf}	1.47^{Af}	1.31^{Be}	132^{Ae}	128^{Bf}	11.7^{Af}	10.1^{Be}	109^{Ae}	105^{Bd}
S.E.		3.01		0.06		3.16		0.64		1.78	

Data averaged over 2 years (2018 and 2019 growing seasons). Values belonging to the same parameter without common letters in row for AM fungi inoculation (upper case letter) and in columns for irrigation level of each biochar rate (lower case letter) are statistically different according to LSD (0.05). RDM, root dry matter; SD, stem diameter; PH, plant height; HD, head diameter; DM, days to maturity; S.E., standard error.

Table 3. Correlation coefficients between features measured on sunflower plants at flowering stages and yield and yield characteristics of sunflower seeds.

	Proline	SOD	APX	CAT	POX	RWC	Chl	ABA	H_2O_2	MDA	PN
BY	0.13	0.25	0.28	0.20	0.26	0.75**	0.66*	0.43	–0.53*	–0.54	0.67*
Seed yield	0.10	0.09	0.15	0.18	0.21	0.53*	0.53*	0.33	–0.54*	–0.58*	0.60*
TSW	0.01	0.01	0.02	0.04	0.10	0.24	0.33	0.12	–0.33	–0.40	0.58*
Seed protein	0.15	0.20	0.22	0.26	0.21	0.69*	0.50	0.19	–0.55*	–0.58*	0.66*
Oil content	0.10	0.07	0.09	0.12	0.13	0.49	0.44	0.23	–0.60*	–0.59*	0.61*
IV	0.11	0.09	0.05	0.07	0.11	0.46	0.47	0.12	–0.67*	–0.49	0.44
FFAs	–0.07	–0.05	–0.09	–0.10	–0.08	–0.55*	–0.66*	–0.03	0.59*	0.60*	–0.53*

Pearson's r-value corresponds to 2018 and 2019 growing seasons, n=108. BY, biological yield; TSW, thousand seed weight; SOD, superoxidase dismutase; APX, ascorbate peroxidase, CAT, catalase; POX, peroxidase activity; RWC, relative water content; Chl, chlorophyll; ABA, abscisic acid; H_2O_2, hydrogen peroxide; MDA, malondialdehyde content; PN, net photosynthesis rate. The significance level is (*) or (ns) significant at P<0.05, or P>0.05, respectively.

the SP significant positive correlations with RWC (r=0.69*) and PN (r=0.66*), while H_2O_2 content and malondialdehyde content negatively affected the SP (r= –0.55* and –0.58*, respectively). The oil content ranged from 0.51 % to 0.37 % and was mainly affected by the irrigation level (0.49%, 0.42%, and 0.38% in 50^{MAW}, 30^{MAW} and 10^{MAW}, respectively). Regarding the biochar level, the greatest differences were observed in 50^{MAW}, while no differences were detected among the biochar level under 10^{MAW} irrigation. Similarly, the AMF inoculation only affected the oil content only in the 50^{MAW} (on average 0.50 *vs* 0.48 % in $^+$AMF and $^-$AMF, respectively) and 30^{MAW} (on average 0.44 *vs* 0.39 % in $^+$AMF and $^-$AMF, respectively) irrigation levels, while no differences were observed in the 10^{MAW} irrigation level regardless the biochar rate (Figure 2). As expected, the oil content was positively correlated with the PN (r=0.66*), while it was negatively affected by the H_2O_2 and MDA (r= –0.60* and –0.59*, respectively, Table 3). The iodine value (IV) was higher in the 50^{MAW}, intermediate in the 30^{MAW} and lower in the 10^{MAW} on average 129.2, 108.2 and 96.0 g I 100 gr oil^{-1}, respectively) irrigation level and in $^+$AMF compared with $^-$AMF (on average 115.1 *vs* 107.1 g I 100 gr oil^{-1}, respectively). Conversely, among the biochar treatments, the greater differences were observed in $^+$AMF, especially under the 50^{MAW} irrigation level, while no differences were observed under $^-$AMF treatments among at each irrigation level (Figure 2). The results showed an inverse relationship between IV and H_2O_2 content (r=–0.67*). The FFAs content increased as the irrigation level decreased and was lower in 50^{MAW}, intermediate in 30^{MAW} and higher in 10^{MAW} (on average 0.37, 0.54 and 0.65 mg KOH g oil^{-1}, respectively). Additionally, the FFAs were reduced in $^+$AMF compared to $^-$AMF (on average 0.48 *vs* 0.55 mg KOH g oil^{-1}, respectively), while the biochar levels generally showed few differences among each irrigation level, except under $^-$AMF under 30^{MAW} and 10^{MAW} irrigation levels where the FFAs content increased as the biochar rate decreased ($B_h > B_m > B_l$,). The FFAs content was negatively affected by the RWC (r= –0.55*), Chl (r= –0.66*) and PN (r= –0.53*). Conversely, it showed a positive relationship with the H_2O_2 content (r=0.59*) and MDA (r=0.60*).

Discussion

Nowadays, there is an urgent need to individuate appropriate agricultural practices to reduce negative impacts on cultivated plants caused by adverse climatic conditions, especially under climate change scenarios that may lead to extreme climatic events. This study aimed to evaluate the agronomic response of sunflower plants grown in semi-arid environmental conditions when subjected to different biochar rates and AMF inoculation. Induced water

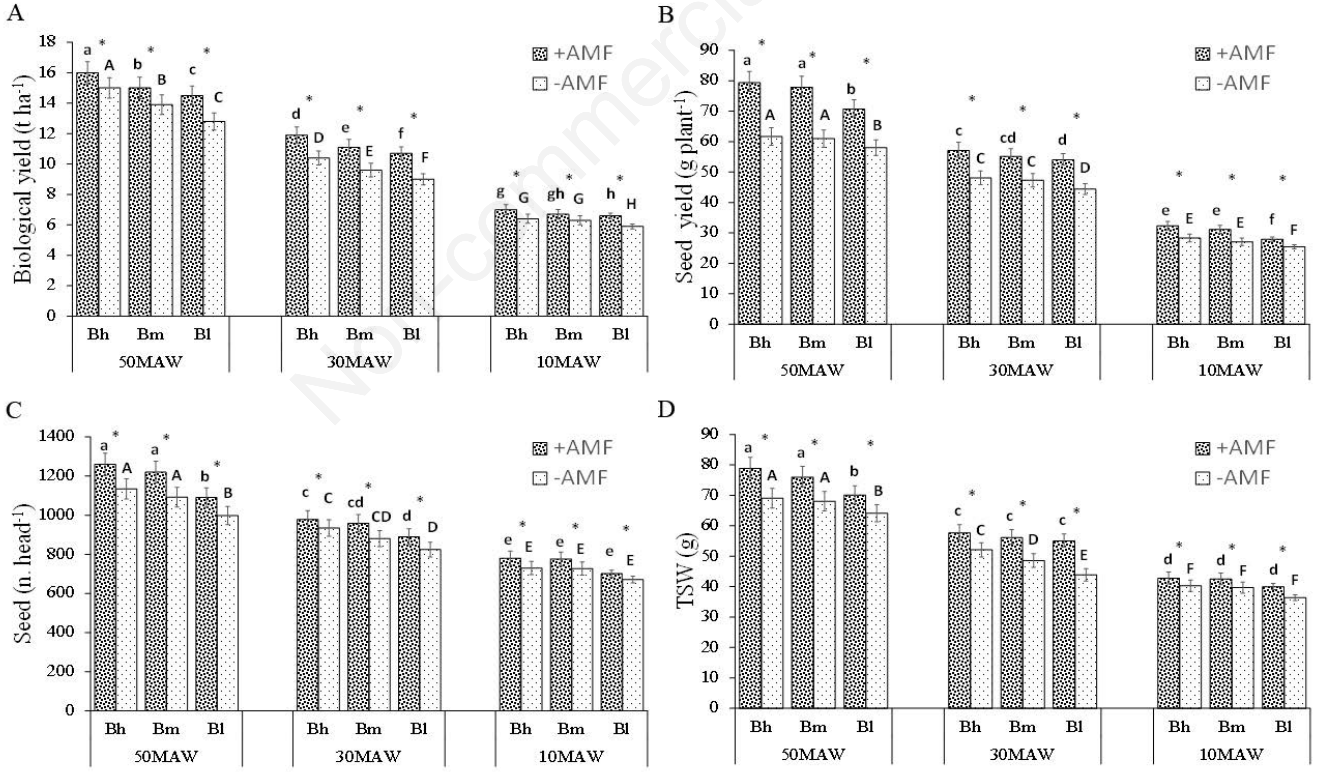

Figure 1. The interaction effect of Irrigation level × AM fungi × biochar rate on biological yield (A), seed yield (B), number of seeds (C) and thousand seed weight (D) at sunflower harvesting stage. Data averaged over 2 years (2018 and 2019 growing seasons). Values belonging to the same parameter without common letters are statistically different according to LSD (0.05) in lower case letter under $^+$AMF treatments and in upper case letter in –AMF treatments, TSW, thousand seed weight. The asterisk indicates the differences between $^+$AMF and $^-$AMF in each comparison.

shortage by means of different irrigation schedules subjected sunflower plants to different levels of water stress. The results showed that biochar application and AMF inoculation could be adopted to mitigate the negative effect of deficient irrigation on seed yield and quality. The deep roots of sunflower plants allow them to reach and catch water in deep soil layers giving them drought-tolerant properties. However, when the drought conditions persisted for a long period of time, it had a significant negative effect on sunflower plant growth, especially when severe deficit irrigation was applied (10^{MAW}). The results showed as drought conditions affect sunflower plants through their negative influence not only on plant development, but also on agronomical performance including seed yield and quality. Similarly, Hussain *et al*. (2018) observed that drought conditions affect plants at every organizational level.

Water stress generated by severe deficit irrigation subjected sunflower plants to both reduced water potential and turgor of cells, which gradually led to growth inhibition associated with less carbon assimilation, imbalanced mineral nutrition, and accumulation of abscisic acid (Hussain *et al*., 2018; Ghobadi *et al*., 2013). According to the results of Garofalo and Rinaldi (2015), water stress in sunflower resulted in a reduction of LAI and total dry biomass at the flowering stage, which reduced plant canopy area and photosynthetic activities compared with sunflower plants grown without stress. Similarly, Pagter *et al*. (2005) showed that drought stress reduced LAI by reducing the production and development of leaves. In this study, the application of biochar significantly increased the LAI in both $^-$AMF and $^+$AMF treatments, probably due to direct factors, such as increased soil water availability, and indirect factors, such as increased nutrient availability, as suggested by Gavili *et al*., 2019 and Suppadit *et al*., 2012. In addition, Kammann *et al*. (2011) reported that the application of biochar increased LAI and biomass of quinoa crops subjected to limited water availability. The porous structure of biochar increases soil surface area and contributes to greater water absorption and availability at the root level (Ali *et al*., 2017). Baiamonte *et al*. (2015) reported that the soil aggregate stability increased because of biochar application, and therefore promoted water accumulation in the soil, especially in drought conditions. Biochar is also well-known for its ability to enhance the retention of nutrients released by organic matter mineralization, through cation and anion exchange processes that keep the nutrients available at the root level (Rogovska *et al*., 2014).

In this study, sunflower plants benefitted from AMF inoculation by showing higher values of TDM compared with no-inoculated plants, probably due to fungal hyphae that allow the plants to exploit smaller soil pores, including also those added by biochar, and take-up available water and nutrients more efficiently compared with $^-$AMF plants, as observed in chicory by Safahani

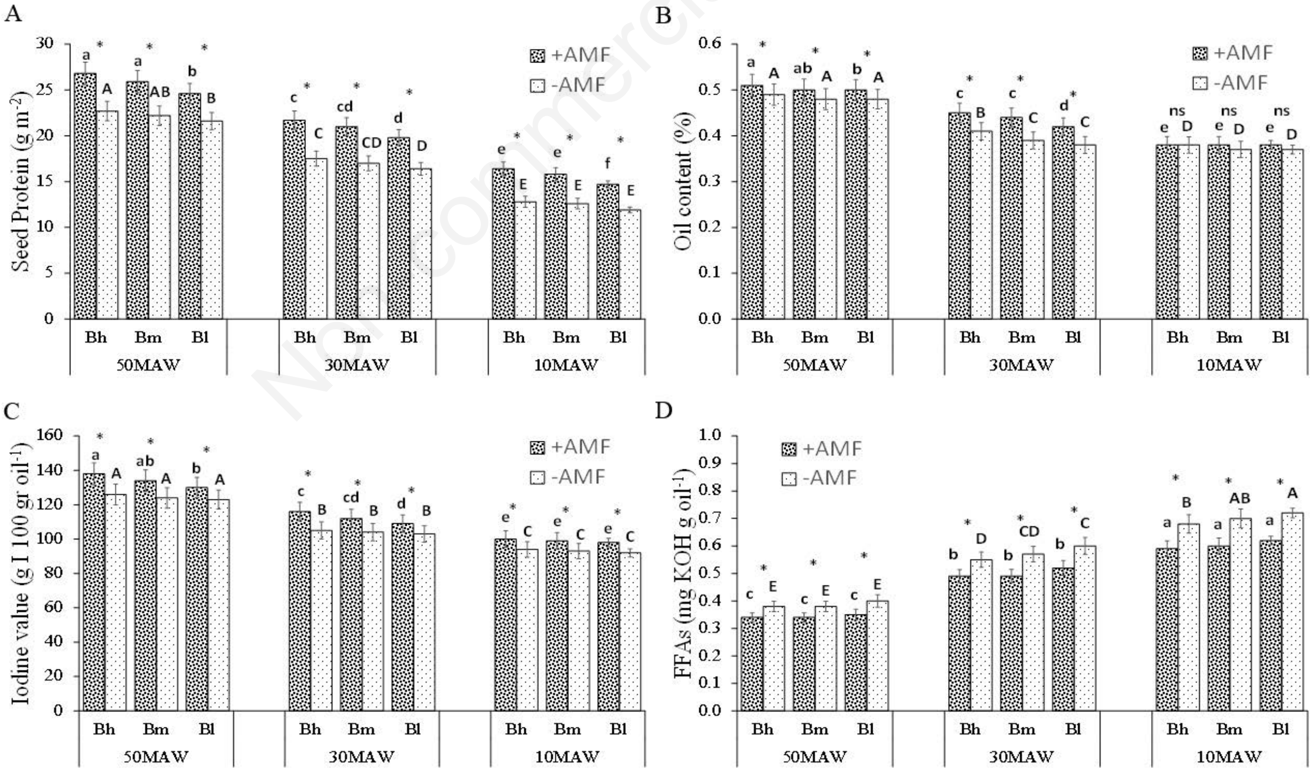

Figure 2. The interaction effect of Irrigation level × AM fungi × biochar rate on seed protein (A), oil content (B), iodine value (C) and free fatty acids (D) at sunflower harvesting stage. Data averaged over 2 years (2018 and 2019 growing seasons). Values belonging to the same parameter without common letters are statistically different according to LSD (0.05) in lower case letter under $^+$AMF treatments and in upper case letter in $^-$AMF treatments, TSW, thousand seed weight. The asterisk indicates the differences between $^+$AMF and $^-$AMF in each comparison.

Langeroodi *et al*. (2020). Moreover, the inoculation of AMF led to higher root biomass compared to non-inoculated plants confirming the results of Ren *et al*. (2019). The greater root system allows the plants to explore a greater portion of the soil and have better access to soil nutrients, especially nitrogen, potassium and phosphorous (Safahani Langeroodi *et al*., 2020). The increased sunflower root system of $^+$AMF plants combined with biochar application improved nutrient status due to enhanced availability of nutrient elements and an improved soil-saturated hydraulic conductivity and soil structure (Gavili *et al*., 2019).

Considering that the flowering stage is a critical phase for determining fertile flowers, it is conceivable to observe limited achene yield under water-stressed plants. Similarly, García-López *et al*. (2014) reported different yield responses of sunflower to various irrigation regimes and water stress, especially when irrigation water was reduced to 60% of optimal irrigation. In addition, Fatemi (2014) reported pollen infertility and reduced carbon assimilation due to water stress conditions. In this study, sunflower yield reduction was also affected by physiological and morphological attributes, indeed the content of H_2O_2 and MDA shows negative relationships with total biomass, achene yield and its quality parameters. Essahibi *et al*. (2018) reported that reduced water availability induces oxidative stress in the plants with an increase in free radical content. In addition, Sairam *et al*. (2001) showed that plant cells exposed to drought conditions produce malondialdehyde and are subjected to membrane lipid peroxidation, which led to the decline of cell membrane stability. The inoculation of AMF promotes antioxidant mechanisms by stimulating gene expression or increasing the content of antioxidant enzymes such as POD and SOD (Ren *et al*., 2019). The results also influenced yield components, in terms of head diameter, the number of achenes per capitulum and 1000-achene weight, were strongly hampered under reduced availability of irrigation water (Khan *et al*., 2000). In this study, biochar and AMF played an important role in increasing the growth and yield of sunflower plants, especially under severely deficit irrigation. The correlation analysis showed how increased RWC and chlorophyll content were positively correlated to biomass accumulation (He *et al*., 2017). Similarly, Hashem *et al*. (2019) observed chickpea plants subjected to biochar application and AMF inoculation and found a significant enhancement in net photosynthetic rate. Moreover, the combined effects of biochar and AMF promoted plant growth by affecting resource use efficiency for the same kind of plants (Banerjee *et al*., 2013).

Sunflower plants subject to drought conditions showed significant reductions in oil content and composition (Hussain *et al*., 2018; Ali *et al*., 2009). The results showed a negative relationship between H_2O_2 and seed protein, oil content and IV. Under drought conditions, the synthesis of reactive oxygen species, such as hydrogen peroxide (H_2O_2), may cause oxidative stress and alteration to biochemical processes that are linked to lipids and proteins synthesis (Wada *et al*., 2019; Lang *et al*., 2018; Shabbir *et al*., 2016). In particular, the fatty acid composition (oleic and linoleic acids) changes due to accelerated embryo development and enzymatic processes that are stimulated by drought conditions (Rondanini *et al*., 2003; Baldini *et al*., 2002). The combined effect of biochar and AMF inoculation improved the availability of potassium (K) in sunflower plants, which plays an essential role in developing the resistance mechanisms of plants (Shafiq *et al*., 2014). In this study, sunflower plants subject to severe deficit irrigation showed a reduction of IV associated with an increase of FFAs in achene oil. Similarly, Shehzad *et al*. (2020) found a decrease in IV in sunflower oil ascribable to drought stress. Accordingly, the reduced FFAs content observed in well-irrigated sunflower plants is indicative of its oxidative stability, as reported by Bozdogan *et al*. (2019). Similarly, the application of biochar associated with AMF inoculation supported the reduction of FFAs content showing a positive role in enzyme activation for an increased level of oleic acid (Seleiman *et al*., 2019).

Conclusions

This study showed that the application of biochar in AMF inoculated plants could sustain the cultivation of sunflower subject to severe drought conditions. Indeed, sunflower plants subjected to biochar application and AMF inoculation showed increased tolerance to drought stress compared to untreated plants. Results indicated that the combination of biochar and AMF have a beneficial effect on the growth and physiological response of sunflower promoting seed production and quality. Therefore, the adoption of biochar and AMF inoculation in sunflower cultivation can be viewed as a viable environmental-friendly option to balance crop productivity in semi-arid environments. Further research is required to improve our understanding of irrigation and fertilization schedules. These preliminary results could be extended to other crops with similar requirements to sunflower.

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