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Effects of Biochar, Foliar Application of Amino Acid, and Drought Stress on Physiological and Morphological Traits, Yield Componenets, and Water Use Efficiency in *Spinacia Oleracea*

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Abstract
In this research, attempts have been made to investigate the effects of drought stress, foliar application of amino acid and biochar fertilizer on the morphological traits of spinach subjected to the conditions of the research greenhouse of Gonbad Kavous University within the timerange of 2021-2022. The experiment was conducted as a factorial in the form of randomized complete blocks with 3 replications. The experiment factors included 5 levels of drought stress of 0, 10, 20, 30 and 40 percent Maximum allowable depletion(MAD), 2 levels of amino acid foliar application (0 and 2 Lit/ha) and 2 levels of biochar fertilizer (0 and 4% weight of soil). The results of data analysis variance indicated that the effect of drought stress is significant at a level of 1% on the length of the longest root (cm), length of the largest leaf (cm), chlorophyll (mg.g^{-1} fresh leaf), total water use (mlit) and water use efficiency (g.lit^{-1}). Also, the effect of amino acid on the total water use and that of biochar on the length of the largest leaf and chlorophyll content were significant at a level of 0.01. Moreover, the effects of biochar and amino acid in antioxidant (µg.ml^{-1}) were significant at a level of 5%. A comparison of mean results showed that the highest value of the length of the largest leaf (cm), dry weight (gr), plant fresh weight (gr) is associated with the biochar application treatment. The highest amount of total use water was also found in the treatment without biochar. The highest and lowest amounts of chlorophyll content were found to be associated with the treatments with (14.52 mg.g^{-1} fresh leaf) and without (11.92 mg.g^{-1} leaf leaf) biochar application, respectively. Furthermore, the comparison of mean results showed that the highest amounts of chlorophyll, dry weight (gr) and plant fresh weight (gr) are corresponding to the amino acid application treatment.
Keywords: Amino acid, Biochar, Spinach, Water stress, Water use efficiency.

1. Introduction
Water scarcity accounts for a critical and vital issue for most countries located in dry and desert areas. One of the most effective non-destructive methods for determining a plant's water status is the use of vegetation temperature, which is a reliable index for measuring water stress (Fitzgerald et al., 2006). The plant water stress index has been employed in order to plan irrigation and evaluate factors such as plant water stress, conduction and resistance of leaf stomata, photosynthesis, leaf water potential, and red pepper transpiration rate.

Water stress is one of the most important non-living environmental stresses that causes a decrease in crop yield, especially in arid and semi-arid regions of the world (Khezrie-Afravi et al., 2010). Being situated in the arid and semi-arid regions of the world, Iran is faced with water limitations in terms of the amount of inappropriate distribution of precipitation, which can be prevented to some extent by planning and using the facilities in a proper way (Naseri et al., 2016). During their growing period, plants are faced with many environmental stresses, each of which might have different effects on growth and yield according to the level of sensitivity and growth stage of the plant. The results of the research conducted by Ghaemi et al. (2017) showed that water stress has a significant effect on morphological characteristics of the plant, so that with a decrease in soil moisture level, the plant height, number and surface of leaves decrease in all growth stages, and the stem diameter in the growth stages, especially the severe stress, as well as the root length increase in the flowering stage.

Peng et al. (2022) investigate the impacts of water deficit and post-drought irrigation on transpiration rate, root activity, and biomass yield of Festuca arundinacea during phytoextraction investigate. Two drought stress levels (D_1, slight stress and D_2, moderate stress) were carried out at one of five plant growth stages (G_1, germinating; G_2, tillering; G_3, jointing; G_4, booting; and G_5, flowering). The results showed that drought stress, regardless of level, significantly decreased the transpiration rate of F. arundinacea by 38.9%–85.7%.

Rezaei Sokht-Abandani et al. (2020) investigate the effect of drought stress, in different levels of Nitrogen and Potassium Fertilizer on some physiological and agronomical traits of Maize hybrid (Zea mays L. CV. Single cross 704). Spinach with the scientific name *Spinacia oleraceae* L. is an annual and long day, accounting for one of the most important leafy greens that is consumed fresh or processed. This plant has a high nutritional value, so that it is rich in vitamins and mineral elements among most commonly consumed fruits and vegetables (Pivast, 2018). Leskovar (1998) examined the effect of various amounts of irrigation water on the growth and development of spinach in the early stages of growth. The results of this research showed that the depth of irrigation water and its frequency might affect the physical and chemical properties of the growth environment for the vegetables and cause changes in the physiological indices of the plant such as rooting, stem and root length increasing. These can have significant effects on the yield of vegetables. The soil moisture must be high enough to make the buds of spinach seeds green. Therefore, this plant needs a lot of water and is a drought-sensitive plant. Spinach needs abundant water and should be irrigated during the growing period in dry lands. In addition to increasing the crop yield, irrigation also delays the premature flowering of the plant.

Biochar is a carbon material obtained by heating plant remains and waste in an environment containing limited or no oxygen. One of the significant features of biochar is its stability after adding to the soil, which depends on the producing temperature of this material and even the conditions and type of soil (Lehmann and Joseph, 2009). This characteristic of biochar is very important for carbon sequestration in soil. On the other hand, biochar is a mostly stable and resistant organic carbon compound that is produced by heating biomass under low oxygen (preferably no oxygen) conditions (Lehmann et al., 2011). Increasing the available water due to the addition of biochar is due to a change that biochar has caused in particle size distribution and soil porosity due to the high specific surface area.

Adding biochar leads to a decrease in bulk density (Chan et al., 2007; Laird et al., 2010). One of the most important physical parameters of the soil, which strongly affects the crop yield, is the water available to the plant. Recent studies have shown that biochar increases the water storage capacity in the soil. It has been reported that using biochar, through improving plant water status, leads to an increment in the efficiency of nutrients use, nutrients preservation and increasing availability of elements for the plants. Major et al. (2010) reported that the crop yield increase due to the application of biochar in the soil might be due to the direct impact of the existing nutrients in the biomass, which are converted into biochar during the thermal decomposition process, and indirectly leads to an improvement in the physicochemical and biological characteristics of the soil.

Amino acids affect the growth and crop yield through increasing the tolerance to environmental stresses, increasing the chlorophyll concentration, and as a result, influencing photosynthesis. In another study, it was shown that amino acids directly and indirectly affect the physiological activities, growth and development of the plant (Abd El-Aal et al., 2010) also reported that amino acid increases the fresh and dry weights of the plant due to its important role in plant metabolism and protein accumulation (Shehata et al., 2011).

Pour Youssef et al. (2013) showed that the use of biofertilizer and amino acids during eight leaves + tassel appearance stage increase the biological and grain yield in corn S.C.260. Rezakhani and Haj Seyed Hadi (2017) conducted a research on the effect of animal manure and amino acids foliar application on the growth characteristics, grain yield and essential oil content of coriander and reported that the highest plant height, leaf area index and biomass yield are associated with the amino acid foliar application treatment.

Foliar spraying by seaweed on turnip has led to an increment in the photosynthetic pigments and increased phosphorus and potassium contents of the leaves and as a result increased green surface of the plants (Hakim Che Behishat et al., 2011).

Silambarasan et al. (2022) investigated that Plant growth-promoting actinobacterial inoculant assisted phytoremediation increases cadmium uptake in Sorghum bicolor under drought and heat stresses. Multiple abiotic stress tolerances were found in these two actinobacterial strains, including Cd stress (CdS), drought stress (DS) and high-temperature stress (HTS). These findings suggest that RA04 and RA07 strains could be effective bio-inoculants to accelerate phytoremediation of Cd polluted soil even in DS and HTS conditions.

Liu et al. (2022) reports on the mechanisms of alleviating salinity stress by cerium oxide nanomaterials (CeO2 NMs) in maize. Soil-grown maize plants were irrigated with deionized water or 100 mM NaCl solution as the control or the salinity stress treatment. These results will provide new insights for the application of CeO_2 NMs in management to reduce the salinity-caused crop loss.

El-Badri et al. (2022) to examine the promising effects of nanopriming via bioSeNPs on the expression level of aquaporin genes, seed microstructure, seed germination, growth traits, physiochemical attributes and minerals uptake of two rapeseed cultivars under salinity stress conditions.

This comprehensive data can add more knowledge to understand the mechanisms behind plant-bioSeNPs interaction and provide physiological evidence for the beneficial roles of nanopriming using bioSeNPs on rapeseed germination and seedling development under salinity stress conditions.

The results of the study conducted by Ismailpour et al. (2019) showed that the use of seaweed extract leads to an improvement in the growth of basil under drought stress conditions through increasing the amount of proline, creating osmotic regulation, reducing chlorophyll decomposition and reducing membrane leakage.

Application of biochar under low irrigation conditions increases water use efficiency and crop quality and has been reported as a helpful strategy to increase crop yield in soils affected by salinity stress (Akhtar et al., 2015).

Phares and Akbara (2022) investigated about compost or inorganic NPK fertilizer with biochar influenced soil quality, grain yield and net income of rice. Journal of Integrative Agriculture.

An et al. (2022) reported that biochar application with reduced chemical fertilizers improves soil pore structure and rice productivity.

According to the literature review, no research has been so far conducted on the effect of biochar fertilizer and amino acid foliar application on the resistance of spinach to water stress, which is the aim of the present investigation.

2. Materials and Methods

The current research has been conducted in the research greenhouse of Gonbad Kavous University during 2021-2022. The experiment was conducted in the form of randomized complete block design (RCBD) with three replications. Four levels of irrigation (main factor) was 10, 20, 30, 40 and 50% MAD, two levels of amino acid (Azomino) was none use and use of 2 lit/ha, and two levels of biochar was none use and use of 4% biochar. In this research, local spinach was used. 60 pots (with 10 kg soil) was used and soil had a silty loam texture. Table 1 lists the characteristics of the examined soil.

Table 1. Characteristics of the examined soil

pH	7.1
EC (µmohs/cm)	1003
Ca (ppm)	300
Mg (ppm)	200
Na (ppm)	865.5
K (ppm)	140.5
Cl (ppm)	1200
FC (%)	20
PWP (%)	8
Soil texture	Silty clay loam
P_b (g/cm^3)	1.3

2.1. Water use efficiency

The water use efficiency is obtained from the following equation:

$$Water\ use\ efficiency = \frac{Plant\ fresh\ weight\ (gr)}{total\ water\ use\ (lit)} \quad (1)$$

2.2. Data analysis

Statistical analysis of data has been performed using statistical software SAS Ver. 9.4. In order to compare the mean data, the least significant difference (LSD) test has been employed at a level of 5%.

3. Results and Discussion

The results of analysis of variance (ANOVA) showed that the effect of amino acid on total water use was significant at 1% and proline and antioxidant contents at 5% probability level. The effect of biochar on length of the largest leaf, chlorophyll, total water use, fresh and dry weight of plant and water use efficiency was significant at 1% and proline and antioxidant contents at 5% probability level. The effect of drought stress on the length of the largest leaf and longest root, chlorophyll, total water use and proline was significant at 1% probability level.

Furthermore, none of the examined traits were influenced by the interaction of factors (Table 2).

Table 2. Results of ANOVA (mean square) of the examined traits of spinach

Trait Source of variation	Degree of freedom	Length of the longest root	Length of the largest leaf	Chlorophyll	Total water use	Plant fresh weight	Plant dry weight	Proline	Antioxidant	Water use efficiency
Replication	2	3.120	1.89	2.57	0.042	0.92	0.0102	17.82	8.019	0.082
Amino acid (A)	1	1.568	6.029	22.92	0.855**	7.46	0.081	110.2*	40.425*	0.044
Biochar (B)	1	3.902	20.21**	101.4**	0.304**	31.3**	0.502**	116.3*	16.014*	2.232**
Drought stress (DS)	4	38.18**	7.91**	19.96**	2.098**	5.83	0.106	185.5**	25.395	0.703**
B×A	1	0.088	0.094	0.049	0.018	0.06	0.0005	5.184	0.062	0.009
DS×A	4	0.103	0.295	1.168	0.009	0.09	0.0010	1.541	1.094	0.001
DS×B	4	0.583	1.012	0.530	0.001	0.23	0.0040	0.509	0.377	0.013
DS×B×A	4	0.526	0.249	2.143	0.004	0.14	0.0029	0.428	0.299	0.008
Error	38	1.067	1.692	1.807	0.027	1.06	0.1840	16.47	5.639	0.063
CV(%)	-	8.49	9.13	10.17	3.65	11.65	11.59	10.13	11.54	12.71

* and ** denote the significance at levels of 5 and 1%, respectively.

3.1. Comparison of the mean values of the largest leaf, chlorophyll content, total water use, fresh and dry weights under the influence of biochar application

3.1.1. Length of the largest leaf

A comparison of the mean results indicated that the length of the largest leaf in the biochar treatment is 14.83 cm, being more than that obtained in the non-biochar treatment with a value of 13.67 cm (Table 3). The use of biochar in the soil has led to an increase in the length of the leaves. Moreover, among the beneficial effects of the use of biochar in agricultural soils, one can mention the improvement of water retention in the soil and improved plant growth such as length of the leaf.

3.1.2. Chlorophyll

According to the results, application of biochar has increased leaf chlorophyll content by 21.81% (Table 3). Considering that the greenness index is related to the nitrogen content of the plant, it can be stated that the increasing nitrogen level due to the use of biochar. Adding biochar to the soil increases nitrogen absorption by the plant as a consequence. Therefore, the amount of leaf chlorophyll content increases as well. Investigating the effect of cow manure biochar and moisture stress on the growth characteristics and water use efficiency of spinach in greenhouse conditions, Gavili et al. (2015) concluded that the use of biochar enhances the greenness index by 6%.

Akhtar et al. (2015) reported that the biochar produced from the combination of rice husk and flax seed significantly increases nitrogen accumulation in the plant and consequently the greenness index of wheat.

3.1.3. Total water use

The present findings showed that the highest and lowest amounts of water use were corresponding to the treatments without (4.578 lit) and with (4.435 lit) biochar application, respectively (Table 3). Due to the high porosity as well as high specific surface area, biochar retains more water and prevents evaporation and loss of soil water and consequently reduces the amount of water used.

As concluded by Gavili et al. (2015), application of biochar leads to a significant decrease in the total water amount consumed by the plant, which is probably due to the increase in the water storage capacity in the soil and the need for less water amount during the growing season.

3.1.4. Fresh weight

The results showed that the highest plant fresh weight (9.56 gr) is associated with the biochar application treatment, while the lowest amount belonged to the treatment with no biochar application with an estimated value of 8.12 gr (Table 3). According to the previous researches, the increase in corn yield in biochar treatments can be attributed to the availability of nutrients and improvement of soil physical characteristics such as the bulk density reduction (Chan et al., 2007; Zhang et al., 2010).

3.1.5. Dry weight

Plant dry weight in treatments of with an without biochar application were 1.26gr and 1.08gr, respectively (Table 3). This indicates an increase in the plant dry weight by 16.67% with the use of biochar. This is mainly due to the positive effect of biochar on the water retention and nutrients in the soil. This positive effect of biochar application has led to an improvement in the plant growth conditions.

Major et al. (2010) stated that the increase in yield due to the biochar application in the soil might be due to the direct effect of the nutrients in the biomass, which are converted into biochar during the thermal decomposition process, and also the indirect effect of improving physico-chemical and biological characteristics of the soil.

3.1.6. Water use efficiency

According to the results presented in Table 3, the highest water use efficiency has been obtained from the treatment of biochar application while the lowest amount was achieved in the absence of biochar.

Akhtar et al. (2015) reported that the application of biochar obtained from the combination of rice husk and flax seed increases the water use efficiency in all irrigation treatments compared to the condition without any biochar.

This shows the different reactions of traits to the studied factors. In other words, the factors have influenced the investigated traits and caused their increase or decrease.

Table 3. Comparison of the mean values of the largest leaf, total water use, chlorophyll, fresh and dry weights and water use efficiency under the effect of biochar application

Trait / Biochar	Length of the largest leaf (cm)	Chlorophyll ($mg.g^{-1}$ fresh leaf)	Total water use (lit)	Plant fresh weight (gr)	Plant dry weight (gr)	Water use efficiency ($g.lit^{-1}$)
Application	14.83^a	14.52^a	4.435^b	9.56^a	1.26^a	2.274^a
Non-application	13.67^b	11.92^b	4.578^a	8.12^b	1.08^b	1.788^b
$LSD_{5\%}$	0.68	0.70	0.086	0.54	0.07	0.132

3.1.7. Chlorophyll content

According to the obtained results, the highest (13.84 $mg.g^{-1}$ fresh leaf) and lowest (12.60 $mg.g^{-1}$ fresh leaf) amounts of Chlorophyll was achieved from treatmens of application and none application of amino acid, respectively (Table 4). The more the nutritional and environmental conditions such as light, are suitable for plant growth, the more the plant's ability to produce chlorophyll in the leaves and energy. Amino acid provides improved nutritional conditions for the plant and would have a positive effect in this regard.

Researchers have stated that the application of zinc micronutrient foliar application improves the chlorophyll level in the plant under drought stress and increases the plant photosynthesis as well (Ebrahim et al., 2005).

3.1.8. Total water use

According to the mean values (Table 4), the highest amount of total water use was related to the amino acid application treatment (4.626 lit) and the lowest to none application (4.387 lit).

By creating water balance in the plant, amino acids reduce damage caused by adverse plant conditions. This accelerates the plant growth and increases water consumption as a consequence.

3.1.9. Plant fresh weight

The comparison of mean results showed that the highest amount of plant fresh weight is corresponding to the amino acid application treatment with 9.19 gr while the lowest value was obtained as 8.49 gr in the treatment of none application of amino acid (Table 4).

Amino acids directly and indirectly affect the physiological activities and growth of the plant. Knowing that the plant needs higher water use for the growth, the plant fresh weight meets an increasing trend in the treatment with amino acid application.

According to the literature, amino acid increases the plant weight and dry weights due to its important role in plant metabolism and protein accumulation (Shehata et al., 2011).

3.1.10. Dry weight

As would be observed from the present findings, the highest (1.21 gr) and lowest (1.13 gr) values of dry weight was beloned to the treatments of none application and application of amino acid, respectively (Table 4).

Amino acids influence the nitrogen absorption from the soil and increase the vegetative growth, and consequently, lead to an increment in the dry weight of the plant. Hakim Che Behishat et al. (2013) concluded that use of glycine amino acid significantly affected dry weight of corn S.C.704.

3.1.11. Antioxidant

The results showed that the highest (21.39 $\mu g.ml^{-1}$) and lowest (19.75 $\mu g.ml^{-1}$) antioxidant levels are corresponding to the application and non-application of amino acid, respectively (Table 4). Ourghi and Javanmard (2014) conducted a study on the effect of amino acid foliar application on the qualitative traits of *Brussels sprouts* under greenhouse conditions and concluded that the highest antioxidant activity is obtained in the foliar application treatment of amino acid at a concentration of 900 $mg.l^{-1}$.

Table 4. Comparison of the mean values associated with the longest root, length of the largest leaf, dry weight and antioxidant under the influence of amino acids

Trait / Amino acid	Chlorophyll ($mg.g^{-1}$ fresh leaf)	Total water use (lit)	Plant fresh weight (gr)	Plant dry weight (gr)	Antioxidant ($\mu g.ml^{-1}$)
Non-application	12.60^b	4.387^b	8.49^b	1.13^b	21.3927^a
application	13.84^a	4.626^a	9.19^a	1.21^a	19.7510^b
LSD $_{5\%}$	0.70	0.086	0.54	0.07	1.24

Table 5 presents a comparison of the mean values of the length of the longest root, length of the largest leaf, chlorophyll, total water use, water use during the growth period, plant fresh weight, dry weight, proline and water use efficiency under the influence of drought stress conditions.

3.1.12. Length of the longest root

The present achievements showed that the maximum length of the longest root is related to severe stress treatment (14.20 cm) while the lowest value is associated with the slight stress treatment (9.43 cm) (Table 5). It seems that when the plant is faced with drought stress, the root tends to a lower depth to adsorb water and increases its length.

Therefore, it is obvious that length of the longest root is achieved under severe stresses, especially in resistant plants to avoid drought.

3.1.13. Length of the largest leaf

It was found that the maximum and minimum lengths of the largest root are related to the severe and slight stress treatments with estimated values of 15.20 cm and 13.26 cm, respectively (Table 5). The leaf area limitation can be the first sign of defense to deal with drought, so the reduction of water potential during the dehydration period has caused the reduction of leaf length.

The reseach conducted by Azedo-Silva et al. (2004) on Aloe vera proved that although the stomatal resistance significantly increases under water deficiency conditions, but ultimately, the stomatal cells became smaller due to water stress and irrigation limitation, and the amount of photosynthesis decreased with leaf growth.

3.1.14. Chlorophyll

It was found that the highest and lowest chlorophyll contents are related to slight (14.78 $mg.g^{-1}$ fresh leaf) and severe (11.68 $mg.g^{-1}$ fresh leaf) stress treatments, respectively (Table 5).

Gavili et al. (2015) carried out a study on spinach plant and concluded that the greenness index reduction might be due to the beginning of the leaves deterioration at the end of the growing season.

3.1.15. Plant fresh weight

The results of mean values obtained under stress conditions indicated that the highest (9.73 gr) and lowest (8.06 gr) plant fresh weights are associated with the slight and severe drought stress treatments, respectively (Table 5).

Pourqasemian and Moradi (2016) examined the effect of drought stress on some growth and biochemical parameters in pot marigold and reported that the fresh weight increase significantly with an increasing drought stress level.

3.1.16. Plant dry weight

According to the obtained results, the highest (1.29 gr) and lowest (1.06 gr) plant dry weights were related to the slight and severe drought stress treatments, respectively (Table 5).

Furthermore, Pourqasemian and Moradi (2016) found that the dry weight in marigolds significantly increases with an increase of the drought stress. under stress conditions, the dry weight reduction might be due to the turgor pressure of the cell caused by the reduction of the plant leaf area (Lawlor, 2002).

3.1.17. Total water use

The results of mean values showed that the highest and lowest amounts of total water use are related to slight and severe stress treatments with estimated amounts of 5.094 and 4.080 lit, respectively (Table 5). When the plant is under the influence of slight drought stress, it is irrigated more frequently compared to severe stress conditions. The more frequent irrigation is done, the higher the amount of water consumed because more water is allocated to evaporation.

Water stress may reduce the size of stomata, which reduces the rate of transpiration as a consequence. Lack of water causes a decrease in water use.

3.1.18. Proline

According to Table 5, the highest amount of proline has been achieved in severe stress conditions (46.107 $mg.g^{-1}$) while the lowest value was obtained from slight stress treatment (36.380 $mg.g^{-1}$).

Being adsorbed through leaves and roots, the drought stress affects various processes of plant growth and resistance to stresses and unfavorable environmental conditions, including extreme cold and heat, drought, and salinity, so the amount of proline decreases.

In another study conducted by Danaee and Abdossi (2022), the effects of drought stress and nitric oxide on the growth indicators and enzyme activity of savory were investigated. Their results showed the increasing trend of proline under drought stress conditions.

3.1.19. Water use efficiency

As would be observed from Table 5, the highest water use efficiency were related to the treatments of of 3 and 4 MAD with 2.209 and 2.186 $g.lit^{-1}$, respectively, while the lowest amount was obtained in the treatment without stress (1.613 $g.lit^{-1}$).

Tabatabai et al. (2013) conducted a research on the effect of water stress on the growth, yield and water use efficiency of pepper in greenhouse conditions and reported that a decrease of water use by 20, 40 and 60% of the water requirement, leads to a reduction in the water use efficiency of about 24.22, 56.10 and 35.29 %, respectively.

Investigating the effect of cow manure biochar and moisture stress on the growth characteristics and water use efficiency of spinach under greenhouse conditions, Gavili et al. (2015) concluded that the water use efficiency in moisture treatments of 70 and 100 % of the field capacity are statistically the same, indicating that the application of stress up to a level of 70% of the farm capacity, causes a significant decrease in plant water use by 24%.

Golestani Far et al. (2017) indicated that with the increase of drought stress level from 20% of soil moisture depletion to 60%, the water use efficiency of rye increases by 95.33%. This is mainly due to the fact that rye has consumed more water under normal conditions compared to that of drought stress, and as a result, the water use efficiency decreased due to an increment in the amount of water used.

Saeidnejad et al. (2012) also stated that the amount of water use efficiency under moderate stress of 66% and severe stress 33% increases by 62.19 and 11.29%, respectively compared to the 100% control treatment.

3.1.20. Antioxidant

According to the results, the amount of antioxidants has increased with increasing drought stress level, so that the highest content level was due to the treatment of severe water stress (22.62 $\mu g.ml^{-1}$) while the lowest one was obtained in the treatment of 10% of the allowable moisture depletion (18.73 $\mu g.ml^{-1}$).

Sayari et al. (2021) also confirmed the significant effect of the drought stress on antioxidant enzymes. Rezaeinia et al. (2018) investigated the effect of drought stress on the activity of some antioxidant enzymes and physiological traits of chickpea genotypes. According to their study, the enzyme activity was reduced at 30% stress level compared to that of 65%. The activity of antioxidant enzymes under drought stress conditions was higher in tolerant plants than sensitives.

Table 5. Comparison of the mean values associated with the longest root, length of the largest leaf, chlorophyll, total water use, water use during the growing season, plant fresh weight, dry weight proline and water use efficiency under the influence of drought stress

Trait / Stress level	Llr (cm)	Lll (cm)	Chl.(mg.g^{-1} fresh leaf)	Total water use (lit)	Pfw (gr)	Plant dry weight (gr)	Proline (mg.g^{-1})	Water use efficiency (g.lit^{-1})	Antioxidant (μg.ml^{-1})
10%	9.43^{d}	13.79^{c}	14.79^{a}	5.094^{a}	8.21^{b}	1.09^{c}	36.380^{c}	1.613^{d}	18.729^{c}
20%	11.68^{c}	14.96^{ab}	13.91^{ab}	4.757^{b}	9.07^{a}	1.19^{ab}	37.341^{b}	1.910^{c}	19.855^{bc}
30%	12.58^{b}	15.20^{a}	13.60^{b}	4.41^{c}	9.73^{a}	1.29^{a}	38.736^{b}	2.209^{a}	20.479^{bc}
40%	12.98^{b}	14.04^{bc}	12.13^{c}	4.191^{d}	9.14^{b}	1.23^{a}	41.668^{a}	2.186^{ab}	21.181^{ba}
50%	14.20^{a}	13.26^{c}	11.68^{c}	4.08^{d}	8.06^{b}	1.06^{bc}	46.107^{a}	1.985^{bc}	22.616^{a}
LSD $_{5\%}$	0.85	1.08	1.11	0.136	0.85	0.11	3.35	0.208	1.96

Llr= Length of the longest root Lll= Length of the largest leaf chl= Chlorophyll Pfw=Plant fresh weight

4. Conclusion

Among the beneficial effects of using biochar in agricultural soils, one can point out to the improvement of water retention in the soil and improvement of plant growth as well. It seems that this feature accounts for one of the positive effects of biochar application in the soil on the leaf length. Moreover, amino acids directly and indirectly play a role in the physiological activities and growth of the plant. Use of amino acid leads to the plant growth and development.

Knowing that the plant needs more water to grow, the fresh weight of the plant increases in the amino acid treatment. The highest amount of total water consumption, water consumption during the growth period and proline content was obtained from the treatment without biochar application.

Due to its high porosity as well as high specific surface area, biochar holds more water and prevents the loss of soil water from evaporation. For this reason, a reduction in the amount of water use was observed in the biochar treatment.

As would be observed, the use of biochar has led to an increased leaf chlorophyll content. According to the present achievements, there has been a positive correlation between nitrogen content and lifestyle index, and since the adding biochar improves the nitrogen level of the soil and as a result increases nitrogen adsorption by the plant, the chlorophyll content of leaves ill be increased.

5. Disclosure Statement

No potential conflict of interest was reported by the authors

6. Refrences

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