



## Assess Effect of Different Level of Biochar and Humic Acid on Crop Production and Nutrition Content of Maize Affected Water Stress Condition

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### RESEARCH ARTICLE

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### ABSTRACT

**BACKGROUND:** Water deficit stress is one of the most important agricultural problems in arid and semi-arid regions. Biochar has a great ability to absorb and store nutrients and chemical fertilizers, even keeping elements such as phosphorus and nitrogen and preventing their leaching, increases the growth of plants.

**OBJECTIVES:** The purpose of this research was to study the effect of Biochar and Humic acid amounts on the absorption of elements and the seed yield of corn hybrid (SC.704) under water deficit stress in Ahvaz region.

**METHODS:** Current study was conducted in the form of a split split plot experiment based on randomized complete block design with three replications during the cropping years 2016-2018 and 2018-2019 in Ahvaz. The main plot includes water deficit stress with three levels of irrigation (irrigation after draining 30, 40 and 50% of the field capacity, respectively, optimal irrigation, mild stress and severe stress). Also the sub-plot includes biochar with 2 levels (no use of biochar and the application of 4 t.ha<sup>-1</sup>) and sub-subplot included humic acid with 4 levels (no application of humic acid, application of 2, 4 and 6 liters of humic acid per hectare).

**RESULT:** The results of analysis of variance showed that the effect of water stress, biochar and humic acid on all measured traits was significant. Also interaction effect of treatments was significant (instead effect of water stress × biochar on seed yield, iron and zinc seed content and water stress × humic acid on seed yield) Under severe stress conditions, seed yield decreased (36%). Humic acid had a positive and significant effect on all studied traits. The maximum seed yield (8995.18 kg.ha<sup>-1</sup>) was related to the irrigation treatment after draining 40% of the field capacity and applying 4 t.ha<sup>-1</sup> of biochar.

**CONCLUSION:** In general, in conditions of moderate moisture stress, the use of 4 liters of humic acid per hectare increased the seed yield by 42% compared to the treatment of severe stress and no use of humic acid.

**KEYWORDS:** Iron, Nitrogen, Protein content, Zinc, Seed yield.

## 1. BACKGROUND

Water deficit stress is one of the factors that limiting production in crops including corn. Some growth stages of plants are highly sensitive to water shortage. So that in maize seedling stage, pollination and seed filling is more sensitive to water deficit (Lobell *et al.*, 2014). Ghasemi-Golzani *et al.* (2018) reported in corn that the highest seed yield (628 gr.m<sup>-2</sup>) was obtained from the treatment of 60 mm of evaporation from the pan and the lowest of this trait was obtained from the treatment of 120 mm of evaporation from the pan. Organic materials are one of the important soil modifiers and improve the physical, chemical and biological properties of soils. Biochar is a carbon-rich product that is produced during the thermal decomposition of biomass such as wood, fertilizers, leaves, straw and stubble, as well as agricultural waste in anaerobic or hypoaerobic conditions (Lehmann *et al.*, 2007). The use of biochar in the soil causes the absorption of large amounts of water. Due to the great ability of this material to absorb and store nutrients and chemical fertilizers, even keeping elements such as phosphorus and nitrogen and preventing their leaching, increases the growth of plants (Burek *et al.*, 2011). In the study, the application of 10 tons per hectare of biochar caused a 56% increase in seed yield, improved soil properties and increased wheat biomass compared to its non-application (Mahmoud *et al.*, 2019). Ullah *et al.* (2018) reported in corn that biochar increased seed and biological yield and macro and micro seed nu-

trients. The use of natural fertilizers such as humic acid can improve the physical condition and increase water retention in the soil without harmful environmental effects (Mirhajian, 2012). Szczepanek and Wilczewski (2016) stated in the response of corn plants to the application of humic acid that humic acid increased cob weight and seed characteristics such as seed weight and seed yield compared to the control treatment.

## 2. OBJECTIVES

The purpose of this research was to study the effect of biochar and humic acid amounts on the absorption of elements and the seed yield of corn hybrid (SC.704) under water deficit stress in Ahvaz region.

## 3. MATERIALS AND METHODS

### 3.1. Field and Treatments Information

This experiment was conducted in Ahvaz, located in the southwest of Iran, with a latitude of 31 degrees and 20 minutes north and a longitude of 48 degrees and 40 minutes east and a height of 22.5 meters above sea level. The average annual rainfall of the region is 250 mm and the minimum temperature in winter is 3 degrees Celsius and the maximum temperature in summer is 49 degrees Celsius. Before conducting the experiment, samples were taken from the field soil at a depth of 0-30cm and the physical and chemical characteristics of the soil and biochar were presented in tables 1 and 2.

**Table 1.** Chemical characteristics and some other important properties of the soil of tested field

Crop year	Depth (cm)	Soil texture	Zn (mg.kg <sup>-1</sup> )	Fe (mg.kg <sup>-1</sup> )	K (ppm)	N (%)	O.C (%)	EC (ds.m <sup>-1</sup> )	pH
2017-2018	0-30	Sandy loam	1.82	12.1	188	0.13	0.89	3.94	7.62
2018-2019	0-30	Sandy loam	1.85	12.44	175	0.11	0.91	3.74	7.3

**Table 2.** Chemical characteristics and some other important properties of biochar

pH	4.83
EC (ds.m <sup>-1</sup> )	3.88
Organic carbon (%)	64.45
H (%)	4.88
N (%)	0.53

This research was carried out in the form of a split-split plot based on randomized complete blocks design with three replications during the cropping years 2016-2017 and 2017-2018. The main plot includes water deficit stress with three levels of irrigation (irrigation after draining 30, 40 and 50% of the field capacity, optimal irrigation, mild stress and severe stress, respectively), the sub-plot includes biochar with 2 levels (no use of biochar and the application of 4 tons in A hectare) and sub-subplot included humic acid with 4 levels (no application of humic acid, application of 2, 4 and 6 liters of humic acid per hectare). The experiment consisted of 72 plots. Each experimental plot was six meters long and had seven planting lines with a distance of 75 cm and the distance between plants on the row was 18 cm with a density of 75,000 plants per hectare. The distance between the two sub-plots was one meter and the distance between the main plots was 1.5 meters.

### 3.2. Farm Management

The operation of preparing the bed included plowing with iron plow, disc and finally leveling operation with trowel. The basic fertilizer used in the farm includes nitrogen fertilizer from the source of urea in the amount of 150 kg per hectare divided in two stages (50% at the same time as planting and 50% in the six-leaf stage) and phosphorus fertilizer based on 60 kg. Pure phosphorus was sourced from triple super-

phosphate during land preparation. Hybrid cultivated was single cross 704, which was a late variety. In early August 2017 and 2018, seeds were sown manually on piles at a depth of four centimeters of soil, slightly above the furrow. After sowing, the experimental field was irrigated immediately. All the biochar in the specified treatment was added to the soil at a depth of 20 cm before planting. The application of different amounts of humic acid at the stage of 6-8 leaves in 3 treatments (2, 4 and 6 liters per hectare) along with irrigation water was applied in the field. Weed control was done by manual weeding. Until the five-leaf stage, irrigations were carried out based on draining 30% of moisture from the field capacity of the soil according to the depth of root development (50 cm) in all treatments, and from this stage onwards, water stress treatments were applied in the field.

### 3.3. Measured Traits

The ripeness of the seeds was determined by creating a black layer at the base of the seeds, and the final harvest was done by removing 50 cm from the beginning and end of the lines from a surface equivalent to two square meters. In order to measure the seed yield in each experimental plot, after removing 0.5 meters from both ends of the lines, all the cobs in the three middle lines with a length of two meters were removed manually, and after drying and separating the seeds, it was done manually. In order to measure the raw protein percentage of seeds, first, using Kjeldahl apparatus in the laboratory, the total nitrogen percentage of the seeds was calculated and then by multiplying the nitrogen percentage of the seeds by a factor of 6.25, the amount of protein in the seeds was obtained (Keeney and Nelson,

1982). To measure the amount of iron and zinc elements, first, the sample was placed in a furnace at a temperature of 550 degrees Celsius for 6 to 12 hours to turn it into ash. Then the sample was taken out, first a few drops of distilled water and then 2-5 ml of 2 M hydrochloric acid were added to it and it was placed at 70 degrees Celsius for one hour. After that, the sample was brought up to volume in a 50 cc flask with distilled water and using an atomic absorption device (Perkin 400) and installing a special lamp for each element, the amount of absorption at wavelengths of 3.24 nm for iron and 21.9 nm was read (Emami, 1996).

#### 3.4. Statistical Analysis

Data analysis of variance was done by SAS statistical software and LSD test was used to mean comparison at 5% probability level.

## 4. RESULT AND DISCUSSION

### 4.1. Seed Zinc concentration

The results showed that the simple effects of water deficit stress, biochar and humic acid and the mutual effects of water deficit stress and biochar on the seed zinc concentration were significant at the 1% probability level (Table 3). In this research, the highest concentration of zinc in the seed belonged to the treatment of 6 liters per hectare, which was not statistically significantly different from the treatment of 4 liters per hectare, and the lowest concentration of seed zinc was obtained from the treatment of no application of humic acid (Table 4). It can be stated that humic acid increases the absorption of elements such as zinc, nitrogen and iron by increasing the root growth and the surface of the filaments. It has been reported that the

main factor in increasing zinc absorbability as a result of using organic fertilizers is the formation of organic complexes (Schmidt, 2003). Irrigation treatment after draining 30% of field capacity and applying 4 tons per hectare of biochar (which had no statistically significant difference with irrigation treatment after draining 40% of field capacity and using 4 tons per hectare of biochar) had a positive and 36.5% effect on the increase in the concentration of seed zinc compared to the irrigation treatment after draining 50% of the field capacity and not using biochar (Table 5). The reason for this is the increase in water absorption, ventilation and availability of food, as well as the increase in the activity of microorganisms in the presence of biochar, which was consistent with the results of Inal *et al.* (2015) in corn plants. Other researchers such as Khorram Qahfarokhi *et al.* (2015) have pointed out the positive role of humic acid in increasing the concentration of seed zinc, which was consistent with the results of this research.

### 4.2. Seed iron concentration

The effect of water deficit stress, biochar and humic acid on seed iron concentration was significant (Table 3). According to the results of the comparison table, the irrigation treatment after draining 30% of the field capacity and the application of 4 tons per hectare of biochar had a positive effect of 13.27% on the increase of seed iron concentration compared to the irrigation treatment after draining 50% of the field capacity and not using biochar (Table 5). In this study, the application of biochar under mild stress conditions increased the iron concentration in corn seeds.

**Table 3.** Result of analysis of variance effect of treatments on studied traits

S.O.V	df	Seed yield	Seed zinc content	Seed iron content	Seed nitrogen content	Seed protien content
Year	1	2054.1 <sup>ns</sup>	2.07 <sup>ns</sup>	1.44 <sup>ns</sup>	0.55 <sup>ns</sup>	3.21 <sup>ns</sup>
Year × Replication	4	17.04	0.84	0.57	0.04	1.06
Water stress (W)	2	2854370 <sup>**</sup>	256.2 <sup>**</sup>	6045 <sup>**</sup>	40.01 <sup>**</sup>	274.3 <sup>**</sup>
Y × W	2	5214 <sup>ns</sup>	10.16 <sup>ns</sup>	3.43 <sup>ns</sup>	0.17 <sup>ns</sup>	1.18 <sup>ns</sup>
E <sub>a</sub>	8	18256	30.22	320.17	3.87	8.33
Biochar (B)	1	160574 <sup>**</sup>	190.5 <sup>**</sup>	3802.1 <sup>**</sup>	34.13 <sup>**</sup>	200.05 <sup>**</sup>
Y × B	1	480.25 <sup>ns</sup>	1.41 <sup>ns</sup>	0.06 <sup>ns</sup>	2.86 <sup>ns</sup>	0.15 <sup>ns</sup>
W × B	2	202574.1 <sup>**</sup>	205.7 <sup>**</sup>	4000.5 <sup>**</sup>	0.34 <sup>ns</sup>	2.12 <sup>ns</sup>
Y × W × B	2	600.3 <sup>ns</sup>	0.05 <sup>ns</sup>	0.71 <sup>ns</sup>	0.63 <sup>ns</sup>	0.88 <sup>ns</sup>
E <sub>b</sub>	12	14247.4	18.08	245.08	1.2	5.11
Humic Acid (H)	3	175871 <sup>**</sup>	264.14 <sup>**</sup>	5059.63 <sup>**</sup>	25.51 <sup>**</sup>	170.3 <sup>**</sup>
Y × H	3	102 <sup>ns</sup>	1.19 <sup>ns</sup>	3.51 <sup>ns</sup>	0.07 <sup>ns</sup>	2.25 <sup>ns</sup>
W × H	6	400547 <sup>**</sup>	7.64 <sup>ns</sup>	18.74 <sup>ns</sup>	0.28 <sup>ns</sup>	1.11 <sup>ns</sup>
Y × W × H	6	193.4 <sup>ns</sup>	0.13 <sup>ns</sup>	29.84 <sup>ns</sup>	0.35 <sup>ns</sup>	1.05 <sup>ns</sup>
B × H	3	251 <sup>ns</sup>	3.02 <sup>ns</sup>	11.45 <sup>ns</sup>	1.41 <sup>ns</sup>	0.67 <sup>ns</sup>
Y × B × H	3	174.4 <sup>ns</sup>	0.06 <sup>ns</sup>	0.34 <sup>ns</sup>	0.05 <sup>ns</sup>	0.18 <sup>ns</sup>
W × B × H	6	230.8 <sup>ns</sup>	2.05 <sup>ns</sup>	1.62 <sup>ns</sup>	1.09 <sup>ns</sup>	0.09 <sup>ns</sup>
Y × W × B × H	6	574 <sup>ns</sup>	0.67 <sup>ns</sup>	2.91 <sup>ns</sup>	0.84 <sup>ns</sup>	0.01 <sup>ns</sup>
E <sub>c</sub>	72	13800	12.14	228.91	0.05	2.21
CV (%)	-	16	11.39	9.31	15.97	17.86

<sup>ns</sup>, \* and <sup>\*\*</sup>: non-significant, significant at 5% and 1% of probability level, respectively.

Such an increase can be related to the release of nutrients such as iron by the biochar added to the soil and as a result improving soil fertility. In this regard, Lehmann *et al.* (2013) also reported that the use of biochar increases the absorption of iron, zinc and copper and, as a result, increases plant growth. The results showed that the seed iron concentration with the application of 6 liters per hectare had a significant increase of 10.66% compared to the absence of humic acid application (Table 4). By having a large amount of weak

acid groups in its molecular structure, acid humic can correct alkaline pH. By adjusting pH by humic substances, iron deposition in soils is prevented (Karakurt *et al.*, 2009). The results of Pinton *et al.* (1999) showed that humic acid increased nitrate absorption and ATPase enzyme activity in the plasma membrane of root cells, and also increased nutrient absorption and corn seed yield, which is consistent with the results of this research.

**Table 4.** Mean comparison results of the studied traits affected different levels of water stress, biochar and humic acid

Treatment	Seed yield (kg.ha <sup>-1</sup> )	Seed zinc content (mg.kg <sup>-1</sup> )	Seed iron content (mg.kg <sup>-1</sup> )	Seed nitrogen content (%)	Seed protein content (%)
<b>Water stress</b>					
<b>30% Field Capacity (Control)</b>	9000.24	37.85	170.29	1.08	6.11
<b>40% Field Capacity</b>	7270.14	29.38	163.09	1.39	8.45
<b>50% Field Capacity</b>	5750.61	24.54	154.1	1.75	10.40
LSD 5%	450.17	2.45	1.21	0.05	0.41
<b>Biochar</b>					
<b>Non use (Control)</b>	6475.25	27.01	153.36	1.23	7.13
<b>4 t.ha<sup>-1</sup></b>	8205.42	34.17	171.62	1.58	9.51
LSD 5%	370.05	2.81	1.57	0.04	0.66
<b>Humic Acid</b>					
<b>Non use (Control)</b>	5600.56	25.58	151.75	1.16	6.04
<b>2 L.ha<sup>-1</sup></b>	6010.36	29.62	159.62	1.3	7.38
<b>4 L.ha<sup>-1</sup></b>	8800.14	32.45	168.54	1.56	9.85
<b>6 L.ha<sup>-1</sup></b>	8950.28	34.71	169.87	1.60	10.01
LSD 5%	341.34	1.95	1.08	0.02	0.84

\*In each column, the means of each treatment that have common letters are not significantly different from each other based on LSD test.

#### 4.3. Seed nitrogen percentage

The results obtained from the compound variance analysis table confirm that the stress treatments of water deficit, biochar and humic acid were significant on the amount of seed nitrogen at the probability level of 1% (Table 3). The results showed that the highest amount of seed nitrogen was allocated to the irrigation treatment after draining 50% of the field capacity (1.75%) and the lowest amount of seed nitrogen was allocated to the irrigation treatment after draining 30% of the field capacity (1.08%) (Table 4). It can be stated that the increase in nitrogen concentration in plants under water stress is due to the rapid accumulation of amino acids

that have not been converted into proteins (Salehi *et al.*, 2003). Also, in the optimum irrigation treatment (control), the used nitrogen was exposed to washing and was out of reach of the roots and the amount of absorption was reduced, but with the reduction of the amount of water needed by the plant, nitrogen was absorbed more efficiently. In this regard, Gouili *et al.* (2016) stated that the increase in nitrogen concentration could be due to the decrease in the dry weight of the plant and as a result, the increase in nitrogen accumulation in the plant, which was consistent with the results of this research.

**Table 5.** Mean comparison interaction effect of different levels of water stress and biochar on zinc and iron content and seed yield

Water stress	Biochar	Seed zinc content (mg.kg <sup>-1</sup> )	Seed iron content (mg.kg <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )
30% Field Capacity (Control)	Non use (Control)	32.11	166.15	8146.88
	4 t.ha <sup>-1</sup>	37.23	172.01	9164.46
40% Field Capacity	Non use (Control)	29.05	161.23	6295.53
	4 t.ha <sup>-1</sup>	36.2	170.1	8995.18
50% Field Capacity	Non use (Control)	23.64	149.17	5467.71
	4 t.ha <sup>-1</sup>	25.33	156.31	5972.25
LSD 5%		1.88	1.37	360.44

\*In each column, the means of each treatment that have common letters are not significantly different from each other based on LSD test.

On the other hand, the application of 4 tons per hectare of biochar (1.58%) significantly increased the concentration of nitrogen in the plant by 22.15% compared to the control (Table 4). In relation to the effect of biochar on the availability of nitrogen in the soil, recent findings have shown that biochar is like a source of fertility and maintains the nutritional balance in the soil ecosystem by providing and maintaining nutrients, including nitrogen, which increases plant growth and productivity. will be the product (Farrell *et al.*, 2014). In this regard, Solomon *et al.* (2007) also showed high nitrogen content in soils rich in biochar. In this research, by increasing the level of humic acid consumption in the soil, the content of this element in the seed increased, so that the highest seed nitrogen content was obtained in the treatment of application of 6 liters per hectare of humic acid, which was not significantly different with the treatment of 4 liters per hectare, and the lowest Its content was obtained in the control treatment (Table 4). Adding organic matter to the soil affects plant growth and soil fertility and increases the

level of available nitrogen in the soil. It has also been reported that organic fertilizers stimulate nitrogen fixation in the soil and transfer it from roots to aerial parts and seeds (Nikbakht *et al.*, 2008), which was consistent with the results of this research.

#### 4.4. Seed protein percentage

The simple effects of water deficit stress, biochar and humic acid was also significant on seed protein percentage at the probability level of 1% (Table 3). Based on the results of this research, the highest and lowest percentage of seed protein was related to irrigation treatments after draining 50% and 30% (control) of field capacity, respectively (Table 4). The higher percentage of protein in water deficit stress conditions compared to optimal irrigation conditions can be related to the decrease in the length of the growth and development period in treatments with water limitation, which caused a decrease in the ratio of carbohydrates to protein and as a result, an increase in protein percentage in these treatments.

Be It has been reported that water stress leads to disturbances in the process of photosynthesis, enzyme activity and protein synthesis, which affect the movement of metabolites towards the seed (Talooth *et al.*, 2006). Also, the lowest amount of protein was obtained from the treatment of no application of biochar, which showed a decrease of 25.02% compared to the treatment of application of 4 tons per hectare of biochar (Table 4). In this research, the higher amount of seed protein is related to the ability of biochar to preserve nutrients and prevent their leaching (Mosa-naei *et al.*, 2017). These results were consistent with the reports of Baradaran Najjar *et al.* (2016). The highest percentage of protein related to the treatment of 6 liters per hectare and the lowest percentage of protein was allocated to the treatment of no application of humic acid (Table 4). In this research, the increase in the accumulation of nutrients in the seed can be considered as the reason for the increase in protein due to humic acid spraying (El-Bassiony *et al.*, 2010). This increase is due to the improvement of soil structure and, as a result, the increase of soil water holding capacity, good ventilation and proper drainage, which expands root growth and improves nutrient absorption (Saleh, 2003).

#### 4.5. Seed yield

The results indicated that seed yield showed a significant reaction under the stress of water deficiency, biochar and humic acid and their mutual effects (Table 3). The highest seed yield was obtained from the application of 4 tons per hectare of biochar under irrigation conditions after 30% of the field capacity was depleted (which was not statistically significant with the application of 4 tons per hectare

of biochar under irrigated conditions after 40% of the field capacity was depleted) and the lowest seed yield was obtained from the non-application of biochar under irrigation conditions after 50% of the field capacity was drained (Table 5). In this research, the plant was able to tolerate the lack of available water well and without reducing the yield in moderate water stress conditions compared to proper irrigation conditions through biochar. In the research conducted by Ebrahim *et al.* (2015), treatments containing biochar with irrigation based on 80% water requirement had higher yield than treatments without biochar and irrigation based on 100% water requirement. The highest seed yield was obtained from the irrigation treatment after draining 30% of the field capacity and applying 6 liters per hectare of humic acid, which showed a 42% increase compared to the irrigation treatment after draining 50% of the field capacity and not using humic acid (Table 6). The increase in seed yield is indicative of the ability of humic acid to affect plant reproduction under water stress conditions. It seems that different levels of humic acid led to an increase in seed yield by providing nutrients (Wang *et al.*, 2014), which was consistent with the results of this research.

## 5. CONCLUSION

In general, the results of the experiment showed that under optimal moisture conditions, the increase in humic acid levels was associated with a significant increase in seed yield and absorption of elements and the application of biochar had a very positive effect on seed yield. In the conditions of severe water stress (irrigation after draining 50% of the field capacity), the decrease in the absorption and increase in the wastage of humic acid caused by the lack



of water in the soil led to a decrease in the positive effect of the increase in humic acid on the increase in seed yield. Also, biochar was able to reduce the effects of water deficit stress due to its porous structure. Due to the absence of observed adverse effects of humic acid on the plant and the increase yield, it seems that it is better to use 4 tons of biochar per hectare in order to increase yield, if plant will encounter to limited irrigation conditions during the growth period, In order to increase yield, 4 tons of biochar and 4 liters of humic acid per hectare should be applied.

**Table 6.** Mean comparison interaction effect of different levels of water stress and humic acid on seed yield

Water stress	Humic Acid	Seed yield (kg.ha <sup>-1</sup> )
30% Field Capacity (Control)	Non use (Control)	7362.57
	2 L.ha <sup>-1</sup>	8000.41
	4 L.ha <sup>-1</sup>	9243.75
	6 L.ha <sup>-1</sup>	9700.37
40% Field Capacity	Non use (Control)	6100.5
	2 L.ha <sup>-1</sup>	6601.32
	4 L.ha <sup>-1</sup>	7321.91
	6 L.ha <sup>-1</sup>	9670.42
50% Field Capacity	Non use (Control)	5618.36
	2 L.ha <sup>-1</sup>	5960.58
	4 L.ha <sup>-1</sup>	6212.79
	6 L.ha <sup>-1</sup>	6291.23
LSD 5%		332.06

\*In each column, the means of each treatment that have common letters are not significantly different from each other based on LSD test.

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#### FOOTNOTES

**AUTHORS' CONTRIBUTION:** All authors are equally involved.

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