J. Agr. Sci. Tech. (2023) Vol. 25(3): ??-??

Effect of Biochar Amendment and Irrigation Treatments on Biochemical Attributes and Morphological Criteria of Basil (*Ocimum basilicum* L.) Using Central Composite Design

A. Noroozi[1], P. Rezvani Moghaddam[1]*, M. Hashemian[2], and S. Khorramdel[1]

ABSTRACT

Three different levels of biochar addition in soil (0, 1, 2 kg m^{-2}) and simultaneous irrigation treatments (50, 85, and 120% of crop Evapotranspiration (ET_c)) were applied to basil (*Ocimum basilicum* L.) for two consecutive years (2018 and 2019). Central Composite Design (CCD) was used as an experimental optimization method, and 13 given experiments were carried out. The study was performed at the research farm of the Ferdowsi University of Mashhad, Iran. The effects of these treatments were evaluated on biochemical attributes (total chlorophyll, total phenol, and total soluble carbohydrates) and morphological criteria (biological yield, height, seed yield, and harvest index). Then, all the results were statistically analyzed. The results revealed that biochar amendment in the soil decreased all examined biochemical characteristics. Meanwhile, biochar in the soil strengthened the morphological properties of the basil plant. Also, the basil plant significantly responded to the amount of irrigation levels. High levels of water treatments reduced total phenol and total soluble carbohydrates and raised all other measured factors. Statistical analysis shows no significant relationship between 2-way-interaction (biochar×irrigation) and measured factors, except total soluble carbohydrates.

Keywords: Biological yield, Chlorophyll, Crop evapotranspiration, Harvest Index, Total soluble carbohydrates.

INTRODUCTION

Biochar is a carbon-based material made from renewable sources such as green waste, wheat straw, wood, and rice hull (Huang *et al*., 2020). Biochar application to soil has gained much attention due to environmental and agronomical issues, including climate change, sustainable soil management, and soil pathogen control (Ebrahimi *et al*., 2021a and 2021b). Biochar in the soil can improve soil fertility, cation exchange capacity, soil pH, nutrient retention, and water holding capacity (Pandey *et al*., 2016; Nobile *et al*., 2020), resulting in higher crop yield. Also, there is evidence that the organic carbon content of biochar highly depends on the material source, and it can reach 90% (Leng *et al*., 2019). The higher carbon content of modified soil using biochar could affect plants' morphological and biochemical attributes.

Water is essential for plant quality and productivity. Therefore, investigating the optimum level of irrigation for achieving the best plant quality and maximum plant yield is necessary because of global warming, the limitation of water resources, and increasing competition between industrial and agricultural consumption. (Ekren *et al*., 2012; Bekhradi *et al*., 2015).

The *Ocimum basilicum* L. and many other aromatic plants have been cultivated to treat

[1] Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, P. O. Box: 91779 48974, Mashhad, Islamic Republic of Iran.
[2] Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.
*Corresponding author; e-mail: rezvani@ferdowsi.um.ac.ir

[DOI: 10.22034/jast.25.3.535]

ailments from ancient times (Purushothaman *et al*., 2018). An increase in global demand for plant-based pharmaceuticals, health products, food supplements, flavor additives, nutraceuticals, and cosmetics has caused worldwide attention to cultivating these species. It is also essential to consider all the above criteria for producers to provide the best plant characteristics and maximum productivity (Pandey *et al*., 2016).

The optimization of biochar content and levels of irrigation are necessary. In the traditional form, one factor's influence is monitored in an experiment when others are kept constant, which is called One-Variable-At-a-Time (OVAT). This technique has two main drawbacks. First, interactive effects amongst the variables is not considered, and second, optimization of the experiments needs a high number of experimental runs. These disadvantages cause an imperfect selection of parameters affecting the response. Another popular methodology has been developed, namely, Response Surface Methodology (RSM) to overcome these problems. This optimization method represents the relationship between the adequate factor levels in three dimensions by a surface. Amongst all RSM techniques, Central Composite Design (CCD) method is the most common because of its simplicity and high performance (Izadiyan and Hemmateenejad, 2016). To the best of our knowledge, there are only a few reports on optimizing biochar usage and irrigation that aim to obtain maximum plant quality and post-harvesting yield in *Ocimum Basilicum* L. Still, no research article was found concerning the RSM optimization via the CCD method.

In this study, application of CCD based on RSM is presented for modeling the biochar amendment and irrigation treatments and its impact on secondary metabolites and morphological attributes.

MATERIALS AND METHODS

Experimental Site

The field experiment was conducted in 2018 and 2019 from May to September at the Research Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad (36.16 °N and 59.36 °E, Elevation 985 m) Khorasan Razavi Province, Iran. The area has a long summer climate, with maximum temperatures ranging from 24.5 to 38°C and minimums from 13.7 to 23.2°C, and the climate is semi-arid. Table 1 gives meteorological information about the research station during the growth period for both years. Table 2 demonstrates topsoil's physical and chemical properties (0–30 cm) before the experiment.

Biochar Amendment

The applied biochar, purchased from Rafsanjan Pars Activated Carbon Company (Iran), was obtained from pistachio base granule activated carbon. Biochar had total C, ash, and volatile substances, respectively 75, 15, and 5%. Also, it had maximum moisture of 5%. Three different amounts of ground biochar (0, 1, and 2 kg m^{-2}) were added and mixed with the top 30 cm of soil.

Irrigation Treatments

CropWat software (version 8.0 windows) was used to calculate reference evapotranspiration according to Penman-Monteith based on climatic data. Reference evapotranspiration data are given in Table 1. Then, crop Evapotranspiration (ET_c) was calculated by the below equation (Allen *et al*., 1998).

$ET_c = ET_0 \times K_c$

Where, ET_c= Crop Evapotranspiration; ET_0= Reference Evapotranspiration given by CropWat software, and K_c= Crop coefficient (from FAO paper 56, Refer to mint as the closest group member to the basil plant).

[DOI: 10.22034/jast.25.3.535]

Table 1. Meteorological information of the research station during the growth period in 2018 and 2019.

	Parameter	Temp (°C)		Average temp	Relative humidity (%)	Wind Speed (m s^{-1})	Sun hours (h)	Average rainfall (mm)	ET_0 (mm)
	Month	Max	Min						
2018	May	27. 6	13.7	20.74	38	2.8	8.5	57.82	5.88
	June	34.2	19.8	27.11	24	2.9	11.5	2.12	8.15
	July	38	23.1	31.12	18	3.3	13	0	9.78
	August	34	19.8	27.04	23	2.9	12	0	8.07
	September	30.3	14.6	22.59	24	2.3	10.3	0.11	5.98
2019	May	24.5	14.7	21.53	51	3.2	9.5	43.74	5.41
	June	34	18.5	26.7	27	3	12	23.02	8.15
	July	37.8	23.2	30.96	23	3.4	12.7	0	9.65
	August	34	19.4	26.92	24	3.3	12.4	0	8.5
	September	30	15.2	22.49	30	3.1	10.4	0.01	6.54

Table 2. The physical and chemical properties of topsoil (0–30 cm) at the experimental site.[a]

	EC (dS.m^{-1})	pH	TOC (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Sand (%)	Silt (%)	Clay (%)	TNV (%)	SP (%)
Soil	3.60	7.74	0.798	0.08	28	210	34	51	15	16.32	43.39

[a] EC: Electrical Conductivity; TOC: Total Organic Carbon; SP: Saturation Percentage, TNV: Total Neutralizing Value.

The actual amount of water for each irrigation treatment was estimated by the following equation (James, 1988):

$$\mathrm{I.R_a} = \frac{\mathrm{ET_c}}{(1 - \mathrm{Lf})\mathrm{ER}}$$

Where, $I.R_a$= Total actual Irrigation water applied mm interval^{-1}; ET_c= Crop evapotranspiration; Lf= Leaching factor 10%, and ER= Irrigation system Efficiency of 70% (Singh *et al*., 2021).

Then, three different water treatments were applied: I_1 (50% of ET_c), I_2 (85% of ET_c), and I_3 (120% of ET_c), based on research by Caliskan *et al*. (2017). Flow meters were installed in each hose nozzle, and the volume of irrigation water for each experimental plot was measured intently. Irrigation treatments were started on June 10, 2018, and June 16, 2019, and applied at five-day intervals.

Experiment Design

A rotatable CCD with two factors at three levels was applied to investigate the effect of factors (amount of biochar (kg m^{-2}) and irrigation (%)) on multiple responses, including; morphological properties [Shoot Dry Weight (SDW), plant height, seed yield, and Harvest Index (HI)] and biochemical compositions (total Chlorophyll, phenol content, and total soluble carbohydrates).

In the CCD process, three levels of variables were studied and coded as (-1), (0), and (+1). Values (-1) and (+1) are applied to find the minimum and maximum variables, while (0) was given to the average level. Rotatable CCD estimates the coefficients of a quadratic equation used to predict the response value. The rotatable CCD design matrix for two variables at three levels includes 13 design experiments. Table 3 displays 13 different experimental runs of CCD and the actual equivalent levels of variables. All 13 different treatments were performed, and a quadratic model was fitted to the response data using Minitab software (version 17.3.1). The total number of tests (t) for variables (k) and the number of tests at the center points (r) are given by Aslan (2007):

$$t= 2^k+2k+r= 2^2+(2\times2)+5= 13$$

Growing Conditions and Plant Materials

The basil (*Ocimum basilicum* L.) seeds originated from the city of Mashhad, in the semi-arid climate of the Khorasan Razavi Province, Iran. Basil seeds were sown in the same ecological conditions at the beginning of May 2018 and 2019 for experiments.

Each plot size was 3×2 m, consisting of four rows with 50 cm spacing. Due to the plant's adaption to the new situation, no water stress was applied to the seedlings in the first 30 days. Then, basil plants were harvested to form a monotony among the plants (60 plants in a row).

The plants were harvested on September 22, 2018, and September 19, 2019, when the basil plants fully flowered. One row of plants from each side of the plot was considered as guard and eliminated from all the measurements. The side effects were removed, and residual leaves were used for further measurements.

Biochemical Characteristics

Harvested leaves from each treated plot were air-dried at room temperature. Afterward, dried leaves were ground until a uniform powder was obtained. Powdered leaves were used for all chemical measurements. All experiments were carried out in triplicates.

Chlorophyll

Chlorophyll was extracted from powdered leaves based on a modified method reported elsewhere (Dere *et al*. 1998). In brief, 500 microliters and 96% (V/V) methanol (Merk, Germany) was added to each microtube sample containing 5 mg of powdered leaves.

[DOI: 10.22034/jast.25.3.535]

Samples were homogenized in liquid nitrogen for 5 min. After centrifugation (1,500×g, 10 minutes, room temperature), the supernatant was collected for chlorophyll measurement using a spectrophotometer (model 6305, Jenway UV-Visible). Chlorophyll a and b absorbances were measured at 666 nm, and 653 nm, respectively, and the sum was reported as total Chlorophyll.

Total Phenolic Compounds

Total phenol was approximated using Folin–Ciocalteu (F-C) reagent based on protocol (Ainsworth and Gillespie, 2007). With some modification, briefly, 5 mg of dried powder leaves of each sample was weighed carefully. 500 microliters of 95% (V/V) methanol (Merk, Germany) were added to each sample and homogenized for 5 min. Samples were incubated for 48 hours at room temp. The supernatant was collected after centrifugation (1,500×g, 10 minutes, room temp). Then, 100 microliters of each sample supernatant were added to a microtube. Two hundred microliters of 10% (V/V) F–C reagent and 800 microliters of 700 mM Na_2CO_3 were added to each one, and assay tubes were incubated at 40°C for 30 minutes. To calculate the standard curve, five different concentrations (0, 8.5, 17, 25.5, and 42.5 mMol L^{-1}) of Gallic acid were prepared. The absorbance of all samples, standards, and blank from the assay was read at 765 nm in a spectrophotometer (model 6305, Jenway UV-Visible). The regression equation between Gallic acid standards absorbances was used to estimate total phenolic as Gallic acid equivalents.

Total Soluble Carbohydrates

Extraction of total soluble carbohydrates was carried out using a procedure suggested by Gomez *et al*. (2002) with some changes. First, 5 mg of air-dried leaves powder was weighed in a microtube. Then, a three-part mixture of methanol/water/chloroform, respectively, 100,

Table 3. CCD experimental runs for two variables and two levels (the last four experiments replicate the central point).

Run	The actual level of variables: Amount of biochar (kg m^{-2})	Irrigation levels (%)
1	0	50
2	2	50
3	0	120
4	2	120
5	0	85
6	2	85
7	1	50
8	1	120
9	1	85
10	1	85
11	1	85
12	1	85
13	1	85

100, and 200 microliters was added to the microtube. After prolonged shaking with vortex, tube contents were centrifuged (1,500×g, 10 minutes, room temperature). Next, the methanol/water supernatant was carefully separated into a new microtube for further measurements. The colorimetric method was used to detect phenol-sulfuric acid as a reagent and spectrophotometer as the detector. For this purpose, 10 microliters of the previous solution were added to a new microtube containing 10 microliters of phenol (40% W/V) and 600 microliters of sulfuric acid 98% (W/V). They were shaken and placed in a bain-marie at 25 to 30°C for 15 minutes after 10 minutes. In the end, the absorbance was measured at 480 nm. The amount of sugar was then estimated, referring to the glucose standard curve. Five glucose concentrations (6, 12, 24, 48, and 56 Mm L-1) were used to calculate the standard curve. The linear relation between these points was used to estimate the concentration of total carbohydrates in actual samples.

Morphological Crop Characteristics

Plants were grown for about three months, and after that, various morphological

[DOI: 10.22034/jast.25.3.535]

features were measured. Stem height and seed yield were measured in three replicates per ½ plot. The plant height was measured before harvesting, from the soil surface to the top level of the plant. The leaves and stems dry biomass were determined after air-drying when a constant weight was obtained. The Harvest Index (HI) was calculated as the ratio of harvested seed yield to total Shoot Dry Weight (SDW) (Unkovich *et al*., 2010).

Statistical Analysis

Statistical analysis of data was performed using Minitab software (version 17.3.1) based on RSM analysis. In both years, similar results were obtained. Therefore, data were pooled, and pooled data (2018 and 2019) are reported in this article.

RESULTS

Model Fitting

As the first step, proper selection of biochar and levels of irrigation range were taken into account. The biochar doses ranging from 0–2 kg m^{-2} were considered for CCD. Higher doses were not realistic from a farmer's perspective, and are not cost-effective, so, it was not included (Pandit *et al*., 2018). The irrigation treatment range was created based on 50 to 120% crop water requirements (Caliskan *et al*., 2017). The average of the highest and lowest limits of the variables was given by Minitab software. Results from the statistical analysis by CCD are given in Table 4. Details of the RSM quadratic model and P-values of the relations (linear, square, and 2-way-interaction) between variables for the measured factors (total Chlorophyll, total phenol, soluble carbohydrates, biological yield, height, seed yield, and HI) are given in Table 4. The results of CCD were analyzed based on the F test ($P< 0.01$) for all of the variables (Table 4). An insignificant lack of fit for measured parameters indicates reasonable data analysis (Table 4). The determination coefficient of total phenol, soluble carbohydrates, biological yield, and seed yield were 95.65, 94.80, 99.54, and 96.43, respectively, indicating that the CCD model explains the maximum diversity ratio for these variables. The lower amount of R^2 for total Chlorophyll, plant height, and HI (81.27, 62.39, and 86.55) indicates that additional variables other than biochar and irrigation affect these factors. Also, Figure 1 shows that the actual experimental data and the predicted values obtained from the model are well matched, which validates the regression model.

Figures 2-4 demonstrate the pooled results of chlorophyll, total phenol, and total soluble carbohydrates under different biochar and irrigation levels, obtained in 2018 and 2019.

As Figure 2 shows, the total chlorophyll concentration decreases significantly when soil biochar amendment is elevated. It also shows a linear relationship with the level of irrigation. Statistical analyses revealed that a 2-way-interaction between biochar and irrigation levels shows an insignificant ($P> 0.01$) correlation with total chlorophyll concentration.

The data in Figure 3 clearly show that the total phenol concentration decreases appreciably when soil biochar amendment is elevated. Also, outcomes indicate that reduction in irrigation levels results in higher total phenol concentration. Based on data analysis, simultaneous use of higher levels of biochar and irrigation levels had no significant effect on total phenol concentration.

Figure 4 shows the total soluble carbohydrate concentration. A significant relationship ($P= 0.000$) was obtained between the total concentration of dissolved carbohydrates and biochar. The total soluble carbohydrate concentration slightly decreases and grows in biochar amendment up to 1 kg.m^{-2}. Then, a gentle rise was observed when the biochar level reached 2 kg m^{-2}. Total soluble carbohydrates face a

[DOI: 10.22034/jast.25.3.535]

Table 4. P-value obtained from Central Composite Design (CCD) analysis for each measured factor.

Source	df	Total chlorophyll	Total phenol	Soluble carbohydrates	Biological yield	Height	Seed yield	Harvest Index (HI)
Model	6	0.000**	0.000 **	0.000 **	0.000 **	0.002 **	0.000 **	0.000 **
Blocks	1	0.515 ns	0.001 **	0.038 *	0.000 **	0.045 *	0.890 ns	0.096 ns
Linear	2	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **
Biochar	1	0.000 **	0.000 **	0.000 **	0.000 **	0.849 ns	0.000 **	0.021 *
Irrigation	1	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **
Square	2	0.117 ns	0.000 **	0.000 **	0.000 **	0.324 ns	0.012 *	0.000 **
Biochar×Biochar	1	0.050 *	0.000 **	0.000 **	0.239 ns	0.698 ns	0.008 **	0.012 *
Irrigation×Irrigation	1	0.859 ns	0.000 **	0.000 **	0.000 **	0.232 ns	0.018 *	0.000 **
2-Way interaction	1	0.192 ns	0.312 ns	0.000 **	0.351 ns	0.358 ns	0.978 ns	0.074 ns
Biochar×Irrigation	1	0.192 ns	0.312 ns	0.000 **	0.351 ns	0.358 ns	0.978 ns	0.074 ns
Error	19	-	-	-	-	-	-	-
Lack-of-fit	11	0.055 ns	0.094 ns	0.774 ns	0.136 ns	0.886 ns	0.313 ns	0.716 ns
Pure error	8	-	-	-	-	-	-	-
Total	25	-	-	-	-	-	-	-
R^2	-	81.27	95.65	94.80	99.54	62.39	96.43	86.55

[ns] Non-significant; * Significant at 5% level, ** Significant at 1% level.

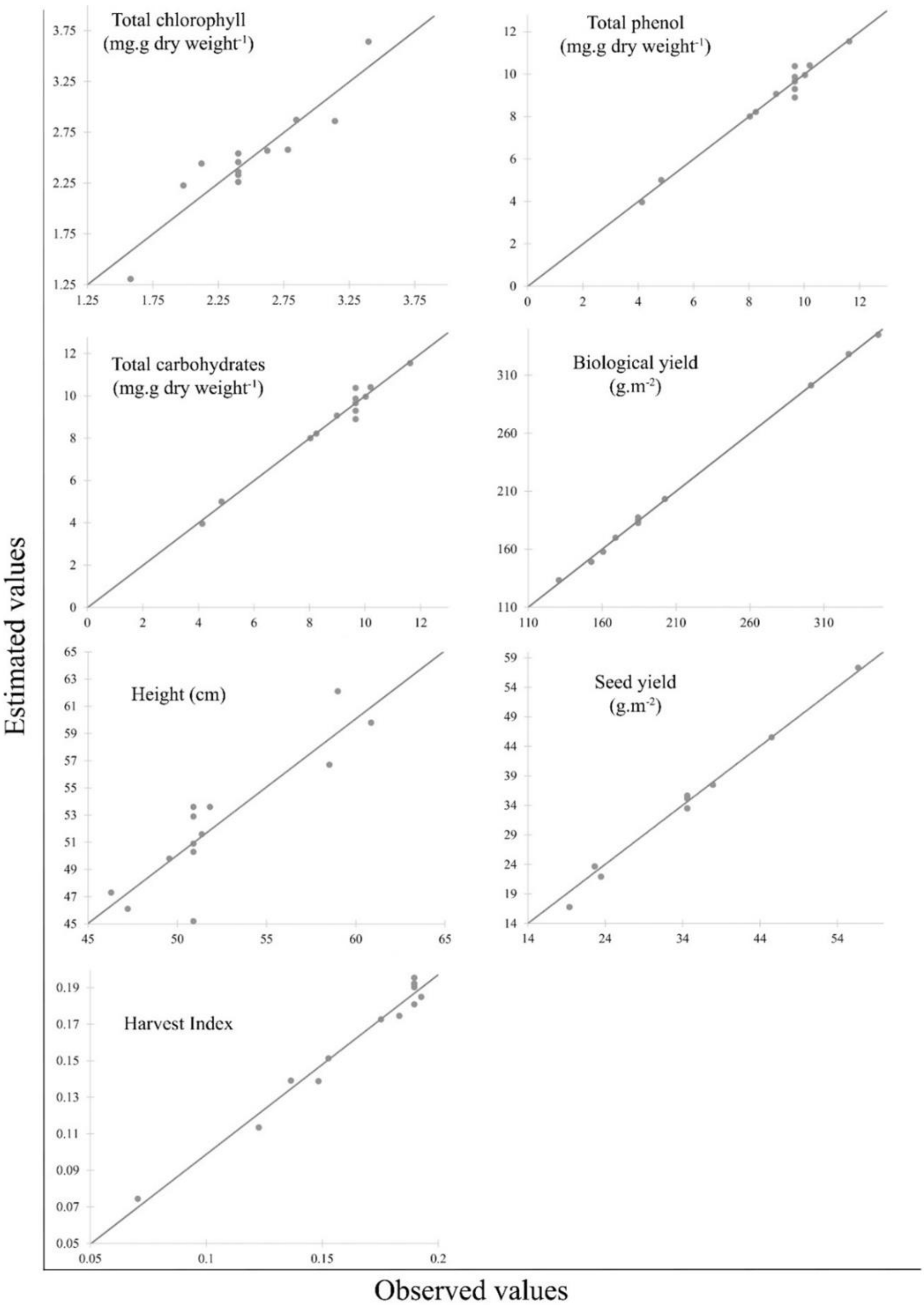

Figure 1. Comparison of estimated and observed values of total chlorophyll, total phenol, total soluble carbohydrates, biological yield, height, seed yield, and harvest index by 1:1 line.

[DOI: 10.22034/jast.25.3.535]

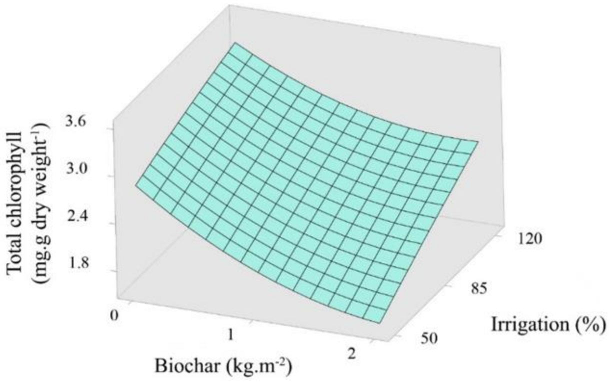

Figure 2. Total chlorophyll response to irrigation and biochar. Total chlorophyll concentration shows a linear relationship with irrigation levels and a reverse relationship with biochar amendment.

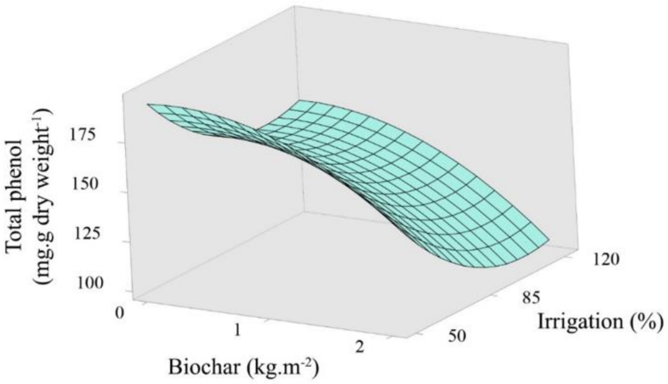

Figure 3. Total phenol concentration, response to irrigation, and biochar. Phenol concentration shows a reverse relationship with both irrigation levels and biochar amendment.

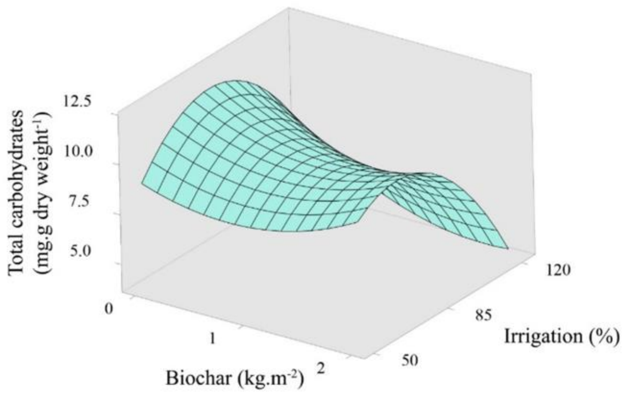

Figure 4. Total soluble carbohydrates response to irrigation and biochar. Biochar amendment in soil less than 1 kg m^{-2} negatively affects total soluble carbohydrates concentration. In contrast, the higher amount of biochar in soil increases total soluble carbohydrates concentration. Irrigation levels up to 85% increase total soluble carbohydrate concentration, while higher levels negatively affect total carbohydrate concentration.

[DOI: 10.22034/jast.25.3.535]

plunge when basil plants are treated with high Irrigation levels (I> 85%).

Experimental results and statistical analysis show a negative and significant 2-way-interaction between soluble carbohydrates concentration and biochar×irrigation levels. This result indicates that as the irrigation level increases, it reduces the positive effect of biochar on soluble carbohydrates concentration.

Morphological Criteria

The growth of basil plants was significantly increased by adding biochar to the soil. Also, higher irrigation levels enhanced vegetative growth in all treatments. As shown in Figure 5, biochar slightly increases the amount of biomass; moreover, higher irrigation levels significantly improve SDW. The level of irrigation similarly affects the SDW. As Figure 5 shows, the highest basil productivity was obtained from the highest Irrigation (I_3) treatment (340 g DW m^{-2}), while the lowest SDW was gained in the I_2 and I_1 treatments by 149 and 130 g DW m^{-2}, respectively. The maximum biological yield was recorded at the highest biochar and irrigation treatments.

There are minimal reports about using biochar and irrigation levels simultaneously.

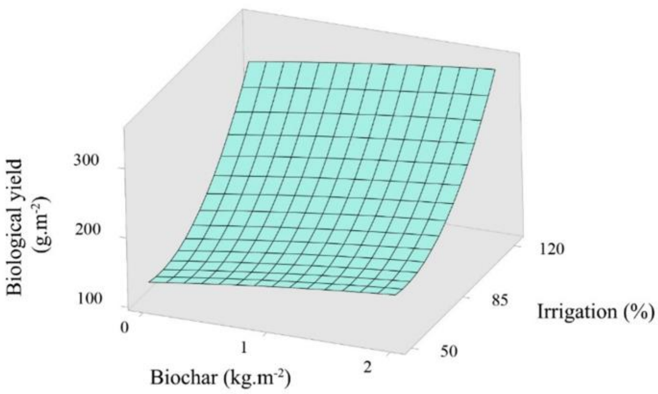

Figure 5. Biological yield response to irrigation and biochar. Both biochar and irrigation levels have a significant and positive effect on SDW.

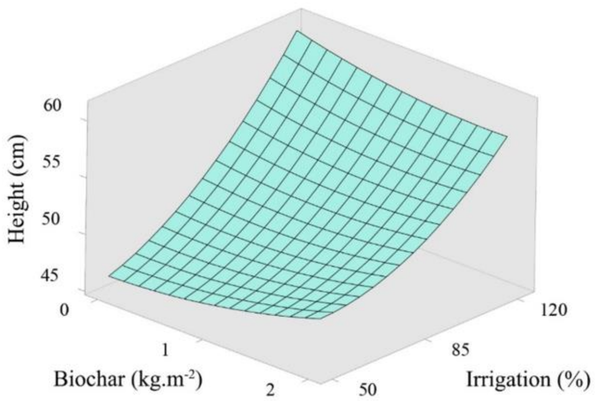

Figure 6. Height response to irrigation and biochar. Biochar amendment does not have a significant effect on plant height. However, irrigation level has a significant positive effect on plant height.

[DOI: 10.22034/jast.25.3.535]

However, based on our results, it has been found that biochar in combination with irrigation has no significant effect (P= 0.351) on dry biomass weight. Treated plots with high levels of biochar addition produce a high dry weight only when irrigation levels are maximum.

Although plant height shows a slight increase in biochar-amended plots (Figure 6), statistical analysis reveals no significant relationship between plant height and the amount of biochar in soil (P= 0.849). Figure 6 illustrates that plant height is significantly affected by the irrigation levels. Although a maximum average of plant height was obtained at I_3 and a higher level of biochar amendment, by about 64 cm, there is no significant statistical relationship between plant height and interaction between biochar and irrigation similar to biological yield.

Seed yield has also been reported as another growth factor. Figure 7 shows that seed yield increases significantly when biochar and irrigation levels are increased. Nevertheless, there is no noteworthy relation between 2-way interactions (P= 0.978).

Harvest Index (HI) is reported in Figure 8. HI increases by a 95% confidence interval when biochar addition to the soil increases. Also, higher irrigation treatments lead to an increase in HI. Incidentally, HI has not been affected by the interaction between biochar and irrigation treatments.

DISCUSSION

Biochar efficiently absorbs various

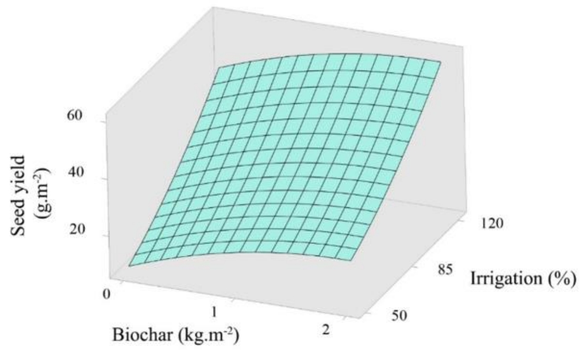

Figure 7. Seed yield response to irrigation and biochar. There is a significant positive relationship between biochar and irrigation levels and seed yield.

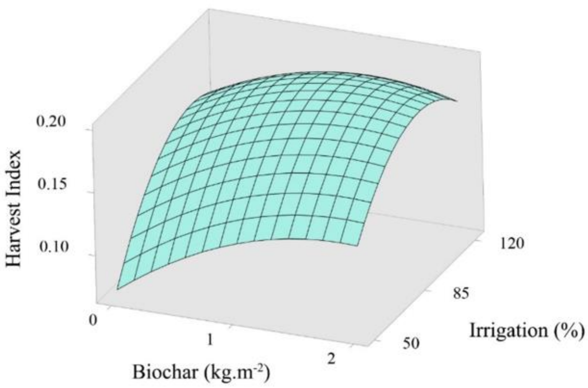

Figure 8. Harvest index response to irrigation and biochar. Harvest Index (HI) significantly rises when biochar in soil and irrigation levels increase.

organic and inorganic contaminants present in the soil because biochar is highly porous, which gives it plenty of room to absorb water and nutrients (Foereid, 2015). This phenomenon causes a reduction in the bioavailability and phytotoxicity of metals from soils (Zhang *et al*., 2013). Nutrients in the soil may have been too absorbed by the biochar at the highest concentration of biochar, making it difficult for the plant to absorb. There are shreds of evidence that soil pH increases when biochar is added to the soil, reducing metal uptake by plants (Kim *et al*., 2015; Nair and Carpenter, 2016). All these may cause a reduction in iron (Fe) and copper (Cu) in the basil plant. Limitation in Fe availability in shoot may cause a reduction in chlorophyll since Fe is an essential element for chlorophyll synthesis (Dickson *et al*., 2016), so, the chlorophyll concentration decreases as biochar amendment rises. The same trend was revealed by other researchers (Chrysargyris *et al*., 2020; Huang *et al*., 2020).

On the other hand, when Cu enters the plant cells, it disturbs the dynamic equilibrium between detoxification and Reactive Oxygen Species (ROS) production. In such a situation, plants tend to produce non-enzymatic molecules such as phenolic compounds as a defense mechanism to avoid oxidative stress and scavenge ROS (Pérez-López *et al*., 2014). Therefore, biochar amendment in soil may reduce Cu accumulation in basil leaves, reducing oxidative stress. Consequently, total phenol concentration decreases when more biochar is added to the soil. Others obtained similar observations (Chrysargyris *et al*., 2020; Ding *et al*., 2020).

Changes in soluble carbohydrate concentration detected in basil leaves grown in soils treated with biochar can be due to the physicochemical properties of biochar. The same observation was reported in similar research (Pirbalouti *et al*., 2017). Some soil criteria like water content and nutrient availability are improved by biochar. The accessibility of essential nutrients such as N, P, K, and Ca, which promote root growth, improves using biochar. These conditions may result in higher SDW (Sun *et al*., 2014). Evidence (Sohi *et al*., 2009) shows that biochar can improve soil physical properties (acquisition and retention of water-soluble nutrients), improving crop production. Lehmann *et al*. (2006) reported a positive effect on plant growth by adding biochar to soil up to 5.5 kg m^{-2}. Others reported the same trend in basil growth under various conditions (Pandey *et al*., 2016; Ding *et al*., 2020). Some research confirmed that biochar efficacy on crop biomass depends not only on the biochar properties but also on the crop species (Nobile *et al*., 2020).

HI can be used as a factor to report reproductivity efficiency. The amount of carbon allocated in the plant can affect HI (Unkovich *et al*., 2010). As seed yield and SDW directly correlate with biochar, the amount of biochar significantly improves HI.

A reduction in chlorophyll under drought conditions is a common phenomenon and it might be due to the subtraction of the main chlorophyll pigment complexes that synthesize by encoding the cab gene family (Allakhverdiev *et al*., 2003). Also, water deficit conditions may ruin some pigment-protein complexes that protect the photosynthetic apparatus or detriment chloroplast lipids and proteins by oxidation (Lai *et al*., 2007). All these situations can reduce chlorophyll concentration. A similar trend is reported in other literature (Pirbalouti *et al*., 2017). Phenolic compounds play an essential role in overcoming stress conditions and adapting plants to the environment (Lattanzio *et al*., 2008). Therefore, when water deficit declines, total phenolic concentration increases. The same trend in various researches has been reported on basil (Pirbalouti *et al*., 2017) and other plants like Lemon balm (*Melissa officinalis* L.) (Manukyan, 2011) and *Rehmannia glutinosa.* (Chung *et al*., 2006).

[DOI: 10.22034/jast.25.3.535]

Carbohydrates are the leading organic soluble components for plant osmotic adjustment. Carbohydrate concentration positively correlated with irrigation deficit. Total carbohydrate concentration increases in leaves, reducing leaf osmotic potential and maintaining turgor (Pirbalouti *et al*., 2017). This is an imperative adaptive mechanism in plants subjected to drought conditions. Several researchers reported similar results (Al Abbasy *et al*., 2015; Al-Huqail *et al*., 2020).

Higher morphological properties (including biological yield, height, seed yield, and HI) are expected when basil plants face higher irrigation treatments (Ekren *et al*., 2012; Bekhradi *et al*., 2015; Pandey *et al*., 2016). In addition, It is a fact that turgor decreases by reducing irrigation water, which is an essential factor for growth and cell development (Hsiao, 1973). Also, irrigation encourages vegetative growth. This phenomenon was reported in other plant species such as rice, wheat, and eggplant (Wang *et al*., 2012; Ebrahimi *et al*., 2021).

CONCLUSIONS

The results revealed that high irrigation and biochar levels would be useful in maximizing morphological properties of basil such as seed yield and harvest index. Also, the higher levels of irrigation treatments always lead to higher and more significant results in both measured properties (morphological and biochemical). Nevertheless, higher levels of biochar amendment in soil cause a significant decrease in total chlorophyll and total phenol. At the same time, it has a positive effect on vegetative properties.

On the other hand, no significant effect was observed in the 2-way-interaction between biochar×irrigation levels and the measured properties, except for total soluble carbohydrates. In conclusion, growing basil under high levels of biochar and irrigation treatment would be valuable to modify the amount of critical biochemical compounds. Also, based on our results, irrigation levels always play a critical role in basil properties, other than biochar content.

ACKNOWLEDGEMENTS

The authors want to thank the financial support of the project by Vice President for Research and Technology (grant number 47028), Ferdowsi University of Mashhad, Iran.

REFERENCES

1. Ainsworth, E. A. and Gillespie, K. M. 2007. Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent. *Nat. Protoc.,* **2(4):** 875-877.
2. Al Abbasy D. W., Pathare, N., Al-Sabahi, J. and Khan, Sh. A. 2015. Chemical Composition and Antibacterial Activity of Essential Oil Isolated from Omani Basil (*Ocimum basilicum* Linn.). *Asian Pac. J. Trop. Dis.,* **5(8):** 645-649.
3. Al-Huqail A., El-Dakak, R. M., Sanad, M. N., Badr, R. H., Ibrahim, M. M., Soliman, D. and Khan, F. 2020. Effects of Climate Temperature and Water Stress on Plant Growth and Accumulation of Antioxidant Compounds in Sweet Basil (*Ocimum basilicum* L.) Leafy Vegetable. *Scientifica*, **2020(3808909):** 1-12.
4. Allakhverdiev S. I., Hayashi, H., Nishiyama, Y., Ivanov, A. G., Aliev, J. A., Klimov, V. V., Murata, N. and Carpentier, R. 2003. Glycinebetaine Protects the D1/D2/Cytb559 Complex of Photosystem II against Photo-Induced and Heat-Induced Inactivation. *J. Plant Physiol.,* **160**(1): 41-49.
5. Allen, R. G., Pereira, L. S., Raes, D. and Smith, M. 1998. *Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements*. FAO Irrigation and Drainage Paper 56, Fao, Rome **300(9):** D05109.
6. Aslan, N. 2007. Application of Response Surface Methodology and Central Composite Rotatable Design for Modeling the Influence of Some Operating Variables

of a Multi-Gravity Separator for Coal Cleaning. Fuel, **86(5-6):** 769-776.
7. Bekhradi, F., Luna, M. C., Delshad, M., Jordan, M. J., Sotomayor, J. A., Martínez-Conesa, C. and Gil, M. I. 2015. Effect of Deficit Irrigation on the Postharvest Quality of Different Genotypes of Basil Including Purple and Green Iranian Cultivars and a Genovese Variety. *Postharvest Biol. Technol.,* **100**: 127-135.
8. Caliskan, O., Kurt, D., Temizel, K. E. and Odabas, M. S. 2017. Effect of Salt Stress and Irrigation Water on Growth and Development of Sweet Basil (*Ocimum basilicum* L.)." *Open Agric. J.* **2(1):** 589-594.
9. Chrysargyris, A., Prasad, M., Kavanagh, A. and Tzortzakis, N. 2020. Biochar Type, Ratio, and Nutrient Levels in Growing Media Affects Seedling Production and Plant Performance. *J. Agron.,* **10(9):** 2-21.
10. Chung, I. M., Kim, J. J., Lim, J. D., Yu, C. Y., Kim, S. H. and Hahn, S. J. 2006. Comparison of Resveratrol, SOD Activity, Phenolic Compounds and Free Amino Acids in Rehmannia Glutinosa under Temperature and Water Stress. *Environ. Exp. Bot.,* **56(1):** 44-53.
11. Dere, S., Gunes, T. and Sivaci, R. 1998. Spectrophotometric Determination of Chlorophyll-A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents. *Turk. J. Bot.,* **22(1):** 13-18.
12. Dickson, R. W., Fisher, P. R., Padhye, S. R. and Argo, W. R. 2016. Evaluating Calibrachoa (*Calibrachoa×hybrida* Cerv.) Genotype Sensitivity to Iron Deficiency at High Substrate pH. *HortSci.,* **51(12):** 1452-1457.
13. Ding, Z., Zhou, Zh, Lin, X., Zhao, F., Wang, B., Lin, F., Ge, Y. and Eissa, M. A. 2020. Biochar Impacts on NH3-Volatilization Kinetics and Growth of Sweet Basil (*Ocimum basilicum* L.) under Saline Conditions. *Ind. Crops. Prod.,* **157**: 112903.
14. Ebrahimi, M., Souri, M. K., Mousavi, A. and Sahebani, N. 2021. Biochar and Vermicompost Improve Growth and Physiological Traits of Eggplant (*Solanum melongena* L.) under Deficit Irrigation. *Chem. Biol. Technol. Agric.,* **8(1):** 1-14.
15. Ebrahimi, M., Souri, M. K., Mousavi, A. and Sahebani, N. 2021. Can Vermicompost and Biochar Control *Meloidogyne javanica* on Eggplant?. *J. Nematol.,* **23(9):** 1053-1064.
16. Ekren S., Ç. Sönmez, *et al*. 2012. The effect of Different Irrigation Water Levels on Yield and Quality Characteristics of Purple Basil (*Ocimum basilicum* L.). *Agric. Water Manag.,* **109**: 155-161.
17. Foereid, B. 2015. Biochar in Nutrient Recycling: The Effect and Its Use in Wastewater Treatment. *Open J. Soil Sci.,* **5(02):** 39.
18. Ghasemi Pirbalouti, A., Malekpoor, F., Salimi, A. and Golparvar, A. 2017. Exogenous Application of Chitosan on Biochemical and Physiological Characteristics, Phenolic Content and Antioxidant Activity of Two Species of Basil (*Ocimum ciliatum* and *Ocimum basilicum*) under Reduced Irrigation. *Sci. Hortic.,* **217**: 114-122.
19. Gomez, L., Rubio, E. and Auge, M. 2002. A New Procedure for Extraction and Measurement of Soluble Sugars in Ligneous Plants. *J. Sci. Food Agric.,* **82(4):** 360-369.
20. Hsiao, T. C. 1973. Plant Responses to Water Stress. *Annu. Rev. Plant Physiol.,* **24(1):** 519-570.
21. Huang, L., Gu, M., Yu, P., Zhou, C. and Liu, X. 2020. Biochar and Vermicompost Amendments Affect Substrate Properties and Plant Growth of Basil and Tomato. *J. Agron.,* **10(2):** 224.
22. Izadiyan P. and Hemmateenejad, B. 2016. Multi-Response Optimization of Factors Affecting Ultrasonic Assisted Extraction from Iranian Basil Using Central Composite Design. *Food Chem.,* **190**: 864-870.
23. James, L. G. 1988. Principles of Farm Irrigation Systems Design. John Wiley and Sons Limited, Chichester.
24. Kim H. -S., K. -R. Kim, Kim, H. –J., Jung-Hwan Yoon, J. –H., Yang, J. E., Ok, Y. S., Owens, G. and Kye-Hoon Kim, K. –H. 2015. Effect of Biochar on Heavy Metal immobilization and Uptake by Lettuce (*Lactuca sativa* L.) in Agricultural Soil. *Environ. Earth Sci.,* **74(2):** 1249-1259.
25. Lai Q. -X., Bao, Z. -Y., Zhu, Z. –J., Qiong-qiu Qian, Q. –Q. and Mao, B. –Z. 2007. Effects of Osmotic Stress on Antioxidant

[DOI: 10.22034/jast.25.3.535]

Enzymes Activities in Leaf Discs of P SAG12-IPT Modified Gerbera. *J. Zhejiang Univ. Sci. B.,* **8(7):** 458-464.

26. Lattanzio V., Kroon, P. A., Quideau, S. and Treutter, D. 2008. Plant Phenolics: Secondary Metabolites with Diverse Functions. In: "Recent Advances in Polyphenol Research", (Eds.): Daayf, F. and Lattanzio, V. Wiley-Blackwell, Oxford, UK, Ames, Iowa, **1:** 1-35.
27. Lehmann, J., Gaunt, J. and Rondon, M. 2006. Bio-Char Sequestration in Terrestrial Ecosystems: A Review. *Mitig. Adapt. Strateg. Glob. Chang.,* **11(2):** 403-427.
28. Leng, L., Huang, H., Li, H., Li, J. and Zhou, W. 2019. Biochar Stability Assessment Methods: A Review. *Sci. Total Environ.,* **647**: 210-222.
29. Manukyan, A. 2011. Effect of Growing Factors on Productivity and Quality of Lemon Catmint, Lemon Balm and Sage under Soilless Greenhouse Production: I. Drought Stress. *Open Access J. Med. Aromat. Plants,* **5(2):** 119-125.
30. Nair, A. and Carpenter, B. 2016. Biochar Rate and Transplant Tray Cell Number Have Implications on Pepper Growth during Transplant Production. *Horttechnology,* **26(6):** 713-719.
31. Nobile, C., Denier, J. and Houben, D. 2020. Linking Biochar Properties to the Biomass of Basil, Lettuce, and Pansy Cultivated in Growing Media. *Sci. Hortic.,* **261**: 109001.
32. Pandey, V., Patel, A. and Patra, D. D. 2016. Biochar Ameliorates Crop Productivity, Soil Fertility, Essential Oil Yield and Aroma Profiling in Basil (*Ocimum basilicum* L.). *Ecol. Eng.,* **90**: 361-366.
33. Pandit, N. R., Mulder, J., Hale, S. E., Zimmerman, A. R., Pandit, B. H. and Cornelissen, G. 2018. Multi-Year Double-Cropping Biochar Field Trials in Nepal: Finding the Optimal Biochar Dose through Agronomic Trials and Cost-Benefit Analysis. *Sci. Total Environ.,* **637**: 1333-1341.
34. Pérez-López, U., Pinzino, C., Quartacci, M. F., Ranieri, A. and Sgherri, C. 2014. Phenolic Composition and Related Antioxidant Properties in Differently Colored Lettuces: A Study by Electron Paramagnetic Resonance (EPR) Kinetics. *J. Agric. Food Chem.,* **62(49):** 12001-12007.
35. Purushothaman, B., PrasannaSrinivasan, R., Suganthi, P., Ranganathan, B., Gimbun, J. and Shanmugam, K. 2018. A Comprehensive Review on *Ocimum basilicum*. *J. Nat. Remedies,* **18(3):** 71-85.
36. Singh, Y., Jat, H. S. and Jat, S. L. 2021. Wheat Productivity Enhancement through Climate Smart Practices. Chapter 15. In: "*Improving Cereal Productivity Through Climate Smart Practices*". Woodhead Publishing Series in Food Science, Technology and Nutrition, PP. 255-268.
37. Sohi, S., Lopez-Capel, E., Krull, E. and Bol, R. 2009. Biochar, Climate Change and Soil: A Review to Guide Future Research. *CSIRO Land Water Sci. Rep.,* **5(09):** 17-31.
38. Sun, Z., Bruun, E. W., Arthur, E., de Jonge, L. W., Moldrup, P., Hauggaard-Nielsen, H. and Elsgaard, L. 2014. Effect of Biochar on Aerobic Processes, Enzyme Activity, and Crop Yields in Two Sandy Loam Soils. *Biol. Fertil. Soils,* **50(7):** 1087-1097.
39. Unkovich, M, Baldock, J. and Forbes, M. 2010. Chapter 5: Variability in Harvest Index of Grain Crops and Potential Significance for Carbon Accounting: Examples from Australian Agriculture. *Adv. Agron.,* **105:** 173-219.
40. Wang, J., Pan, X., Liu, Y., Zhang, X. and Xiong, Zh. 2012. Effects of Biochar Amendment in Two Soils on Greenhouse Gas Emissions and Crop Production. *Plant Soil,* **360(1-2):** 287-298.
41. Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N. S., Pei, J. and Huang, H. 2013. Using Biochar for Remediation of Soils Contaminated with Heavy Metals and Organic Pollutants. *Environ. Sci. Pollut. Res.,* **20(12):** 8472-8483.

[DOI: 10.22034/jast.25.3.535]

تأثیر تیمارهای بیوچار و آبیاری بر خصوصیات بیوشیمیایی و مورفولوژیکی ریحان (*Ocimum basilicum* L.) با استفاده از طرح مرکب مرکزی

چکیده

تاثیر سه سطح مختلف بیوچار (۰، ۱، ۲ کیلوگرم در متر مربع) همزمان با اعمال تیمارهای آبیاری (۵۰، ۸۵ و ۱۲۰ درصد تبخیر و تعرق گیاهی (ETc)) در دو سال متوالی (۲۰۱۸ و ۲۰۱۹) بر روی گیاه ریحان (*Ocimum basilicum* L.) مورد بررسی قرار گرفت. طرح مرکب مرکزی (CCD) به عنوان یک روش بهینه سازی تیمار های آزمایشی با ۱۳ تیمار استفاده شد. این مطالعه در مزرعه تحقیقاتی دانشگاه فردوسی مشهد انجام شد. اثرات این تیمارها بر ویژگی‌های بیوشیمیایی (کلروفیل کل، فنل کل و کربوهیدرات‌های محلول) و خصوصیات مورفولوژیکی (عملکرد بیولوژیکی، ارتفاع بوته، عملکرد دانه و شاخص برداشت) ارزیابی شد. سپس تمامی نتایج مورد تجزیه و تحلیل آماری قرار گرفت. نتایج نشان داد که استفاده از بیوچار در خاک تمامی خصوصیات بیوشیمیایی مورد مطالعه را کاهش داد. در این میان بیوچار موجود در خاک، خصوصیات مورفولوژیکی گیاه ریحان را تقویت کرد. همچنین گیاه ریحان بطور معنی داری به سطوح آبیاری پاسخ داد. سطوح بالای تیمارهای آبیاری باعث کاهش فنل کل و کربوهیدرات های محلول شد و سایر صفات اندازه گیری شده را افزایش داد. تجزیه و تحلیل آماری نشان داد که هیچ رابطه معنی داری بین برهمکنش دو طرفه (بیوچار×آبیاری) و صفات اندازه گیری شده به جز کربوهیدرات های محلول وجود ندارد.

[DOI: 10.22034/jast.25.3.535]