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Role of biochar soil amendment for alleviation of adverse effects of water stress on *Dimorphotheca ecklonis* plants

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Water is a key factor for plant growth and development. Plants need an adequate amount of soil moisture for their optimum growth and yield. It plays very important role in building up plant metabolism. Soil amendment is one of the important methods that use to overcome water stress conditions. Biochar has been the most amendment, which used due to its potential role in many fields such as increasing microbial activity, better crop yield, C sequestration and increasing soil carbon. In the present study, two field experiments were established at the Experimental Farm of El-Kassasin Horticultural Research Station, Ismailia Governorate, Egypt, during the two successive seasons of 2015/2016 and 2016/2017.The objective of this research was to determine the effect of three soil moisture levels (W1=50, W2=35 andW3= 20% depletion of the available soil water) and three amounts of biochar (Bio1= 0.5, Bio2= 1and Bio3=1.5 ton biochar/fed) on growth and flowering characters as well as some biochemical characters of *Dimorphotheca ecklonis* (cape marigold) plant. The results indicated that the highest significant increases in cape marigold growth, flowering characters, NPK contents and uptake and non-reducing sugars were observed under the combined effect of the highest soil moisture level and 1.5ton of biochar/ fed. Results indicate also that biochar could be used to enhance plant growth and flowering characters as well as improving soil properties especially under water stress conditions.

Keywords: Different soil moisture levels; *Dimorphotheca ecklonis,* Biochar application, Growth, Yield, NPK , Carbohydrates content.

INTRODUCTION

Water is essential for all living organisms, and it plays very important role in building up plant metabolism. Water quality and availability can be a limiting factor in plant development and growth. Water deficit created when insufficient irrigation water prevents a plant from completion of life cycle and normal growth. Depending on the extent and duration of drought stress, a range of plant processes occurring at molecular, cellular, biochemical and whole-plant levels may be altered (Manivannan et al., 2007).Drought is an abiotic stress that limits development and growth of plants by aggravating physiological disorders and reduces photosynthesis rate. It has most drastic effects on plant growth and productivity than any other environmental stresses. Water stress severity is expected to increase in the upcoming years due to changing climate conditions (Handmer et al., 2012). There is a wide opportunity for small landholders in a developing country to cultivate flower crops to increase profit margin (Younis et al., 2016). A different school of thought exists to cope with water stress conditions that includes; efficient irrigation systems, mulches, cultivars selection, and use of media having maximum water retention. Drought tolerance differ even amongst the member of the same species (Younis et al., 2017) therefore; screening of the most drought resistant plants is a realistic approach for maximum water use efficiency under changing climatic situations.

Dimorphotheca ecklonis DC. (Cape marigold) is well-known ornamental plants cultivated throughout the world. It is a half - hardy annual plant, which produces large, vividly colored flowers on top of long and slender stems. It belongs to family, Asteraceae (Compositae). It can grow up to 2 feet high and bears large, white flowers with a bluish center. Cape marigolds are loose mound heavily covered with flowers during cool seasons or in cool climates. Flowers are 3 to 4 inches in size its colure may be white, yellow, rose, or salmon. The reverse sides of the petals are colored in shades of blue or lavender. Cape marigolds are sometimes called Osteospermum, depending on which botanist classified closely related plants. Also called African daisy and star of the veldt, they are not closely related to true marigolds (Abdel-Wahid et al., 2005).

Biochar, a porous pyrogenic material, has been applied to soil systems and has improved both the chemical and physical characters of the soil. Biochar is a byproduct of gasification, from the thermo chemical decomposition of organic materials at high temperatures in the absence of oxygen. Biochar used as a soil conditioner in agriculture. Over time, the application of biochar can increase surface area, and soil fertility by increasing the cation exchange capacity as well as increase water retention that can reduce nutrient leaching from soils (Lehmann et al., 2006). Biochar have a substantial impact on the retention and release of PO4, NO3, and K in the soil (Altland and Locke, 2013). Biochar increase the soil pH in acid soils (Jeffery et al., 2015) and increase plant nutrient availability (Major et al., 2010). These factors may increase yields of horticultural crops, agricultural crops, and microbial mass (Jin, 2010). Using soil moisture measurements combined with a low cost substrate, which increases water-holding capacity may reduce the water requirement for high-value crops and mitigate nutrient and water leaching (Jahromi and Walker, 2018). The objective of this research was to provide a preliminary assessment of the effect of biochar amendment on growth, flowering characters, as well as some biochemical characters of cape marigold underwater stress conditions.

MATERIALS AND METHODS

Water Treatments:

The following three water treatments were applied throughout the entire growth period of the plant life:

W1= water stress maintained around 50% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level was reached.

W2= water stress maintained around 35% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level was reached.

W3= water level maintained around 20% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level was reached.

These drip irrigation treatments applied after 15 days from transplanting and the field capacity was measured before each irrigation to restore the soil to the appropriate moisture regime by adding a calculated amount of water. The general principal stated by Boutraa and Sanders (2001) was used for the water treatment application.

2. Biochar treatments:

The following biochar treatments were used during the experiment:

Bio1= 0.5 ton biochar/ fed.

Bio2= 1 ton biochar/ fed.

Bio3=1.5 ton biochar/fed.

3. Plant cultivation and watering procedure:

Two field experiments were established at the Experimental Farm of El-Kassasin Horticultural Research Station, Ismailia Governorate, Egypt, during the two successive seasons of 2015/2016 and 2016/2017.The experimental unit area was 21 m^2 (4.2 x 5 m) and each unit contained six rows with 5 m length and 70 cm width for each, four inner rows were possessed for flowering determination, whereas the two outer rows were for determination of plant growth characters. Two rows were left between the experimental plots of irrigation treatment to avoid the overlapping. All possible combinations between all treatments were tested. The normal agricultural practices of *Dimorphotheca* plants under drip irrigation system of this area were followed according to the recommendations of Agriculture Ministry. Seeds were obtained from Agriculture Research Center, Giza, Cairo, and directly sown in nursery on 15th September in foam trays, then the seedlings were transplanted (with 3-4 true leaves about 40 days) on25th October in both growing seasons. The distance between the seedlings was 30 cm. The mechanical and chemical analyses of the soil were determined according to the standard method described by Klute (1986) and shown in Table1.

Design of the Experiment

This experiment included 9 treatments which were the combination between three soil moisture levels (50,35 and 20% depletion of the available soil water) and three biochar treatments (0.5,1 and 1.5 ton biochar/fed).Treatments were arranged in a split plot design with three replicates; different soil moisture levels were assigned at random in the main plots, while sub-plots were devoted to the different biochar treatments.

Data Collection

A. Growth measurements

A random sample of six plants from each plot was taken after 90 days from transplanting and the following data were recorded: plant height (cm), number of leaves/plant, number of stems/plant and dry weight of leaves, stems and whole plant (g). A random sample of other six plants from each plot was taken and dried at 70 ^{0}C till constant weight and the dry weight of aerial parts (stem + leaves and whole plant) was determined.

B. Flowering characters:

At the flowering stage a random sample of six plants were taken at random to estimate the following characters:

1- Number of days to flowering
2- Flower ray length (cm)
3- Flower ray width (mm)
4- Flower disk diameter (mm)
5-No of ray /inflorescence
6-Peduncle length (cm)
7-Peduncle diameter (mm)
8-No of inflorescences/plant
9-Inflorescence fresh weight (g)
10-Inflorescence diameter (cm)

C. NPK contents and uptakes:

Total nitrogen, phosphorus and potassium were determined in dry weight of whole plant according to A.O.A.C. (2005), and NPK uptake were calculated as NPK contents on dry weight basis (Kg/fed.)

D. Sugar contents:

Total sugar, reducing and non-reducing sugar were determined in fresh inflorescence samples after harvest, calorimetric according to the methods described by (**A.O.A.C., 2005**).

Table 1: Physical and chemical analysis of the experimental soil

(a) Physical

Particle size distribution				Textural class	Ca Co_3%	O.M%
Coarse sand %	Fine sand %	Silt %	Clay %			
5.38	78.53	10.08	6.01	Sandy	1.20	0.80

(b) Chemical

pH*	F. C. %	Ec* dsm^{-1}	Sp	Ion concentration in paste extract ($mmol_c/l$)						Available** (mg/kg)		
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO^{-3}	So^{-4}	N	P	K
8.1	12	0.83	27	3.90	2.70	1.85	0.55	5.85	3.15	58.60	9.50	190.51

Samples of the soil were obtained from 25 cm soil surface.
* pH in paste, and EC in paste extract.
** N: K_2SO_4 extract, P: Na-bicarbonate extract, K: NH_4OAc extract.

Statistical analysis

The collected data were subjected to statistical analysis of variance using the normal (F) test and the means separation were compared by using Least Significant Difference (LSD) at 5% level according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Morphological characters:

Data dealing with the morphological characters of *Dimorphotheca ecklonis* plant includes mean values of; plant height, number of stems/plant and number of leave/ plant as affected by water stress and different biochar amounts during the two growing seasons are presented in (Table 2).Obtained data demonstrated that increasing soil moisture level from W1to W3 caused significant increases in the previously mentioned growth parameters of *Dimorphotheca ecklonis* plant, where the highest growth records were observed in plants grown under the highest soil moisture level W3. While, the lowest means were found under W1 treatment in both growing seasons. These results are in agreement with those obtained by El-Juhany and Aref (2005) who recorded decrease in the number of leaves of *Conocarpus* species under drought conditions. In addition, (Kanwal et al., 2012) reported decrease in plant height under severe water stress conditions. (Riaz et al., 2016) obtained similar results, where they recorded significant decline in lateral branches of *Conocarpus erectus* with higher levels of water stress. This corresponds with the findings of (Younis et al. 2017) who indicated that under severe drought conditions, cell elongation may suppressed due to water flow interruption from the xylem to elongating cells. They added also that growth reduction in *Abelmoschus esculentus* under drought conditions could be linked with the decrease in photosynthesis rate, or due to stomatal closure as the early effect of water stress in leaves. Other possible reason for the reduction in growth characters under water deficit condition is related with more leaf senescence (Bhatt and Rao 2005).

Data in Table 2 also showed that, when different amounts of biochar were introduced into the soil, the response of *Dimorphotheca ecklonis* plant began to change. When the volume of biochar was increased from 0.5 to 1.5 ton, a significant increase in growth of cape marigold plant was observed. Comparing the effectiveness of different biochar doses showed that the greatest growth means were obtained under Bio3 treatment and with significant difference, followed by Bio2 treatment where the difference between the treatments was insignificant and the lowest means appeared in Bio1 treatment in the two growing seasons. The positive effect of biochar treatment could be attributed to the increase in the soil organic matter content and EC and to the decrease in soil pH (Luo et al., 2017).In addition, biochar application could improve the plant growth by improving the soil biological activity and physicochemical properties (Pandit et al., 2018).They stated that biochar caused alleviation of moisture stress through improving soil water retention, thus increasing plant growth and development.

Under all soil moisture levels, there was less increases in growth parameters of stressed cape marigold plants with 0.5-ton biochar as compared to the plants treated with the highest dose of biochar (1.5 ton). The data of interaction figured out also that W3XBio3 treatment proved to be the most effective treatment in increasing the growth parameters of cape marigold plant compared to the other treatments in both growing seasons. Followed by W3XBio2 treatment where the difference between the two treatments was mostly insignificant. Similarly, Masinde et al. (2006) pointed out that under 35 and70%depletionof soil moisture levels; 5kg biochar was found to be having enough moisture to increase both transpiration rate and leaf area. Moreover, (Ippolito et al., 2012) illustrated that 3-7% of plant moisture content was increased due to 2% addition of biochar that further accelerate photosynthesis rate as well. Similar observations were recorded by Artiola et al., 2012; Akhtar et al., 2014; Batool et al., 2015; Paneque et al. ,2016 and Kanwal et al., 2018.

Dry weights:

Results obtained on dry weights of different plant parts depicted that the dry weights of *Dimorphotheca ecklonis*stems, leaves, and the whole plant were reduced significantly by increasing drought stress level or by decreasing soil moisture level (Table3). Plant gained the maximum dry weights under W3 treatment compared with other treatments and with significant differences. While, W1 treatment showed the highest reduction in plant dry weights compared to the other two treatments.

Table 2: Effect of different soil moisture levels, biochar treatments and their interactions on growth parameters of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017seasons.

Characters / season		Plant height (cm) 1st	Plant height (cm) 2nd	No of stems/plant 1st	No of stems/plant 2nd	No of leaves /plant 1st	No of leaves /plant 2nd
Effect of different soil moisture levels							
W1		52.15	53.32	16.85	17.27	92.71	92.84
W2		65.25	68.72	22.09	22.51	111.37	114.89
W3		77.77	77.10	24.74	25.51	142.80	146.89
$LSD_{0.05}$		4.21	3.09	2.75	3.88	5.14	4.61
Effect of biochar treatments							
Bio1		61.67	63.81	19.80	20.46	109.08	110.93
Bio2		65.45	66.33	21.29	21.74	115.83	119.76
Bio3		68.06	68.99	22.59	23.09	121.97	123.93
$LSD_{0.05}$		3.22	4.32	1.97	2.87	6.21	5.64
Effect of interaction							
W1	Bio1	50.45	51.32	15.11	15.48	88.47	85.32
	Bio2	52.69	53.32	17.24	17.21	92.21	93.45
	Bio3	53.32	55.32	18.20	19.11	97.45	99.74
W2	Bio1	60.21	65.79	20.66	21.49	104.33	108.32
	Bio2	65.42	68.94	22.29	22.99	111.06	116.34
	Bio3	70.13	71.43	23.32	23.04	118.72	120.01
W3	Bio1	74.34	74.32	23.64	24.41	134.44	139.14
	Bio2	78.24	76.74	24.33	25.01	144.21	149.48
	Bio3	80.72	80.23	26.24	27.11	149.74	152.04
$LSD_{0.05}$		5.17	4.87	3.47	3.28	4.99	5.07

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Table 3: Effect of different soil moisture levels, biochar treatments and their interactions on dry weights of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017 seasons.

Characters / season		Dry weight of stems (g)		Dry weight of leaves (g)		Dry weight of plant (g)	
		1st	2nd	1st	2nd	1st	2nd
Effect of different soil moisture levels							
W1		18.58	19.30	73.92	74.01	92.50	93.31
W2		24.36	25.00	83.49	81.99	107.85	106.99
W3		26.81	28.06	87.65	94.07	114.46	122.13
$LSD_{0.05}$		1.09	1.01	2.47	2.08	4.31	3.79
Effect of biochar treatments							
Bio1		22.46	23.47	80.26	79.19	102.72	102.66
Bio2		23.45	24.08	81.46	83.07	104.91	107.15
Bio3		23.85	24.81	83.33	87.81	107.19	112.62
$LSD_{0.05}$		0.41	0.61	1.64	2.06	2.04	1.99
Effect of interaction							
W1	Bio1	17.95	18.77	72.67	72.14	90.62	90.91
	Bio2	18.79	19.24	73.10	73.77	91.89	93.01
	Bio3	19.01	19.89	75.99	76.12	95.00	96.01
W2	Bio1	23.21	24.21	82.11	76.21	105.32	100.42
	Bio2	24.66	25.01	83.79	82.22	108.45	107.23
	Bio3	25.21	25.77	84.57	87.54	109.78	113.31
W3	Bio1	26.21	27.42	86.01	89.22	112.22	116.64
	Bio2	26.89	27.99	87.49	93.21	114.38	121.20
	Bio3	27.34	28.77	89.44	99.77	116.78	128.54
$LSD_{0.05}$		2.11	1.09	3.88	2.49	3.79	2.88

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Similar results were obtained by Basu et al., 2010; Rizwan et al., 2015; Guzman et al., 2016; Anjum et al., 2017; they all reported decrease in plant dry weights due to increase in water stress. The decrease in the dry weights of different plant parts may be due to the considerable decrease in photosynthesis, plant growth, and canopy structure as indicated by leaf senescence during drought stress conditions (Bhatt and Rao, 2005). Severe water stress may result in disturbance of metabolism, arrest of photosynthesis, and finally decrease in dry weights (Ali et al., 2017).

In addition, they illustrated that sever water stress affected the biomass accumulation and plant growth by inhibiting stomatal conductance and leaf expansion causing lower photosynthetic rates and plant dry weights.

Biochar application affected dry weights of cape marigold stems, leaves and whole plant significantly (Table 3). The obtained data illustrated that the biochar treatment caused progressive increasein dry weights of different studied plant parts significantly. The data in the same table also revealed that the influence of a rate 1.5-tonbiochar (W3 treatment)gave the highest significant dry weights production compared to the other biochar treatments. In this respect, our data are similar to those of Wang et al. 2016 and Ali et al., 2017. The increment in dry weights of cap.

marigold due to biochar application may be attributed to that the application of biochar not only improve the availability of nutrients but also promote vegetative growth by improving the photosynthetic pigments especially under water stress condition and this led to increase in plant dry weights (Lehmann et al., 2006).

Our study showed also that the biochar application improved the dry weights of cape marigold plant especially in drought-stressed plants. Biochar significantly improved the dry weights of cape marigold under different soil moisture levels. However, biochar application at a rate of 1.5 ton gave the highest dry weights records under different soil moisture levels compared to the other treatments. Furthermore, W3XBio3 showed the maximum dry weights means compared to the other treatments. Followed by W3XBio2 treatment where the difference between the two treatments was insignificant. This result may contribute to the increase in relative water contents (RWC), water use efficiency (WUE), and to the decrease in stomatal density of drought-stressed plant leaves (Paneque et al., 2016).

Flowering quantity and quality:

Data concerning with the cape marigold yield and its components which including: number of days to the flowering, flower ray length (cm), ray width (mm), flower disk diameter (mm), number of rays /inflorescence, Peduncle length (cm), Peduncle diameter (mm), number of inflorescences/plant, Inflorescence fresh weight (g) and inflorescence diameter (cm)at harvest stage affected by water stress and different biochar amounts; in the two successive seasons are columned in Table (4 and 5). It is clear that the highest soil moisture level (W3 treatment) increased significantly all yield and flower characters of *Dimorphotheca ecklonis* plant as compared with the other treatments in both growing seasons. On the other hand, the lowest values of these characters were obtained by W1treatment in both seasons. A data obtained in the present investigation also indicated that the application of the lowest soil moisture level resulted in significantly earlier first flowering against to the highest soil moisture level. Whereas, the maximum days required for first flowering (151.92 and 152.59 days) were recorded in W3 treatment. This might be due to water deficit condition in W1 treatment, reduced vegetative growth parameters, and stimulate reproductive growth, which caused reduction in plant life cycle and earlier flowering symptoms (Chawla 2006). The application of highest soil moisture level also increased significantly quality and quantity of cape marigold flowers. Where, W3 treatment, gave the maximum number of flowers ray/inflorescence(22.31 and 21.76), the biggest diameter of flower (11.04 and 11.02mm),the greatest flower ray length (2.11 and 2.16cm) and width(13.04 and 13.05cm),the highest number of inflorescences/plant (107.50 and 111.97), the maximum inflorescence diameter (6.93 and 6.95cm), the heaviest inflorescence fresh weight (1.72 and 1.74g),the tallest peduncle length (18.1 and18.62g) and largest peduncle diameter (1.65 and 1.62mm) for both seasons respectively. It is an established fact that increasing water supply increases the degree of ionization and solubility of nutrients in the soil resulting into increased availability of nutrients and improved lipid synthesis with optimum soil moisture supply.

Table 4: Effect of different soil moisture levels, biochar treatments and their interactions on flowering characters of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017 seasons.

Characters / season		No of days to flowering		Flower ray Length (cm)		flower ray width (mm)		Flower disk diameter (mm)		No of ray flowers/inflo.	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Effect of different soil moisture levels											
W1		137.23	135.32	2.04	2.06	12.46	12.48	10.47	10.58	17.63	17.60
W2		145.62	145.52	2.08	2.11	12.85	12.81	10.70	10.76	19.07	19.23
W3		151.92	152.59	2.11	2.16	13.04	13.05	11.04	11.02	22.31	21.76
$LSD_{0.05}$		4.46	3.87	0.02	0.03	0.02	0.03	0.01	0.04	1.07	1.01
Effect of biochar treatments											
Bio1		142.59	142.62	2.06	2.09	12.70	12.75	10.71	10.75	19.18	18.89
Bio2		144.95	144.49	2.08	2.11	12.79	12.78	10.74	10.78	19.67	19.64
Bio3		147.22	146.33	2.09	2.12	12.85	12.81	10.76	10.82	20.17	20.06
$LSD_{0.05}$		1.78	1.88	0.01	0.01	0.02	0.01	0.02	0.01	0.17	0.09
Effect of interaction											
W1	**Bio1**	134.22	133.41	2.01	2.04	12.33	12.42	10.44	10.55	17.43	17.55
	Bio2	137.33	135.21	2.04	2.06	12.47	12.48	10.47	10.57	17.57	17.58
	Bio3	140.14	137.33	2.06	2.07	12.57	12.55	10.49	10.61	17.88	17.67
W2	**Bio1**	143.33	144.24	2.07	2.09	12.77	12.79	10.66	10.71	18.77	19.01
	Bio2	145.31	145.01	2.08	2.10	12.87	12.81	10.70	10.75	19.02	19.23
	Bio3	148.21	147.32	2.08	2.13	12.92	12.83	10.73	10.82	19.42	19.45
W3	**Bio1**	150.21	150.21	2.09	2.14	13.01	13.04	11.02	10.99	21.33	20.12
	Bio2	152.22	153.24	2.11	2.16	13.04	13.05	11.04	11.03	22.41	22.10
	Bio3	153.32	154.33	2.12	2.17	13.06	13.06	11.06	11.04	23.21	23.07
$LSD_{0.05}$		3.54	2.47	0.03	0.04	0.05	0.04	0.03	0.03	0.09	0.10

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Table 5:Effect of different soil moisture levels, biochar treatments and their interactions on flowering characters of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017 seasons.

Characters / season		Peduncle length (cm)		Peduncle diameter (mm)		No of inflorescences /plant		Inflorescence fresh weight (g)		Inflorescence diameter (cm)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Effect of different soil moisture levels											
W1		12.93	12.66	1.36	1.31	85.22	86.99	1.48	1.50	5.46	5.50
W2		16.13	16.81	1.57	1.54	99.28	98.25	1.66	1.71	5.70	5.76
W3		18.41	18.62	1.65	1.62	107.50	111.97	1.72	1.74	6.93	6.95
$LSD_{0.05}$		0.12	0.13	0.02	0.01	4.32	3.48	0.01	0.01	0.02	0.01
Effect of biochar treatments											
Bio1		15.31	15.27	1.49	1.47	95.81	92.28	1.60	1.63	5.99	6.04
Bio2		15.93	16.28	1.52	1.49	95.42	101.44	1.62	1.65	6.02	6.07
Bio3		16.24	16.54	1.57	1.51	100.77	103.49	1.65	1.67	6.08	6.11
$LSD_{0.05}$		0.03	0.02	0.04	0.03	4.89	3.78	0.01	0.01	0.02	0.02
Effect of interaction											
W1	**Bio1**	12.72	11.79	1.32	1.29	82.47	84.62	1.45	1.47	5.42	5.47
	Bio2	13.01	12.98	1.33	1.31	85.74	87.41	1.49	1.50	5.44	5.49
	Bio3	13.07	13.21	1.42	1.34	87.45	88.94	1.51	1.52	5.53	5.54
W2	**Bio1**	15.21	16.01	1.55	1.52	99.21	82.01	1.64	1.69	5.67	5.72
	Bio2	16.32	17.09	1.58	1.54	93.21	105.43	1.66	1.71	5.71	5.75
	Bio3	16.87	17.33	1.59	1.56	105.42	107.31	1.69	1.73	5.73	5.81
W3	**Bio1**	17.99	18.02	1.61	1.59	105.74	110.21	1.70	1.73	6.88	6.92
	Bio2	18.47	18.77	1.64	1.62	107.32	111.49	1.72	1.74	6.91	6.96
	Bio3	18.77	19.07	1.69	1.64	109.44	114.21	1.74	1.76	6.99	6.97
$LSD_{0.05}$		0.48	0.23	0.10	0.08	5.01	4.02	0.02	0.03	0.04	0.03

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Similar results of positive responses with increasing soil moisture level were found by, Chaves and Oliveira(2004) who stated that the application of water stress at the flowering stage negatively affected the flower quality and flower diameter. In addition, Halepyati et al., (2001)on marigold and Halepyati et al. (2002) on tuberose recorded that the irrigation at 100 % E_{pan} replenishment during entire crop of tuberose produced maximum rachis length ,spike length, number of flowers per spike and flower yield.

Moreover, Younis et al.,(2017) reported on marigold decreasing trend in number of flowers under water stress conditions.

Based on pooled analysis different biochar treatments significantly affect the flowering stage of *Dimorphotheca ecklonis* plant in both seasons(Table 4 and 5).The minimum days required for first flowering (142.59 and 142.62 days) were observed in Bio1 treatment followed by Bio2 treatment (144.95 and 144.49 days) then Bio3 treatment (147.22 and 146.33 days) for both seasons respectively. The flower yield and characters significantly increased because of biochar application. However, the maximum flowers yield and the best flower characters were obtained at Bio3 treatment in both growing seasons. These positive responses of flowers cha racters and yield due to biochar treatment could be due to the possible role of biochar through better root proliferation, as well as better uptake of water and nutrients and higher photosynthetic activity, which enhanced higher food accumulation, that might have resulted in better plant growth and subsequently higher yield. The present result was also similar to the findings of Chandrikapure et al.,1999, Gayithri et al.,2004 and Kumawatet al.,2017.

From the present data, it is quite clear that both quality and quantity of cape marigold flowers were significantly affected by the combined application of different soil moisture levels and different biochar treatments. A comparison among the best treatments of combined application of different soil moisture levels and biochar treatments indicate that the quality and quantity of cape marigold flowers far exceeded in case of combined application of W3XBio3compared with the other treatments in both growing seasons. Followed by W3XBio2 treatment where the difference between the two treatments was mostly insignificant.

Mineral ions content and uptake:

The analysis of variance (ANOVA) (Table 6&7) showed that drought stress had significant effects on NPK contents and their uptake by cape marigold root in both growing seasons. Increasing severity of drought significantly decreases the uptake of NPK and their content in cape marigold plant in both growing seasons. Furthermore, the highest NPK concentration and uptake were noticed under the highest soil moisture level (W3 treatment), while the minimum uptake and accumulation were obtained under W1 treatment as compared to the other soil moisture levels in both seasons. The decreased levels of each of N, P and K contents and their uptake in response to drought stress were ascertained by the work of each of Bieet al. (2004);Koyro(2006); Khalil and Abdel-Kader (2011) and Bista et al.(2018).Such reductions in the concentrations of these elements in different cells and tissues could primarily due to soil water deficiency that markedly decrease the flow of these elements in the soil, their uptake by stressed root cells and also its ability to translocate through the different tissues and organs (Khalil and Abdel-Kader 2011).While the observed reduction in NPK uptake might be due to the damage in nutrient-uptake proteins within root tissue. Previous studies have illustrated that water stress can affect the expression of nutrient-uptake-protein genes in root tissue (Wang et al., 2017).Similar results recorded by Kovács (2005) who illustrated also the importance of mass flow of water to promote plant NPK uptake from the soil, and its effect on maize production. In addition, water deficiency decreasing the activity of enzymes involved in nutrient assimilation, which could then slow nutrient uptake from the soil (Robredo et al., 2011).Rouphael et al. (2012) also indicated that water deficiency might also reduce the expression of nutrient-uptake proteins in roots. Water stress could also reduce soil nutrient contents by decline soil microbial activity (Sanaullah et al., 2012).Plots treated with biochar treatments showed significant increase in the concentration of NPK in cape marigold plant and their uptake by root in both growing seasons. It was also clear from obtained data that increasing biochar doses caused gradual increase in NPK values. Moreover, the lowest significant increase in both NPK concentration and their uptake appeared under the lowest biochar dose (0.5 ton/fed).Such improvements in NPK concentration and uptake because of biochar applications were in agreement with those obtained by Lehmann *et al.,* 2006, Mannan et al., 2016,Pressler et al.,2017and Ali et al., 2017.The increments in microbial activity due tobiochar application might be the reason for the highest nutrient uptake in biochar treated plots.

Table 6: Effect of different soil moisture levels, biochar treatments and their interactions on mineral ions contents of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017 seasons.

Characters / season		N %		P %		K %	
		1st	2nd	1st	2nd	1st	2nd
Effect of different soil moisture levels							
W1		1.09	1.08	0.84	0.86	0.95	0.93
W2		1.15	1.17	0.91	0.94	1.02	1.04
W3		1.25	1.28	1.00	1.01	1.07	1.09
$LSD_{0.05}$		0.03	0.04	0.01	0.03	0.03	0.04
Effect of biochar treatments							
Bio1		1.14	1.14	0.89	0.91	1.00	0.99
Bio2		1.16	1.17	0.91	0.93	1.02	1.02
Bio3		1.19	1.21	0.94	0.96	1.03	1.04
$LSD_{0.05}$		0.02	0.01	0.02	0.01	0.01	0.01
Effect of interaction							
W1	Bio1	1.07	1.03	0.81	0.83	0.93	0.90
	Bio2	1.09	1.07	0.84	0.86	0.96	0.94
	Bio3	1.11	1.13	0.86	0.88	0.97	0.95
W2	Bio1	1.13	1.15	0.89	0.91	1.01	1.01
	Bio2	1.15	1.16	0.91	0.93	1.02	1.04
	Bio3	1.18	1.19	0.93	0.97	1.04	1.06
W3	Bio1	1.22	1.25	0.98	0.99	1.06	1.07
	Bio2	1.24	1.28	0.99	1.01	1.07	1.09
	Bio3	1.28	1.31	1.02	1.03	1.07	1.10
$LSD_{0.05}$		0.04	0.06	0.03	0.03	0.04	0.05

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Table 7: Effect of different soil moisture levels, biochar treatments and their interactions on mineral ions uptake (mg/plant)of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017

Characters / season		N uptake		P uptake		K uptake	
		1st	2nd	1st	2nd	1st	2nd
Effect of soil moisture levels							
W1		1008.59	1005.50	774.31	799.78	882.15	868.19
W2		1244.23	1249.03	981.73	1003.39	1103.88	1110.17
W3		1427.39	1564.41	1141.09	1234.27	1220.98	1327.69
$LSD_{0.05}$		4.65	5.49	5.11	4.31	4.62	5.19
Effect of biochar treatments							
Bio1		1176.29	1183.07	923.72	941.04	1032.02	1026.83
Bio2		1222.36	1263.48	963.71	1007.08	1070.73	1103.52
Bio3		1281.56	1372.39	1009.70	1089.32	1104.25	1175.71
$LSD_{0.05}$		6.21	4.59	4.87	5.31	6.11	4.15
Effect of interaction							
W1	Bio1	969.67	936.37	734.05	754.55	842.80	818.19
	Bio2	1001.60	995.21	771.88	799.89	882.14	874.29
	Bio3	1054.50	1084.91	817.00	844.89	921.50	912.10
W2	Bio1	1190.12	1154.83	937.35	913.82	1063.73	1014.24
	Bio2	1247.18	1243.87	986.90	997.24	1106.19	1115.19
	Bio3	1295.40	1348.39	1020.95	1099.11	1141.71	1201.09
W3	Bio1	1369.08	1458.00	1099.76	1154.74	1189.53	1248.05
	Bio2	1418.31	1551.36	1132.36	1224.12	1223.87	1321.08
	Bio3	1494.78	1683.87	1191.16	1323.96	1249.55	1413.94
$LSD_{0.05}$		6.66	5.49	5.66	4.02	5.62	6.07

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

Table 8: Effect of different soil moisture levels, biochar treatments and their interactions on sugar content in fresh inflorescence of *Dimorphotheca ecklonis* plant during 2015/2016 and 2016/2017.

Characters season		Reducing sugar %		Non reducing sugar %		Total sugar %	
		1st	2nd	1st	2nd	1st	2nd
Effect of different soil moisture levels							
W1		4.27	4.38	1.85	1.87	6.11	6.24
W2		3.81	3.87	2.01	1.98	5.81	5.85
W3		3.44	3.55	2.13	2.07	5.57	5.62
$LSD_{0.05}$		0.05	0.08	0.03	0.04	0.01	0.01
Effect of biochar treatments							
Bio1		3.90	3.98	1.92	1.93	5.84	5.91
Bio2		3.83	3.93	2.01	1.97	5.84	5.90
Bio3		3.79	3.89	2.05	2.01	5.82	5.90
$LSD_{0.05}$		0.06	0.04	0.02	0.03	N.S	N.S
Effect of interaction							
W1	**Bio1**	4.32	4.44	1.74	1.81	6.06	6.25
	Bio2	4.28	4.37	1.88	1.86	6.16	6.23
	Bio3	4.20	4.32	1.92	1.93	6.12	6.25
W2	**Bio1**	3.88	3.91	1.92	1.95	5.80	5.86
	Bio2	3.79	3.87	2.01	1.99	5.80	5.86
	Bio3	3.75	3.82	2.09	2.01	5.84	5.83
W3	**Bio1**	3.49	3.58	2.11	2.04	5.60	5.62
	Bio2	3.43	3.54	2.13	2.07	5.56	5.61
	Bio3	3.41	3.53	2.15	2.09	5.56	5.62
$LSD_{0.05}$		0.18	0.13	0.16	0.08	0.06	0.05

W1 = 50% depletion of the available soil water. W2 = 35% depletion of the available soil water. W3 = 20% depletion of the available soil water. Bio1=0.5 ton biochar/fed. Bio2= 1ton biochar/fed. Bio3= 1.5ton biochar/fed.

According to Pietikäinen et al., (2000), biochar act as a habitat for soil microorganisms involved in N, P, or S transformations. Biochar has also the ability to support the presence of adsorbed bacteria from which the microorganisms may affect soil processes (Pietikäinen et al., 2000).

It was interesting also to note that, there were significant effects of all biochar treatments on NPK content and their uptake under stress and well watered conditions, where the highest significant increase in their means appeared under the highest dose of biochar treatments (1.5ton/fed) under different soil moisture levels.

W3XBio3 treatment revealed the highest significant records compared with the other treatments in both seasons. The biochar application in dry soils might stimulate plant tolerance to drought conditions by enhancing the uptake and accumulation of mineral nutrients in plants cells and tissues. The beneficial effects of the biochar treatment under limited water conditions had been widely recorded by Artiola et al.,2012; Akhtar et al., 2014; Batool et al., 2015; Paneque et al., 2016).

Sugar contents:

The results tabulated in Tables 8 showed in both seasons that, water stress induced significant and progressive increase in reducing sugars content of cape marigold inflorescence. Where, the maximum records (4.27 and 4.38 %) were observed in W1 treatment, while the minimum values (3.44 and 3.55% fresh inflorescence) were obtained under W3 treatment. Opposite trend was observed for the non-reducing sugars, which revealed significant and gradual depress in their content with increasing the soil water stress. The highest non- reducing sugars means (2.13 and 2.07% in fresh inflorescence) were observed under W3 treatment, whereas the lowest means (1.85 and 1.87 % in fresh inflorescence) were noticed under W1 treatment in both growing seasons compared to the other treatments. Furthermore, the total sugars values of cape marigold inflorescence in both seasons accelerated significantly in response to water stress treatment. The data showed that irrigation with the lowest soil moisture level W1 recorded the highest total sugars values (6.11 and 6.24 % in fresh inflorescence) compared with the other treatments. Shortage of the available soil water was reflecting in lowering the plant content of total non-soluble sugars and accelerating the accumulation of reducing and total sugars. Metwally (2002) indicated that stress conditions might cause decline in the plant photosynthesis rate due to the shortage of the available soil moisture that is reflecting in lowering in plant content of total non-reducing sugars or may be due to considerable degradation of polysaccharides (Abbas 2008). Similar results were obtained by Andriotis et al., (2016) and Ali and Elozeiri (2017). While the observed increase in soluble sugars in various plant tissues due to soil water stress are supported the idea of the degradation of polysaccharides which accumulated in leaves during water stress, and have suggested that these sugars might contribute to osmoregulation (Wu and Xia, 2006). The observed increase in soluble sugars could mainly come from the transformation of stored starch to provide energy and carbon skeleton for the synthesis of amino acids, lipids and metabolites needed for plant growth (Mornya et al.,2011).It may also contribute to the osmotic potential of plant tissues and the osmotic adjustment process (Sami et al., 2016). The accumulation of reducing sugars in many plants under stress conditions were on line with those recorded by He et al., 2006, Sami et al., 2016 and Yasseen et al., 2018.

Obtained results in Table 8 presented a clear comparison between the three chosen amounts of biochar application irrespective to water stress. Bio3 treatment revealed the highest significant reduction in reducing sugars content of cape marigold inflorescence (3.79 and 3.89 in both growing seasons respectively), and in total sugars (5.82 and 5.90) but the difference between treatments was insignificant. While, pronounced increases were obtained by Bio3 treatment for the non-reducing sugars (2.05 and 2.01) compared to the other treatments for both growing seasons. The results reached the level of significance in both growing seasons. The beneficial effect of different biochar amounts on non-reducing sugars content might be due to the role of micro and macro elements, which provided by these fertilizers, that stimulate the photosynthetic apparatus and metabolic processes therefore leads to more photosynthesis and carbohydrate synthesis. The beneficial effect of biochar treatment on carbohydrate accumulation were previously observed by Khalil et al., (2002) on *Tagetes erecta*, Hussein et al .,(2006) on *Dracocephalum moldavica*, El-Sherbeny et al., (2005) on *Sideritis montana* L and Hussein et al. ,(2012) on *Plantago ovata.*

The data of interaction figured out in both growing seasons that the best effect of biochar treatments obtained in plots treated with Bio3 treatment compared with the other treatments in the both seasons under the different soil moisture levels (Table 8).Where, Bio3 treatment showed the highest reduction in reducing and total sugars and the highest significant increment in non-reducing sugars under different soil moisture levels for both growing seasons as compared with the other treatments.

CONCLUSION

The results indicated that the highest significant increases in cape marigold growth, flowering characters, NPK contents and uptake and non-reducing sugars were observed under the combined effect of the highest soil moisture level and 1.5ton of biochar/ fed. Results indicate also that biochar could be used to enhance plant growth and flowering characters as well as improving soil properties especially under water stress conditions

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

All authors contributed in collecting and analyzing data. All authors participated in writing every part of this study. All authors read and approved the final version.

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