Biochar Improved Biomass Duration, Membrane Stability Index and Relative Water Content of *Calendula officinalis* L. under Salt Stress Conditions

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Abstract: Pot experiments over two successive seasons 2019-20 to 2020-21 were conducted to study the effect of biochar application on the growth and some physiological parameters of pot marigold grown under salt stress. A factorial experiment block design was performed with 2 levels of biochar (5%, 10%) and 3 levels of saline water (1000, 2000 and 3000 mgl^{-1} of NaCl) in addition to control. Results showed that, application of biochar at both levels mitigated the negative effect of salt stress by enhancing the biomass duration, membrane stability index and relative water content of plants. At high level of salinity, biomass duration was increased by 55 and 31% as well as membrane stability index by 22 and 28% in both seasons, respectively after application of biochar 10%. Also, relative water content was increased by 15% after application of 5% of biochar at first season. Beneficial effect of biochar may be correlated with reduction of salinity in soil solution by 37.9% after application of biochar at 10%, as well as decrease of Ca^{2+}, Na^{+} and K^{+} concentrations. It can conclude that application of 10% biochar was recommended for pot marigold, which irrigated with saline water.

Keyword: Salinity, pot marigold, growth, physiological parameters.

INTRODUCTION

Pot marigold or common marigold, *Calendula officinalis* L., family Asteraceae, is herbaceous, ornamental, and medicinal plant (Cetkovi *et al.,* 2004). It had pharmaceutical properties because of different important phytochemicals, such as carotenoids, flavonoids, terpenoids, quinones, and amino acids (Tanideh *et al*., 2020). These active constituents had a wide range of medicinal applications and biological activities, including spasmogenic, hepatoprotective, genotoxic, spasmolytic, antigenotoxic, anti-oedematous, anti-fungal and anti-bacterial, antidiabetic and anti-HIV, nephron-protective, oropharyngeal mucositis prevention, gastroprotective, and hypoglycemic characteristics (Jan *et al*., 2017). Marigold is a widely garden plant that also grows wild throughout North America and Europe (Bernatoniene *et al*., 2011).

High soil salinity disrupted the plant's minerals-water balance, causing dryness and increasing the concentration of vacuolar cell sap. The minerals-water balance is vital for electrochemical activities (Parida and Das, 2005). Toxicity and ionic competition occurred on cell membrane due to high salinity. The earliest signs of salt overload are wilting of plants and/or leaf "burn" or drying (Alam, 1999). By the time symptoms appear, the plant has crossed its tolerance threshold and is losing vigor and possible its productivity. Salinity causes water and osmotic stress in plants, and the salt ions accumulated in plant organs to toxic levels (Rasool *et al.,* 2013). Water deficiency reduces the leaf turgor, increase stomata closure, and decline of stomatal conductance and lower photosynthetic rates (Chaves *et al.,* 2009). According to Adamipour *et al.* (2019), Ali and Hassan (2018), increasing of salinity reduced the leaf area, fresh and dry weight of flower, shoot and roots while increase the proline content in the plants (Kozminska *et al.,* 2017).

Biochar is a carbon-rich solid organic material produced by heating biomass with little or no oxygen (Tan *et al.,* 2016). Biochar is produced by slow pyrolysis, hydrothermal carbonization, flash carbonization, and gasification (Tan *et al.,* 2015). Biochar used to enrich agricultural soil and trap carbon could lock moisture in the soil (Kroeger *et al*., 2018). Its application to farmland soils is recognized as a way to help agriculture mitigate some of its massive emissions damage. Biochar reduces the need for fertilizer by enriching the soil and allowing it to absorb excess water, reducing the risk of runoff that destroys valuable top soil. Altaf *et al.* (2021) studied the effect of biochar on stock, and geranium as ornamental plants. Results showed that, adding of biochar enhanced the plant height, leaves number per plant, root length, flowers number, flower diameter, leaf chlorophyll content, and days to first flower appearance in both pervious ornamental plants. However, the beneficial effect of biochar on tomato growth, productivity and quality under saline conditions was attributed by its ability to adsorb sodium ions, which minimize the harmful effect of sodium ions and releasing mineral elements such as potassium, calcium, and magnesium into the soil solution. Therefore, biochar has the potential to be widely utilized in agricultural output in conjunction with saline water irrigation to combat the freshwater issue (She *et al.,* 2018). Biochar may be extremely beneficial for alleviating soil salt stress by inhibiting Na^{+} uptake by plants and increasing the essential nutrients for plants (Kahil *et al.,* 2018). Little literatures were studied the impact of biochar on pot marigold grown under salt stress condition. Therefore, the aim of research is studying the effect of biochar application at 5 and 10% on vegetative growth and some physiological parameters on pot marigold under saline irrigation with 1000, 2000 and 3000 mgl-1 of NaCl.

MATERIALS AND METHODS

The experiment was carried out at the Floriculture Nursery, Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, over two successive seasons of 2019-20 and 2020-21.

Plant material, cultivation, and treatments:

Pot marigold (*Calendula officinalis* L.) seeds were purchased from the Ministry of Water Resources and Irrigation nursery in Al-Qanater Al-Khayriya Qalyubia Governorate. During August, seeds were sowed in foam trays filled with a 3:1 mixture of peat moss and vermiculite. Homogenous seedlings (30 days age) were transferred to 20 cm pots filled with 3 Kg of salt-free sand, contained biochar at 5% and 10% (V: V). Irrigation of the plants was performed on a regular basis. Plants were fertilized every two weeks with 300 ml of water dissolved NPK, 19-19-19 to prevent the participation of ions due to NaCl application. After one month of transplanting, pots were irrigated twice weekly with saline water at 1000, 2000 and 3000 mgl^{-1} of NaCl until the end of experiment (120 d).

Soil and water analysis

Soil and water were chemically analyzed at the beginning of the experiment as shown in Table (1). The soil and water reaction (pH) were measured with Backman pH meter, Electrical conductivity (EC) as dS/m or ppm and soluble cations and anions (meq/l) were determined in the soil-paste extract and water sample (Page *et al*., 1982). Soluble Na+ and K+ were determined by a flame photometer, whereas soluble Ca2+ and Mg2+ were determined using the versenate method according to Richards (1954). Cl-, CO3-2 and HCO3- were determined by titration against silver nitrate for chloride and HCl for the other two anions, as described by Jackson (1973). The sulphate was calculated by the differences between cations and anions. Soil was chemically analyzed at the end of the experiment.

Table (1): chemical analysis of soil and irrigated water at the beginning of the experiment

Sample No	EC ds/m	EC ppm	P^H	Cations meq/l				Anions meq/l			
				SO_4^{2-}	Cl^-	HCO^-	CO_3^{2-}	K^+	Na^+	Mg^{2+}	Ca^{2+}
Soil	**2.11**	**1350**	**7.77**	**4.1**	**15.00**	**2.00**	**0.00**	**0.3**	**9.8**	**4.00**	**7.00**
Irrigated water	0.301	**192.6**	7.96	0.40	1.20	1.50	0.00	0.20	1.00	0.90	1.00

At the end of experiment soil was also chemically analyzed.

Preparation of biochar

Biochar prepared by moderate pyrolysis of mango branches at 650 °C in the absence of or with a limited oxygen source. After 24 hours, we adjusted the PH of biochar by splashing it in phosphoric acid at 1 normal for 24 hours.

The recorded data

Biomass duration (BMD) as g/day: it was calculated as the following formula:

BMD = (BM1 + BM2) x (t2 - t1)/2

Where, BM1 and BM2 is the dry weight of plant in g at times t1 and t2 (every 30 d), respectively (Koneni 2016).

Physiological parameters:

The membrane stability index (MSI) was determined using 0.1g of leaf sample, immersed in two sets of test tubes, each containing ten milliliters of distilled water. After heating test tubes containing the sample at 40°C for 30 minutes, the electrical conductivity of the water was determined (C1). The test tubes containing the second set of samples were heated to 100°C for 15 minutes, and the electrical conductivity of the water was determined (C2) according to Sairam *et al.* (2002 and 1994). MSI values were calculated using the following formula:

$$MSI = (1 - \frac{C1}{C2}) \text{ X } 100$$

Relative water content (RWC) was determined in first season only using the approach outlined by Lohe et al. (2015) using the formula below. The fresh leaves were taken to the laboratory for fresh weight estimation. To determine the fresh weight of leaf samples, they were weighed directly on a balance (FW). After 24 hours (overnight) of soaking in distilled water, the leaves were gently wiped with tissue paper and weighed to establish the turgid weight (TW). Finally, the leaves were dried in an oven at 72°C for two days and reweighed to acquire the dry weight (DW).

All mass measurements were made with a precision of 0.001 g using an analytical scale. The following equation was used to determine RWC using the values of FW, TW, and DW:

$$RWC (\%) = \frac{FW - DW}{TW - DW} x\ 100.$$

Statistical analyses

A factorial experiment had three replicates, one pot in each replicate, were laid out in a completely randomize block design. Statistical analyses were done using SPSS version 18.0 statistical software program in which treatments were compared by following LSD test at 5% level of possibility.

RESULTS AND DISCUSSION

Chemical analysis of soil at the end of the experiment

Application of biochar at both concentrations 5 and 10% decreased the electrical conductivity (EC) of soil, especially under high levels of salinity 2000 and 3000 mgl-1 as observed at Table (2). Salinity was decreased under application of biochar at 10% by about 30 and 37.9% after irrigation with saline water in 2000 and 3000 mgl-1, respectively. These findings were coordinated with She *et al.* (2018), who observed the beneficial role of biochar on diminish the salinity of irrigated water. At the same time,reduction of most of cations and anions were observed after application of biochar. Reduction was by (50 and 57 % for Ca; 28,5 and 33% for Mg; 17.8 and 38.5% for Na; 33 and 16.7 for HCO3; 33 and 53% for Cl), after irrigation with 2000 and 3000 ppm of saline water, respectively. Results were agreed with Kahil *et al.,* 2018, who observed inhibiting uptake of Na^+ by plants and increasing the essential nutrients after application of biochar and it may be beneficial tool for alleviating soil salt stress on plants.

Table (2): chemical analysis of soil after application of biochar and saline water at the end of experiment

Sample No	EC ds/m	EC ppm	P^H	Cations meq/l				Anions meq/l			
				Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO^-	Cl^-	SO_4^{2-}
R0Zero	0.96	614	8.20	3.00	2.00	4.30	0.30	0.00	3.80	4.60	1.20
R0 b1	0.80	512	8.21	2.00	2.50	3.20	0.30	0.00	1.50	5.00	1.50
R0 b2	0.99	634	8.18	2.30	3.00	4.40	0.30	0.00	2.20	5.30	2.50
R1Zero	1.55	992	8.35	3.10	5.20	6.40	0.80	0.00	3.10	8.30	4.10
R1 b1	1.73	1107	8.26	4.30	3.40	9.10	0.50	0.00	2.20	11.1	4.00
R1 b2	1.90	1216	8.27	4.00	3.60	10.5	0.90	0.00	4.00	10.4	4.60
R2Zero	1.64	1050	8.47	2.00	3.00	11.0	0.30	0.00	5.20	7.00	4.20
R2 b1	2.85	1824	8.29	8.00	7.00	12.9	0.60	0.00	6.00	15.00	7.50
R2 b2	1.99	1274	8.33	4.00	5.00	10.6	0.40	0.00	4.00	10.0	6.00
R3Zero	2.11	1350	8.40	5.70	4.00	10.6	0.80	0.00	3.40	14.1	3.60
R3 b1	4.33	2771	8.21	11.00	9.00	22.10	1.20	0.00	6.00	30.0	7.30
R3 b2	2.69	1722	8.42	7.00	6.00	13.60	0.40	0.00	5.00	14.00	8.00

R0= irrigation water with 0 mg/lNaCl, R1=1000 mg/lNaCl, R2=2000 mg/lNaCl
R3=3000 mg/lNaCl, b1 = biochar 5%, and b2=10%

Biomass duration

As illustrated in table 3, irrigation of marigold plants with saline water reduced the values of biomass duration (BMD).Analysis of the main effect of salinity revealed that, BMD was reduced by about 9 to 8%, over the first and second season, respectively. Results were agreed with Hozumi (1989), who demonstrated that, relative growth rate, net assimilation rate, crop growth rate and biomass duration may be suitable parameters for plant growth analysis under normal and stress conditions. Also, Kozminska *et al.* (2017), observed the reduction of leaf area, fresh and dry weight of flower, shoot and roots of the plants with increment of salt concentration.

Analysis of the main effect of biochar was shown an enhancement of BMD of plants (Table 3). BMD was increased by 49 and 20% after application of biochar at 5% at both seasons, respectively. However, it was increased by 53 and 20 % after application of biochar at 10% at both seasons, respectively. The obtained data are in accordance with that reported by Teodoro *et al.* (2020), Helliwell (2015). Collalti *et al.* (2019) who explained that BMD of a plant provides an accurate estimate of its total nighttime respiration in relation to time. Thus, it is highly likely that biomass duration indicates anabolic and catabolic rates under stress conditions and can provide predicted values for the total function. Analysis of the interaction of salinity and biochar obvious that BMD was increased by 55 and 31% after application of biochar 10% at high level of salinity in both seasons, respectively. The beneficial effect of biochar on BMD may be attributed with adsorption of salt ions especially Na+ on biochar surface as shown previously at Table (2).

Table (3): Effects of irrigation pot marigold plants with saline water and addition of biochar to growing media on biomass duration

Treat	Biomass duration (2019-20)					Biomass duration (2020-21)				
	0	1000	2000	3000	mean	0	1000	2000	3000	Mean
Biochar 5%	281.21	293.88	222.56	269.20	266.71	175.22	208.54	209.82	199.67	198.31
Biochar10%	300.87	315.29	261.69	279.51	289.34	212.96	204.48	188.59	182.39	197.11
Control	156.53	135.28	226.94	124.73	135.87	166.66	158.87	154.72	125.86	158.28
Mean	246.21	248.15	203.73	224.48		184.95	199.63	184.38	169.31	
LSD 5%	**Salinity = 10.42** **Treatments = 9.02** **Interaction = 18.05**					**Salinity = 18.13** **Treatments = 15.70** **Interaction = 31.39**				

Membrane stability index (MSI)

Analysis of the main effect of salinity showed a decrease of MSI values by 17 and 42% in both seasons, respectively (Table 4). Our observations were in parallel with those observed by Farooq and Azam (2006), who reported that salinity-induced reduction of MSI and relative water content. Also, Gill and Tuteja (2010), reported that cell membrane index is a critical indicator of oxidative stress in plant cells. Under salt stress, plant cellformed high amount of reactive oxygen species, which oxidized the macromolecules such as membrane lipids, causing reduction of membrane stability and disrupting the nutrient balance (Kaya *et al.,* 2009; Gill and Tuteja, 2010). Interaction analysis of salinity and biochar demonstrate an increase by 22 and 28% of MSI after application of 10% biochar and irrigation with 3000 ppm of NaCl in both seasons, respectively.

Table (4): Effects of irrigation pot marigold plants with saline water and addition of biochar to growing media on membrane stability index

Treat	MSI (2019-20)					MSI(2020-21)				
	0	1000	2000	3000	mean	0	1000	2000	3000	Mean
Biochar 5%	87.19	74.35	43.33	69.00	68.47	83.58	69.14	32.83	60.25	61.45
Biochar10%	83.97	52.62	60.96	78.63	69.05	77.47	50.66	39.67	46.73	53.63
Control	79.39	63.98	64.17	61.86	67.35	81.84	46.37	32.39	33.50	48.52
Mean	83.52	63.65	56.16	69.83		80.97	55.39	34.96	46.82	
LSD	**Salinity = 3.41** **Treatments = 2.95** **Interaction = 5.90**					**Salinity = 4.59** **Treatments = 3.97** **Interaction = 7.94**				

Relative water content

Analysis of the main effect of salinity (Fig. 1) demonstrate that increment of salt concentration decreased the values of RWC compared to the control. RWC was reduced by 13% in plants irrigated with high saline water (3000 ppm of NaCl).This finding has been confirmed by numerous researchers, including (El-shawa *et al.,* 2020). All of biochar concentration as potting mix addition resulted in an increase in the value of RWC in the first season, compared to the control treatment (Fig 2). RWC was increased by 9 and 5% after application of 5 and 10% of biochar, respectively. Also, analysis of interaction of salinity and biochar showed an increment of RWC by 15% after application of 5% of biochar at high level of salinity (3000 ppm of NaCl). These findings suggest that adding biochar improved the water status of plants under salt stress, therefore biochar improved the accumulation of biomass as previously reported. Results coordinated with Soltys-Kalina *et al.* (2016), who reported that RWC is the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit.

CONCLUSION

Application of biochar at 10% decreased the electrical conductivity or salinity in soil by about 30 and 37.9% after irrigation with saline water in 2000 and 3000 mgl-1, respectively. At high level of salinity, biomass duration and membrane stability index were increased by 55 and 31%; 22 and 28% in both seasons, respectively after application of biochar 10%. Relative water content was increased by 15% after application of 5% of biochar at first season.

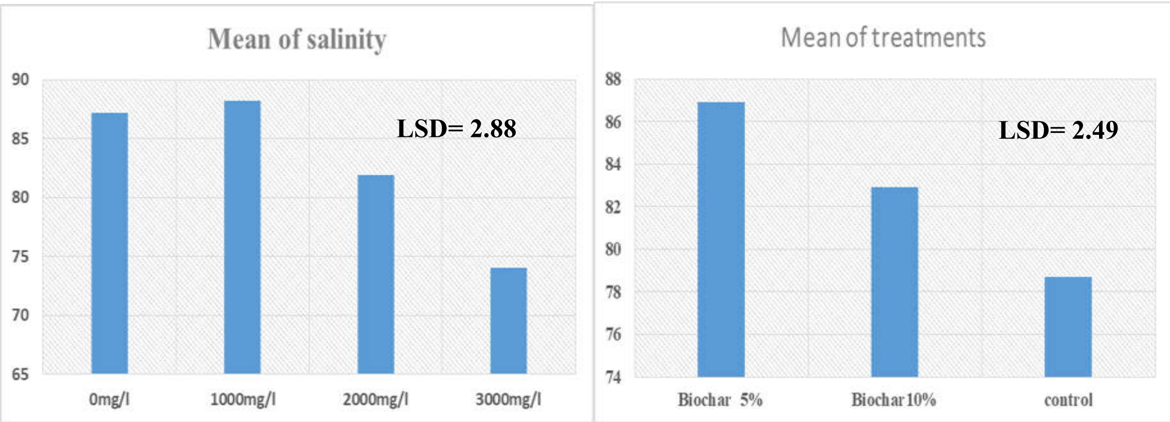

Fig (1): Main effect of salinity and biochar on relative water content

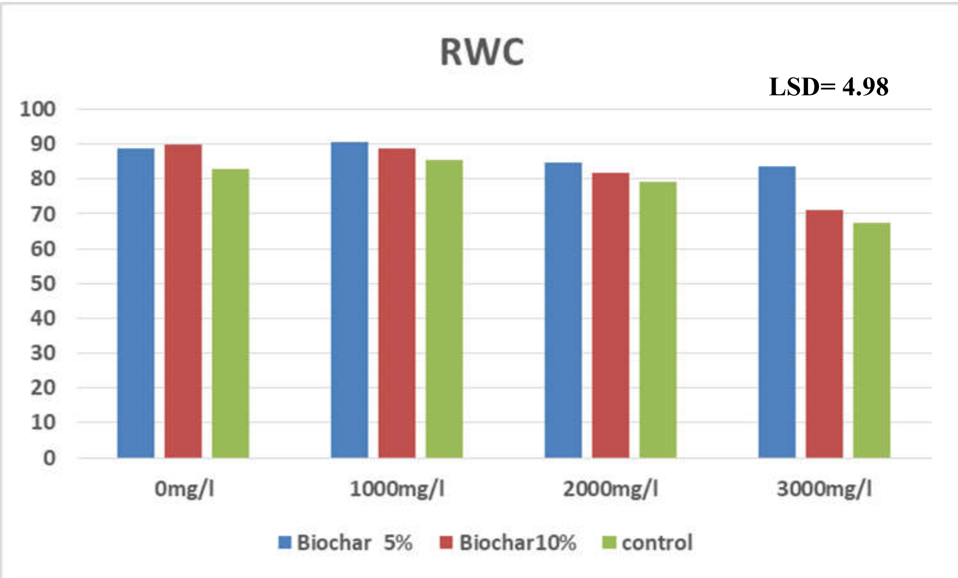

Fig (2): Interaction effect of salinity and biochar on relative water content

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تحسين البيوشار لتعاقب الكتلة الحيوية ودليل ثبات الغشاء ومحتوى الماء النسبي لنبات الأقحوان تحت ظروف الملوحة

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أجريت تجربة أصص على مدار موسمين زراعين ٢٠٢٠/٢٠١٩و ٢٠٢١/٢٠٢٠ لدراسة تأثير إضافة البيوتشار على نمو وبعض الصفات الفسيولوجية لنبات الأقحوان تحت ظروف الملوحة. صممت تجربة عاملية بقطاعات عشوائية كاملة بمستويين من البيوتشار ٥ و ١٠% و٣ مستويات ملوحة (١٠٠٠-٢٠٠٠-٣٠٠٠ جزء في المليون) بالإضافة إلى الكنترول. أظهرت النتائج أن كلا المستويين للبيوتشار قلل التأثير الضار للملوحة على النبات بتنشيط تعاقب الكتلة الحيوية للنباتات وزيادة دليل ثبات الأغشية ومحتوى الماء النسبي. حيث زاد تعاقب تكوين الكتلة الحيوية بنسبة ٥٥ و ٣١% وكذلك زاد دليل ثبات الأغشية بنسبة ٢٢ و ٢٨% في كلا الموسمين على التوالي بعد إضافة البيوتشار بتركيز ١٠% في المستوى العالي من ملوحة ماء الري. كذلك زاد المحتوى المائي النسبي بنسبة ١٥% في الموسم الأول. هذا التأثير الايجابي للبيوتشار ربما يرتبط بانخفاض ملوحة التربة بنسبة ٣٧% مع إضافة البيوتشار بتركيز ١٠% مع نقص تركيز الكالسيوم والصوديوم والكلور في محلول التربة.