

INFLUENCE OF BIOCHAR ON PRODUCTIVITY OF MARJORAM (*Origanum majorana* L.) PLANT GROWN IN SANDY SOIL UNDER DIFFERENT IRRIGATION LEVELS

Comment [AS1]: INFLUENCE OF BIOCHAR AND IRRIGATION ON PRODUCTIVITY OF MARJORAM (*Origanum majorana* L.) UNDER SANDY SOIL CONDITIONS

ABSTRACT

A field experiment was conducted in two successive summer seasons 2021 and 2022 at the Experimental Farm of EL-Quassasin Horticultural Research Station, Ismailia Governorate; Egypt, to investigate the influence of biochar at rates of 0, 2, 4, and 6 tons/fed. at three irrigation levels (1, 1:30, and 2 hours/day) using a drip irrigation system on the growth of marjoram (*Origanum majorana* L.) plant grown in sandy soil. The following characteristics were studied: growth parameters (plant height, number of branches /plant, herb fresh and dry weights/ plant, essential oil (percentage, yield/ plant, and composition), total chlorophyll, as well as leaf mineral content (N, K, and proline). The obtained results revealed that both irrigation and biochar levels affected all the above-mentioned traits. It was clear that plant growth parameters increased as long as increasing irrigation levels and biochar rate. Considering the interaction it was clear that generally the highest values in vegetative growth (plant height, number of branches, and herb fresh & dry weights/ plant), essential oil yield/plant, N%, and K% were attained from the second level of irrigation (1:30 hours/day) combined with the highest rate of biochar (6ton/fed.) treatment. On the other hand, the highest values of essential oil%, p-cymene, trans-Sabinene hydrate, α -terpineol, as well as proline, were recorded in stressed plants (received 1h/ day irrigation and 0 biochar). The existing outcomes show that biochar application might increase water use efficiency and produce a high yield of marjoram. Subsequently, it could be a viable option to alleviate the severity effect of water deficit in marjoram growth which opens opportunities for cultivating arid regions.

Comment [AS2]: Delete

Comment [AS3]: as

Comment [AS4]: Delete

Comment [AS5]: Comma after this

Keywords: *Origanum majorana* L., Biochar, Irrigation, Growth parameters, Essential oil composition

INTRODUCTION

The genus *Origanum* has 42 species distributed widely in North Africa (Ietswaart, 1980 and Meyers, 2005). In ancient times, these species were used as ornamentals, spices, and medicinal plants. Family Lamiaceae, it is found in different Mediterranean countries. One of Egypt's most significant aromatic and medicinal plants, sweet marjoram (*Origanum majorana* L.), has dried leaves that can be used in a variety of food industries as spices and condiments. Marjoram essential oil is used in traditional medicine for treating gastrointestinal disorders, larynx affection, whooping cough, diabetes, respiratory infections, hypertension, allergies, and stomach pain. Its high phenolic and flavonoid content makes it a promising natural source of bioactive compounds. It has remarkable activities as an antimicrobial, antioxidant, anticancer, anti-inflammatory, antimutagenic, nephroprotective, and hepatoprotective (Bouyahya *et al.*, 2021). Marjoram plants from Egypt and Serbia are highly promising due to their high levels of rosmarinic acid, which is the main constituent in their extract, potentially enhancing their anti-oxidant and anti-neurogenerative properties. (Duletić-Laušević *et al.*, 2018) hence, it has great economic importance. Climate change could impact Egypt's agriculture industry and global food security. The agricultural sector, which uses 80% of the country's water budget, is affected by rising crop water demands, global warming, and precipitation decline, among other factors. (Mahmoud, 2017). Drought stress, a severe abiotic stressor, poses a significant threat to agricultural production in the Middle East, particularly in Egypt, negatively impacting growth and crop production in dryland regions worldwide. (Zahedi *et al.*, 2009; Wang *et al.*, 2014a; and Bodner *et al.*, 2015). The United Nations reports that drought significantly impacts rain-fed agricultural lands in the Arab region, leading to land degradation, decreased native plant coverage, reduced yields, and vegetation depletion. (UN, 2015; Hameed, *et al.*, 2020).

Biochar, a solid, black, porous substance produced when biomass like animal manures and agricultural waste is burned under low oxygen conditions, is used for soil remediation, nutrient improvement, microbial carrier, toxic metal remediation, water and wastewater decontamination, industrial catalyst, greenhouse gas emission mitigation, and feed supplement to enhance animal health and nutrient intake effectiveness. (Bolan *et al.*, 2022). Applying biochar under drought stress can increase the water-holding capacity of soil and

Comment [AS6]: No bold in any case

Comment [AS7]: That

Comment [AS8]: Delete

Comment [AS9]: No full stop

Comment [AS10]: Delete

Comment [AS11]: No full stop

Comment [AS12]: No full stop

Comment [AS13]: . It

Comment [AS14]: Delete full stop

improve its physical and biological properties (Ali *et al.*, 2017). Biochar, a sustainable waste treatment option, is gaining attention due to its unique properties such as large surface area, stable structure, high carbon content, and long-term soil cation exchange capacity. Its cost-effective feedstock and simple preparation process make it a practical and environmentally friendly alternative to solid organic waste conversion. (Wang & Wang, 2019).

Comment [AS15]: No full stop

Comment [AS16]: and

MATERIALS AND METHODS

A field experiment was carried out by the Horticulture Research Institute, Agricultural Research Center (HRI, ARC), in the two succeeding summers of 2021 and 2022 at El Qassaseen Research Farm in the Ismailia Governorate (30° 34' 51.7"N 31° 56' 15.6"E and mean altitude above sea level 21 m), to determine the effects of using biochar (0, 2, 4, and 6 ton/fed.), irrigation at three (1, 1.5, and 2 hours per day) and their effects on the sweet marjoram (*Origanum majorana* L.) plant growth parameters (such as plant height, branch number/plant, and herb fresh and dry weights/plant), essential oil (percentage, yield/plant, and composition), total chlorophyll, as well as leaf mineral content (N, K, and proline). Sandy soil conditions are used to grow the sweet marjoram (*Origanum majorana* L.) plant.

Plant materials and cultivation:

Comment [AS17]: delete

Seedlings of marjoram (*Origanum majorana*) plants were obtained from the Farm of Medicinal and Aromatic Plants Research Department, El-Qanater El-Khayria. Seedlings were transplanted at the height of 10- 12cm with 8-10 leaves, in the experimental field at 30 cm apart on March 9th in both seasons. The experimental soil samples were randomly collected from different depths and sent to Soils; Water and Environment Research Institute laboratories (ARC) for physical and chemical analysis according to the methods of Dane & Topp (2020) and Sparks *et al.* (2020) as shown in List 1.

Comment [AS18]: ?

Comment [AS19]: and

Comment [AS20]: Table

List 1. Physical and chemical properties of the experimental soil

Comment [AS21]: Table

		The average of two seasons	
Physical analysis		Fine sand%	62.12
		Coarse sand%	25.71
		Silt%	6.05
		Clay%	6.10
		Soil texture	Sandy soil
Chemical analysis		Organic matter (g.kg-1)	0.28
		EC (dSm ¹)	2.19
		pH	7.89
		Soluble cations (meq. l ⁻¹)	
		Ca ⁺²	4.26
		Mg ⁺²	3.34
		Na+	7.98
		K+	0.88
		Available N mg/kg	7.7
		Available P (mg/kg)	2.55
		Available K	12.1
		Soluble anions (meq. l ⁻¹)	
		HCO ₃	2.51
		Cl ⁻	7.29
		SO ₄ ⁻²	5.36

Before cultivation, the experimental field was plowed and the soil leveled. The main plots were divided into four subplots and biochar was added at 0, 2, 4, and 6 tons/fed. Marjoram plants were fertilized according to the recommendation of the Ministry of Agriculture and Land Reclamation, Egypt.

Biochar is the solid product of burning biomass in pyrolysis and was obtained from Soil, Water, and Environment Research Institute, Agricultural Research Center (ARC), Egypt. It was incorporated into the soil to a depth of 5-10 cm when soil preparation in one dose and at rates were 0, 2, 4, and 6 ton/fed. The biochar composition is presented in (List 2.).

Comment [AS22]: Table

Comment [AS23]: Table

List 2. Physical and chemical properties Biochar

Biochar	PH	Organic Carbone%	N%	P%	K%
	8.6	73%	1.6	6	2.5

Comment [AS24]: Delete

The experimental design and treatments:

The experimental design was split plots, allocated in randomized complete blocks with three replications incorporating 12 treatments. Irrigation was set at the main plots under a drip irrigation system, and subplots were devoted to biochar. The irrigation was applied every other day using a surface drip irrigation system has water flow meters with a 4 l/h discharge. Marjoram plants received all normal agricultural practices whenever they needed as recommended during the two growing seasons.

Data recorded

Two cuts/ year were taken from marjoram plants, the first one was on 18th July and the second was on 26th September, in both seasons and the following characters were measured:

I- Vegetative growth: Plant height (Cm), branches number/ plant, herb fresh and weights (g/plant).

II- Essential oil

1- Essential oil production

According to **British Pharmacopoeia**, (1963), the water distillation method was performed to determine the essential oil % in marjoram fresh herb, then essential oil yield/ plant was calculated.

2- Essential Oil composition (GC- MS analysis)

The obtained essential oil produced in the second cut of the second season was analyzed in the National Research Center, using gas chromatography (Agilent 8890 GC System), coupled to a mass spectrometer (Agilent 5977B GC/MSD) and equipped with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The oven temperature was maintained initially at 50 °C, then programmed from 50 to 220°C at a rate of 5°C/min and from 220°C to 280°C at a rate of 20°C/min, then held for 5 min at 280°C. Helium was used as the carrier gas, at a flow rate of 1.0 ml/ min. The essential oil was dissolved in diethyl ether (20 µL essential oil / mL diethyl ether), and then 1 µL of this solution was injected into the GC with a split ratio of 1:50. The temperature of injection was 230 °C. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 500 amu. The isolated peaks were identified by matching them with data from the library of mass spectra (National Institute of Standards and Technology, NIST).

III- Chemical constituents:

Total nitrogen was measured as described by **AOAC (1980)**, using the modified Micro-Kjeldahl method, potassium was determined using flame photometer as described by **AOAC (1995)**, and proline content (ppm) was analyzed according to the modified method of **Bates et al. (1973)**. Total chlorophyll determination were applied in the third leaf of the plant tip (terminal leaflet) according to the method described by **Lichtenthaler & Wellburn, (1983)**.

Comment [AS25]: No comma

Comment [AS26]: Delete

Comment [AS27]: Delete

Statistical analysis

Data recorded were statistically analyzed, by using computer package program of COSTAT. Mean separation was done using least significant difference (LSD) at 5% level of significance as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

I- Vegetative growth parameters:

The study found significant variations in the vegetative growth parameters of marjoram plants, including plant height, branch count, and herb fresh and dry weights, influenced by varying irrigation levels and biochar rates. (Tables 1-4).

1- Plant height and number of branches/ plant

Data in Table (1) showed that, The study found that the highest irrigation level (2 hours/day) resulted in the tallest plants with mean values of 36.89 cm in the first and second cuts of the first and second seasons, and the maximum number of branch values (16.61, 23.03, 20.11, and 26.72 branches/plant) in the first and second seasons, respectively (Table 1).

Table 1. Effect of irrigation levels, biochar and their interactions on plant height (cm) of marjoram plant during the two successive seasons (2021&2022)

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)
1 hours/ day	29.67	31.89	33.22	34.44	32.31	30.00	32.33	33.33	35.44	32.78
1½ hours/ day	32.00	34.11	37.67	38.56	35.58	32.44	34.78	36.78	39.22	35.81
2 hours/ day	35.11	36.33	38.11	38.00	36.89	35.78	36.22	38.67	38.34	37.25
Mean (B)	32.26	34.11	36.33	37.00		32.74	34.44	36.26	37.67	
L.S.D _(0.05)	I= 0.99		B= 0.88		I*B= 1.50	I= 0.81		B= 0.48		I*B= 0.90
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.
1 hours/ day	31.89	34.11	36.89	38.34	35.31	33.78	35.45	37.11	40.11	36.61
1½ hours/ day	34.78	36.57	38.78	40.00	37.53	34.22	36.56	39.55	41.89	38.06
2 hours/ day	37.56	39.11	39.45	39.22	38.84	38.45	39.11	40.11	40.00	39.42
Mean (B)	34.74	36.60	38.37	39.19		35.48	37.04	38.92	40.67	
L.S.D _(0.05)	I= 1.16		B=0.75		I*B=	I= 0.98		B= 0.97		I*B= 1.63

Comment [AS28]: Delete

Comment [AS29]: 3-6

Comment [AS30]: 3

Comment [AS31]: delete

Comment [AS32]: 3

Comment [AS33]: 2021 and 2022

	1.38	
--	------	--

Accordingly, the tallest plants (37.00, 37.67, 39.19, and 40.67cm) and the highest number of branches (16.67, 22.93, 20.33, and 26.70 branches/ plant) were those which received the highest rate of biochar (6ton/ fed) during the 1st and 2nd cuts of both seasons, respectively.

The study found that the highest level of irrigation with biochar, at a rate of 4 tons per day, led to the tallest plants. The highest mean branch number (17.67 branch/plant) was obtained in the first cut of the first season, while in the second cut and two cuts of the second season, plants treated with a higher rate of irrigation and biochar had the highest branch numbers (23.67, 21.67, and 28.56 branch/plant, respectively).

Table 2. Effect of irrigation levels, biochar and their interactions on number of branches/ plant of marjoram plant during the two successive seasons (2021&2022)

Comment [AS34]: 4

Comment [AS35]: 2021-2022

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)	0 ton / fed.	2 ton / fed.	4 ton / fed.	6 ton / fed.	Mean (I)
1 hours/ day	11.89	12.33	13.33	14.89	13.11	17.67	18.67	20.33	21.67	19.58
1½ hours/ day	12.78	13.89	16.33	17.45	15.11	18.78	19.44	23.22	23.67	21.28
2 hours/ day	15.22	16.00	17.56	17.67	16.61	22.33	22.78	23.55	23.44	23.03
Mean (B)	13.30	14.07	15.74	16.67		19.59	20.30	22.37	22.93	
L.S.D _(0.05)	I= 1.57		B= 0.89		I*B= 1.70	I= 0.58		B= 0.53		I*B= 0.71
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.
1 hours/ day	13.67	15.56	16.67	18.33	16.06	19.67	21.89	23.55	23.78	22.22
1½ hours/ day	15.22	18.14	21.00	21.67	19.01	21.33	24.89	28.11	28.33	25.67
2 hours/ day	18.33	19.78	21.34	21.00	20.11	24.45	26.33	28.11	28.00	26.72
Mean (B)	15.74	17.83	19.67	20.33		21.82	24.37	26.59	26.70	
L.S.D _(0.05)	I= 0.74		B= 0.71		I*B= 1.19	I= 1.72		B= 0.88		I*B= 1.74

1- Herb fresh and dry weights (g/plant)

The study found that marjoram herb plants exposed to drought stress conditions (1h/day) showed a significant reduction in fresh and dry weights, followed by plants irrigated with 1.5h/day. The highest mean values for fresh weight (103.89) and dry weight (38.25, 49.87, 38.85, and 52.34 g/plant) were obtained from plants irrigated for 2h/day in the 1st and 2nd cuts of the first and second seasons, respectively.

Respecting the effect of biochar on marjoram herb fresh and dry weight data illustrated in Table (4,5) showed that increasing the addition rate of biochar resulted in a steady increase in both herb fresh and dry weight/ plant. Moreover, the maximum mean values of fresh weight (102.56, 137.72, 112.56, and 133.61 g/ plant in the two cuts of the 1st and 2nd seasons, respectively) were recorded in plants treated with biochar at 6 tons/ fed. Meanwhile, the highest values of dry weight (37.21, 49.93, 33.41, and 51.26 g/ plant) were obtained from the same treatment in the first and second cuts of the two seasons, respectively.

Comment [AS36]: Change the number

The results showed that biochar application significantly increased herb fresh and dry weights per plant under all irrigation levels, especially 6 tons/fed. The highest values were achieved with plants treated with the average level of irrigation (1:30h/day) plus 6 tons/fed. biochar, resulting in mean values of 108.67, 145.61, 119.50, and 140.06 g/plant for fresh weight and 40.41, 52.55, 41.83, and 54.34 g/plant for dry weight.

Comment [AS37]: 5

Table 3. Effect of irrigation levels, biochar and their interactions on herb fresh weight (g/ plant) of marjoram plant during the two successive seasons (2021&2022)

Comment [AS38]: 2021-2022

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton/fed.	2 ton/fed.	4 ton/fed.	6 ton/fed.	Mean (I)	0 ton/fed.	2 ton/fed.	4 ton/fed.	6 ton/fed.	Mean (I)
1 hours/ day	79.22	82.78	84.56	91.00	84.39	100.11	102.56	111.22	122.44	109.08
1½ hours/ day	92.22	97.78	105.89	108.67	101.14	113.56	132.89	144.99	145.61	134.26
2 hours/ day	97.67	101.89	108.00	108.00	103.89	125.22	134.00	145.34	145.11	137.42
Mean (B)	89.71	94.15	99.48	102.56		112.96	123.15	133.85	137.72	
L.S.D (0.05)	I= 1.30		B= 1.27		I*B=2.14	I= 1.69		B= 1.44		I*B= 2.47
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton/fed.	2 ton/fed.	4 ton/fed.	6 ton/fed.	Mean (I)	0 ton/fed.	2 ton/fed.	4 ton/fed.	6 ton/fed.
1 hours/ day	84.61	88.61	91.89	99.83	91.24	110.00	116.56	119.56	122.22	117.09
1½ hours/ day	100.17	108.61	118.89	119.50	111.79	122.11	128.11	138.03	140.06	132.08
2 hours/ day	108.89	112.22	119.00	118.33	114.61	131.22	133.67	139.11	138.55	135.64
Mean (B)	97.89	103.15	109.93	112.56		121.11	126.11	132.23	133.61	
L.S.D (0.05)	I= 1.62		B=0.85		I*B= 1.67	I=2.26		B= 1.54		I*B= 2.78

Water deficit stress can lead to decreased plant height, branch number, and herb fresh and dry weight. This is due to the adverse effects of water stress on photosynthesis, resulting in reduced CO₂ conductance in leaves due to stomatal closure. The reduction in dry matter may

also be due to cell turgor pressure, as the decrease in leaf area reduces photosynthetic material production, resulting in decreased herb fresh and dry weights (Naeimi *et al.*, 2018 & Mohammadi, *et al.*, 2021). The improvement in marjoram plant growth due to increased irrigation levels can be attributed to the proper moisture conditions maintained during growth, which facilitate cell division, metabolites, and nutrient uptake. (Mohamed *et al.*, 2022, and Farouk & Al-Huqail, 2020) on marjoram plant.

Comment [AS39]: and

Comment [AS40]: and

The study found that applying biochar significantly improved plant height, branches, and herb fresh and dry weight in stressed marjoram plants. This was due to improved soil properties, such as EC, organic matter percentage, and pH, and the increase in soil macro-aggregates. Biochar also acts as a binding agent, forming macro-aggregates that prevent disintegration into smaller ones.

Table 4. Effect of irrigation levels, biochar and their interactions on herb dry weight (g/plant) of marjoram plant during the two successive seasons (2021&2022)

Comment [AS41]: 6

Comment [AS42]: 2021-2022

Biochar (B)	First season 2021										
	First cut					Second cut					
	Biochar (B)										
Irrigation (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)	
1 hours/ day	10.96	11.09	12.03	12.38	11.61	14.91	15.01	16.40	18.17	16.12	
1½ hours/ day	12.00	13.05	14.60	16.08	13.93	16.92	18.91	20.76	21.59	19.55	
2 hours/ day	14.04	15.00	15.85	15.45	15.08	19.38	19.78	21.54	21.53	20.56	
Mean (B)	12.33	13.05	14.16	14.64		17.07	17.90	19.57	20.43		
L.S.D _(0.05)	I= 0.84B= 0.47I*B= 0.91					I= 0.62B= 0.57I*B= 0.97					
Biochar (B)	Second season 2022										
	Biochar (B)										
	Irrigation (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)	0 ton / fed.	2ton / fed.	4 ton / fed.	6ton / fed.	Mean (I)
1 hours/ day	11.29	11.86	12.35	13.50	12.25	15.82	17.55	18.05	18.86	17.57	
1½ hours/ day	13.00	14.34	15.88	16.49	14.93	18.37	18.65	20.85	21.74	19.90	
2 hours/ day	15.53	16.21	16.44	16.31	16.12	20.05	21.23	21.65	21.34	21.07	
Mean (B)	13.27	14.14	14.89	15.43		18.08	19.14	20.18	20.65		
L.S.D _(0.05)	I= 0.59		B= 0.50		I*B= 0.86		I= 0.80		B= 0.43		I*B= 0.8.

This mechanism increases mesopores formation, retaining soil water for plant uptake, improving water use efficiency, plant resistance, and drought resilience, ultimately improving plant growth and yield. (Kim, *et al.*, 2021). Abdelaal *et al.*, (2022) reported similar results with a positive effect of biochar treatments on the growth and yield parameters

Comment [AS43]: No full stop

Comment [AS44]: No full stop

of barley plants via improving stress tolerance of barley to drought-stressed conditions. **Emami, (2022)** reported a positive effect of biochar on echinacea (*Echinacea purpurea* L.) as it caused an enhancement of different physiological characteristics and yield.

Comment [AS45]: No comma

II- Essential oil production

1- Essential oil percentage and yield/ plant:

The study found that the second cut yielded the highest essential oil percentage and plant yield in both growing seasons. The lowest irrigation rate (1h/day) resulted in the highest essential oil content (0.347, 0.493, 0.393, and 0.583%) in the 1st and 2nd cuts of the first and second seasons, respectively. Conversely, plants receiving the highest irrigation rate (2h/day) yielded the highest essential oil content (0.342, 0.663, 0.436, and 0.772 ml/plant in both cuts of the two growing seasons, respectively). These findings suggest that the optimal irrigation rate is crucial for optimal plant growth.

Comment [AS46]: 7

Table 5. Effect of irrigation levels, biochar and their interactions on oil % of marjoram plant during the two successive seasons (2021&2022)

Biochar (B)	First season 2021											
	First cut					Second cut						
	Biochar (B)											
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)		
1 hours/ day	0.323	0.330	0.347	0.387	0.347	0.473	0.477	0.480	0.540	0.493		
1½ hours/ day	0.313	0.320	0.330	0.373	0.334	0.460	0.470	0.477	0.530	0.484		
2 hours/ day	0.303	0.313	0.327	0.370	0.328	0.460	0.463	0.477	0.527	0.482		
Mean (B)	0.313	0.321	0.334	0.377		0.464	0.470	0.478	0.532			
L.S.D _(0.05)	I= 0.006B= 0.004		I*B= 0.007			I= 0.006B= 0.003		I*B= 0.007				
Biochar (B)	Second season 2022											
	Biochar (B)											
	Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	
1 hours/ day	0.373	0.377	0.383	0.437	0.393	0.567	0.573	0.583	0.607	0.583		
1½ hours/ day	0.363	0.370	0.383	0.430	0.387	0.550	0.563	0.577	0.603	0.573		
2 hours/ day	0.353	0.360	0.380	0.427	0.380	0.543	0.553	0.573	0.603	0.568		
Mean (B)	0.363	0.369	0.382	0.431		0.553	0.563	0.578	0.604			
L.S.D _(0.05)	I= 0.007		B= 0.005		I*B= 0.009			I= 0.011		B= 0.005		I*B= 0.011

Comment [AS47]: 7

Also, it can be seen from the data in Table (5) that applying biochar improved both the essential oil percentage and oil yield/ plants. Moreover, the highest rate of biochar (6ton/ fed.) was the superior treatment which gave values of 0.377, 0.532, 0.431, and 0.604% for

the essential oil percentage and 0.386, 0.732, 0.485, and 0.807 ml for oil yield/plant in the two cuts of the first and second seasons, respectively.

With respect to the effect of interaction results in Tables (5) revealed that among all treatments, the highest oil % (0.387, 0.540, 0.437, and 0.607% in the 1st and 2nd cuts of the two seasons, respectively) was recorded in marjoram plants which received the lowest level of irrigation (1h/day) plus the highest rate of biochar (6 tons/fed.). On the other hand, a different trend was observed in essential oil yield/ plant (Table 6), as the most effective treatment was irrigation at 1:30h/day combined with 6 tons/fed. of biochar which recorded mean values of 0.406, 0.772, 0.514, and 0.845 ml/plant in the 1st and 2nd cuts of both seasons, respectively.

Comment [AS48]: 7

Comment [AS49]: 8

Comment [AS50]: 8

Table 6. Effect of irrigation levels, biochar and their interactions on oil yield (ml/ plant) of marjoram plant during the two successive seasons (2021&2022)

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)
1 hours/ day	0.256	0.273	0.293	0.352	0.294	0.474	0.489	0.534	0.661	0.539
1½ hours/ day	0.289	0.313	0.349	0.406	0.339	0.522	0.625	0.691	0.772	0.652
2 hours/ day	0.296	0.319	0.353	0.400	0.342	0.576	0.621	0.693	0.764	0.663
Mean (B)	0.280	0.302	0.332	0.386		0.524	0.578	0.639	0.732	
L.S.D _(0.05)	I= 0.014		B= 0.010		I*B= 0.018	I= 0.012		B= 0.008		I*B= 0.013
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.
1 hours/ day	0.316	0.334	0.352	0.436	0.359	0.623	0.668	0.697	0.741	0.683
1½ hours/ day	0.364	0.402	0.456	0.514	0.434	0.672	0.722	0.796	0.845	0.759
2 hours/ day	0.385	0.404	0.452	0.505	0.436	0.713	0.740	0.798	0.836	0.772
Mean (B)	0.355	0.380	0.420	0.485		0.669	0.710	0.764	0.807	
L.S.D _(0.05)	I= 0.016		B= 0.012		I*B= 0.021	I= 0.013		B= 0.008		I*B= 0.015

1- Essential oil components

Samples of essential oil extracted from six treatments that received different irrigation levels, biochar rates, and their interaction were chosen from the second cut of the second growing season for gas chromatography–mass spectrometry (GC- MS) analysis which showed twelve identified components (Table 7). Essential oil of marjoram plant is characterized by an

Comment [AS51]: 9

enormous variety of chemical composition due to the subsistence of several chemotypes: sabinolic, terpineloic, linoleic, carvacrolic, and mixed. In that study, cis-sabinene hydrate was the prevalent component in the essential oil of marjoram. The other most plentiful components were sabinene hydrate, terpinene-4-ol, γ -Terpinene, trans-Sabinene hydrate, terpineol, and D-limonene. The relative content of specific constituents of marjoram essential oil was affected due to the rate of irrigation and biochar applications. The highest Sabinene hydrate content (27.34%) which is considered the oil main component was obtained under well-watered conditions (2 hours/ day). On the other hand, the lowest rate of irrigation (1h/day) produced the highest mean values of total components (91.89%), trans-sabinene hydrate (8.23%), terpineol (5.25%), β -caryophyllene (3.32%), bicyclo-germacrene (2.74%), and p-Cymene (2.48%).

Comment [AS52]: Comma before this

Table 7. Effect of irrigation levels, biochar and their interactions on oil components in the second cut of the second season of marjoram plant

Comment [AS53]: 9

Biochar (B)	Irrigation (I)			Mean (B)
	1 hours/ day	1½ hours/ day	2 hours/ day	
	Sabinene			
0 ton/fed.	4.64	7.65	6.98	6.42
6 ton/fed.	7.38	7.49	7.88	7.58
Mean (I)	6.01	7.57	7.43	
	α-Terpinene			
0 ton/fed.	4.01	6.48	4.75	5.08
6 ton/fed.	5.12	5.89	4.23	5.08
Mean (I)	4.57	6.19	4.49	
	p-Cymene			
0 ton/fed.	2.78	2.30	1.42	2.17
6 ton/fed.	2.17	2.22	1.60	2.00
Mean (I)	2.48	2.26	1.51	
	D-Limonene			
0 ton/fed.	2.76	3.28	2.58	2.87
6 ton/fed.	3.21	2.75	3.34	3.10
Mean (I)	2.99	3.015	2.96	
	γ-Terpinene			
0 ton/fed.	8.04	10.41	7.58	8.68
6 ton/fed.	8.00	10.07	7.65	8.57
Mean (I)	8.02	10.24	7.62	
	Trans-Sabinene hydrate			
0 ton/fed.	8.63	6.54	4.97	6.71
6 ton/fed.	7.83	5.83	7.17	6.94
Mean (I)	8.23	6.19	6.07	
	α-Terpenolene			
0 ton/fed.	1.91	2.46	1.76	2.04
6 ton/fed.	1.67	2.40	1.66	1.91
Mean (I)	1.79	2.43	1.71	
	Sabinene hydrate			
0 ton/fed.	28.27	21.50	20.96	23.58
6 ton/fed.	24.15	19.49	33.71	25.78
Mean (I)	26.21	20.50	27.34	
	(-)-Terpinen-4-ol			
0 ton/fed.	22.03	21.57	15.44	19.68
6 ton/fed.	18.57	24.59	15.58	19.58
Mean (I)	20.3	23.08	15.51	
	α-Terpineol			
0 ton/fed.	5.87	4.60	4.24	4.90
6 ton/fed.	4.63	5.48	5.23	5.11

Mean (I)	5.25	5.04	4.74	
		β-Caryophyllene		
0 ton/fed.	2.71	2.90	2.03	2.55
6 ton/fed.	3.92	2.64	3.00	3.19
Mean (I)	3.32	2.77	2.52	
		Bicylogermacrene		
0 ton/fed.	1.77	1.96	2.21	1.98
6 ton/fed.	3.71	3.10	2.89	3.23
Mean (I)	2.74	2.53	2.55	
		Total%		
0 ton/fed.	93.42	91.65	74.92	86.66
6 ton/fed.	90.36	91.95	93.94	92.08
Mean (I)	91.89	91.80	84.43	

Respect the application of biochar, it could be noticed that the treatment of 6 ton/fed. exaggerated the mean values of total components (92.08%), sabinene hydrate (25.78%), sabinene (7.58 %), trans-Sabinene hydrate (6.94%), Terpeneol (5.11%), Bicylogermacrene (3.23%), β-caryophyllene (3.19%), and D-limonene (3.10 %) relative to the essential oil component obtained from untreated plants. The composition of marjoram essential oil was also influenced by the combined effect of water level and biochar application rate, it was clear that well-watered plants (2h/day) and treated with 6 ton biochar/ fed. recorded the highest values of total components (93.94%), sabinene hydrate (33.71%), sabinene (7.88%), and d-limonene (3.34%). Overall, the application of biochar is capable of improving the essential oil quality and quantity of marjoram under water-stress conditions. This result is in harmony with those reported by **Edris *et al.*, (2003)** who mentioned that marjoram cultivated in Egypt (German type) mostly belonged to cis-sabinene hydrate/terpinene-4-ol chemotype. **Nurzynska-Wierdak, and Dzida, (2009)** reported that Trans-sabinene hydrate and terpinene-4-ol were the predominant, components of marjoram essential oil. The study results also emphasize the outcomes that the essential oil content produced under drought conditions is either maintained or enhanced, depending on the species and magnitude of the stress. In *Origanum vulgare* L. plant, **Azizi *et al.*, (2009)** stated that water deficiency caused a significant increase in essential oil content which increases the efficiency of irrigation hence this can significantly benefit the farm's performance. Also, **Sangwan *et al.*, (2001)** in mints and sweet basil reported that essential oil content increased as a consequence of drought stress related to the increase in oil glandular hairs density, this could also be true for marjoram. **Aslani, *et al.*, (2023)** in sage (*Salvia officinalis*) mentioned that the relative expression of Sabinene synthase gene increased with increasing the severity of drought stress and the highest record was obtained under severe water deficit conditions (40% FC). In line with this study's results, the improvement of oil yield as a result of applying biochar has been

Comment [AS54]: Delete

Comment [AS55]: No comma

Comment [AS56]: No comma

Comment [AS57]: No comma

Comment [AS58]: No comma

reported in several studies. Mumivand, *et al.*, (2023) reported that biochar application considerably increased essential oil content in *Mentha piperita* L. plant. Beiranvandi *et al.*, (2022) applied biochar treatments on savory plant under water-deficiency conditions, they revealed that it was evidently, biochar exhibits an increase in the essential oil %. Changes in essential oils components % as a result of applying biochar have also been reported by Najafian & Zahedifar, (2018) who demonstrated that the monoterpenoids and sesquiterpenes content in sweet basil oil was significantly influenced by applying biochar and potassium.

Comment [AS59]: Delete

Comment [AS60]: Delete

Comment [AS61]: Delete

Comment [AS62]: and

Comment [AS63]: Delete

III- Pigments and chemical constituents

1- Total chlorophyll

Data in Table (8) showed that chlorophyll biosynthesis was affected by drought and/or biochar applications. Moreover, compared to the highest level of irrigation (2h/day), drought stress decreased total chlorophyll concentrations. The maximum values of total chlorophyll (1.301, 1.409, 1.323, and 1.519 mg/g fresh weight) were obtained with irrigation at 2h/day followed by 1.5h/ day and lastly, 1h/ day, where this effect in the two cuts of both seasons, respectively.

Comment [AS64]: 1

Table 8. Effect of irrigation levels, biochar and their interactions on total chlorophyll (mg/ g FW) of marjoram plant during the two successive seasons (2021&2022)

Comment [AS65]: 10

Comment [AS66]: 2021-2022

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)
1 hours/ day	0.845	0.848	0.978	1.162	0.958	0.957	0.998	1.114	1.270	1.085
1½ hours/ day	0.932	0.936	1.393	1.552	1.203	0.976	1.102	1.537	1.593	1.302
2 hours/ day	0.964	1.125	1.553	1.561	1.301	1.194	1.421	1.439	1.581	1.409
Mean (B)	0.914	0.970	1.308	1.426		1.042	1.174	1.363	1.481	
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.
1 hours/ day	0.862	0.978	0.990	1.214	1.011	1.024	1.162	1.200	1.276	1.166
1½ hours/ day	0.905	1.038	1.544	1.686	1.293	1.108	1.217	1.590	1.695	1.403
2 hours/ day	0.937	1.112	1.555	1.689	1.323	1.379	1.476	1.522	1.699	1.519
Mean (B)	0.901	1.043	1.363	1.530		1.170	1.285	1.437	1.557	

Regarding biochar applications, total chlorophyll concentration was increased due to applying biochar within normal or stressful conditions as compared with the untreated plants (Table 8). The highest values (1.425, 1.481, 1.530 and 1.557 mg/g FW) were a result of using biochar at the rate of 6 tons/fed. in the two cuts of both seasons, respectively.

Comment [AS67]: 10

Concerning the interaction between irrigation intervals and biochar treatments, data revealed that photosynthetic pigments were affected in response to applying different irrigation intervals and different rates of biochar. In the first season the highest values (1.561 and 1.593 mg/g FW in the 1st and 2nd cuts, respectively) were obtained from irrigation at 2h/day and 1:30 h/ day combined with the highest rate of biochar (6 ton/ fed.) whereas, in the second season the highest values (1.689 and 1.699 mg/g FW in the 1st and 2nd cuts, respectively) were recorded in the plants irrigated with 2h/day combined biochar at the rate of 6 ton/fed. Current findings prove that drought results in a dramatic reduction in chlorophyll concentration which implements a vital role in the photosynthesis pathways and biomass assembly process. Previous studies confirmed these results. **Sattar et al., (2020)** reported that chlorophyll contents in maize seedlings were significantly improved with the application of biochar under drought stress. Significant differences were noticed in the interaction treatments of watering regimes with biochar in total chlorophyll, Biochar as a soil amendment, significantly increased total chlorophyll concentration as compared with untreated plants (**El-Gamal et al., 2021**) and **Farouk et al., (2023)** on Borage plants. Drought stress decreased chlorophyll content as a result of inhibiting its biosynthesis or breakdown of chloroplast due to the chloroplast membrane damage, reduced enzymes activity (such as Rubisco), and reduced photochemical efficiency of Photosystem II (**Pandey and Shukla, 2015**).

Comment [AS68]: Delete

Comment [AS69]: Delete

2- Leaf mineral composition

The results of the analysis of leaf mineral composition in marjoram during the two cuts of the second season (Table 9) showed that nitrogen and potassium were affected by drought levels, biochar rates, and their interaction. Based on the results, the highest N (2.51& 2.73%) and K (1.00&1.15%) in the 1st and 2nd cuts, respectively, was related to the highest irrigation level (2h/day) treatment, in comparison with the lowest one (1h/day).

Comment [AS70]: 11

Table 9. Effect of irrigation levels, biochar and their interactions on leaf mineral composition and proline of marjoram plant during the two successive seasons (2021&2022)

Comment [AS71]: 11

Comment [AS72]: 2021-2022

Biochar (B)	Biochar (B)									
	First cut					Second cut				
	Nitrogen content									
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)
1 hours/ day	1.96	2.00	2.10	2.24	2.08	2.10	2.42	2.52	2.90	2.49
1½ hours/ day	2.00	2.24	2.52	2.80	2.39	2.12	2.52	2.80	3.08	2.63
2 hours/ day	2.11	2.53	2.60	2.80	2.51	2.25	2.80	2.82	3.06	2.73
Mean (B)	2.02	2.25	2.41	2.61		2.16	2.61	2.71	3.01	
Potassium content										
1 hours/ day	0.63	0.69	0.75	0.83	0.73	0.92	0.98	1.04	1.10	1.01
1½ hours/ day	0.81	0.89	0.98	1.08	0.94	0.95	1.11	1.14	1.19	1.07
2 hours/ day	.95	0.98	1.01	1.04	1.00	1.09	1.15	1.18	1.18	1.15
Mean (B)	0.80	0.85	0.91	0.98		0.99	1.08	1.12	1.16	

With respect to the effect of biochar, it was obvious that the maximum content of nitrogen (2.61 and 3.01%) and potassium (0.98 and 1.16%) in the 1st and 2nd cuts respectively, resulted from the application of biochar at the rate of 6 ton/ fed. in comparison with other treatments.

Likewise, nitrogen and potassium uptake in marjoram plants was affected by irrigation levels combined with different biochar rate application treatments. It was clear that the moderate level of irrigation (1:30 h/day) plus biochar at the rate of 6 tons/ fed. treatment resulted in the highest content of N (2.80 & 3.08%) and K (1.08& 1.19%) in the first and second cuts, respectively. It could be also noticed that in the first cut, biochar at the highest rate (6ton/ fed.) gave the same record (2.80 %) of nitrogen when combined with both irrigation levels (2 or 1:30 h/day). Similar results were reported by Mohamed, *et al.*, (2022) on marjoram plant, who revealed that biochar applications improved the N uptake, which could be due to improving soil pH, increasing soil N, and modification of microbial colonization. Javan Gholiloo, *et al.*, (2019) on valerian (*valeriana officinalis* L) reported that the reason for potassium content reduction under drought stress could be due to decreasing its solubility and subsequently reducing its absorption by plant roots, additionally, the soil colloids absorb potassium and keep it from absorption by plant roots. García-Caparrós, *et al.*, (2019) Water stress reduces leaf nitrogen concentration in *Lavandula latifolia* and *Thymus mastichina* due to reduced leaf nitrate reductase activity, which is related to photosynthesis and C skeleton availability. Biochar, derived from biomass, can enhance plant macronutrient uptake by providing high amounts of macronutrients (N, P, and K) and micronutrients (Fe, Zn, Cu, and Mn). The chemistry of biochar can also lead to the retention of K due to cation exchange capacity associated with acidic functional groups formed during oxidation on biochar

Comment [AS73]: No comma

Comment [AS74]: No comma

Comment [AS75]: No comma

Comment [AS76]: No comma

Comment [AS77]: No comma

surfaces, making K more available for plants (Agegnehu, *et al.*, 2016 and Ahmadabadi, *et al.*, 2018).

Comment [AS78]: Delete full stop

Comment [AS79]: delete

Comment [AS80]: delete

3- Proline content

Proline plays a vital role in osmotic pressure regulation in plants. It may also act as a potent scavenger of ROS and a cellular component defender against oxidative injury.

In general, proline production and accumulation significantly increase along with environmental stresses including drought stress due to the improvement of the activity of biosynthesizing enzymes, along with a decline in proline catabolism. Conditional on the results in Table (10), water deficit conditions dramatically increased the proline leaf content of marjoram plants, as a result, the highest content of proline (12.33, 10.62, 11.50, and 11.05 $\mu\text{g g}^{-1}$ in the first and second cuts of the two seasons, respectively) were recorded from the lowest irrigation rate (1h/day) conditions.

Comment [AS81]: 12

Outcomes also declared that biochar with different rates decreased the proline content under low irrigation levels in the two cuts of both seasons. Furthermore, the highest rate of biochar (6 ton/ fed.) gave the lowest values of proline 10.01, 8.34, 9.35, and 9.16 $\mu\text{g g}^{-1}$ during the two cuts of the 1st and 2nd seasons.

Table 10. Effect of irrigation levels, biochar and their interactions on proline content (mg/100g) of marjoram plant during the two successive seasons (2021&2022)

Comment [AS82]: 12

Comment [AS83]: 2021-2022

Biochar (B)	First season 2021									
	First cut					Second cut				
	Biochar (B)									
Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)
1 hours/ day	13.5	12.75	11.62	11.45	12.33	11.98	11.17	9.88	9.45	10.62
1½ hours/ day	11.24	11.01	10.94	10.71	10.98	9.23	9.06	8.72	8.54	8.89
2 hours/ day	9.39	8.46	7.99	7.86	8.43	8.22	7.75	7.33	7.03	7.58
Mean (B)	11.38	10.74	10.18	10.01		9.81	9.33	8.64	8.34	
Biochar (B)	Second season 2022									
	Biochar (B)									
	Irrigation (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.	Mean (I)	0 ton / fed.	2ton/ fed.	4 ton/ fed.	6ton/ fed.
1 hours/ day	12.63	11.59	11.04	10.75	11.50	11.24	11.2	11.1	10.86	11.05
1½ hours/ day	10.29	9.76	9.33	9.22	9.65	10.16	9.20	9.20	9.17	9.43
2 hours/ day	8.14	8.12	8.09	8.07	8.11	8.5	8.12	7.41	7.42	7.86
Mean (B)	10.35	9.82	9.49	9.35		9.97	9.51	9.24	9.16	

Regarding the effect of the interaction between irrigation levels and biochar rates on proline content, it was clear that the application of biochar under drought stress conditions caused notable decreases in proline content during the 1st and 2nd cuts of the two seasons (giving 7.86, 7.03, 8.07, and 7.42 $\mu\text{g g}^{-1}$) compared to untreated ones. As expected, proline content in plants increases due to drought stress, as reported in many studies by **Javan Gholiloo, et al.** (2019) on valerian plant, **Shin, et al.** (2021) on lettuce, and **Wani, et al.** (2021). These findings are also in agreement with those reported by **Yildirim, et al.** (2021) who revealed that proline content of cabbage seedlings was significantly influenced by biochar treatments under different irrigation levels as lower irrigation caused an increase in proline. However, when biochar was applied proline content was reduced.

Comment [AS84]: Delete

Comment [AS85]: delete

Comment [AS86]: delete

Comment [AS87]: Delete

Comment [AS88]: delete

Comment [AS89]: delete

Comment [AS90]: DeLETe

Climate change has intensified drought stress, disrupting metabolism and plant development. This accelerates stress responses, causing adverse effects on growth, yield, morphology, and physio-biochemical characteristics of plants. It reduces cell turgor pressure, preventing cell enlargement and division, and increases catalase, peroxidase activity, and reactive oxygen species production. These effects are imponderable or eliminate, making it crucial to address climate change's effects. (Shao *et al.*, 2007, Hafez, *et al.*, 2020, Farooq *et al.*, 2020; Farouk and Omar, 2020; and Farouk & Al-Huqail 2020). In addition, growth-inhibitory hormones (abscisic acid) increase while growth hormones such as auxin) and gibberellin decrease in several plants (De Souza *et al.*, 2006; Maria *et al.*, 2008). On the other, photosynthetic activity decrease due to the closure of the stomata thus reducing plant growth (Ma *et al.*, 2016). Therefore drought stress poses a serious global threat to crop productivity. Consequently, it is globally urgently needed to tackle it by using different measures to improve crop productivity under drought conditions. Among these measures, biochar has been widely used to improve soil health and stimulate crop yield under drought-stress conditions (Wu, *et al.*, 2023). Biochar exhibits excellent adsorption performance due to its surface oxygen-containing functional groups, high surface complexation, and ion exchange mechanisms. It attracts and stores metal ions due to its large number of pores and high surface energy. Biochar also contains high levels of carbon, hydrogen, and oxygen, with low levels of other elements. It improves soil quality by improving structure, aggregate stability, organic matter, nutrient holding capacity, and microbial activities. It also stabilizes soil

Comment [AS91]: ,

Comment [AS92]: Delete

Comment [AS93]: and

Comment [AS94]: Comma after this

Comment [AS95]: Delete

carbon, enhances nitrogen uptake, decreases mineral nitrogen leaching, increases electrical conductivity, water availability, and water use efficiency. This enhances soil nutrient availability and tolerance to abiotic stresses. (Ahmed, *et al.*, 2019, Zhou *et al.*, 2017, Bolan *et al.* 2022 and Osman, *et al.*, 2022). Biochar-mediated soil improvements significantly enhance photosynthetic activity, chlorophyll synthesis, gene expression, stress-responsive proteins, and hormonal balance. They also improve antioxidant activities and osmoprotectants, boosting tolerance against osmotic and ionic stresses, making them a sustainable strategy for enhancing plant growth under drought stress conditions. (Khan, *et al.*, 2021 and Wu, *et al.*, 2023).

Comment [AS96]: Delete

Comment [AS97]: Delete

Comment [AS98]: Delete full stop

Comment [AS99]: Delete

Comment [AS100]: delete

CONCLUSION

In this study, it was obvious that applying biochar with all rates extenuating the harmful impact of water deficit on marjoram plants specifically at the rate of 6ton/fed. which significantly improved the growth, yield, and essential oil productivity of marjoram plants in the two cuts of both growing seasons. In conclusion, according to this study's outcomes, the application of biochar as a soil amendment to reduce the water deficit stress effects on marjoram plant can be suggested in sandy soil. Nevertheless, more research is required to evaluate the role of biochar on soil microorganisms, different types of soils, and various medicinal and aromatic plants in different climate zones, especially in arid and semi-arid areas.

Comment [AS101]: Comma before this

What about time of irrigation

REFERENCES

Abdelaal, K.; Alamrey, S.; Attia, K. A.; ELrobh, M.; ELnahhas, N.; Abou EL-Yazied, A., & Ibrahim, M.A. (2022). The pivotal role of biochar in enhancement soil properties, morphophysiological, and yield characters of barley plants under drought stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(2), 12710-12710.

Comment [AS102]: No bold in any case

Comment [AS103]: Whether italic or not

Agegnehu, G.; Bass, A.M.; Nelson, P. N., & Bird, M. I. (2016). Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas

emissions in a tropical agricultural soil. *Science of the Total Environment*, 543, 295-306.

Ahmadabadi, Z.; Zarei, M.; Yasrebi, J.; Ronaghi, A.; Ghasemi, R.; Saharkhiz, M. J.; ... & Schnug, E. (2018).Influence of arbuscular mycorrhiza fungi, rice-husk-driven biochar and compost on dry matter yield, nutrients uptake and secondary metabolites responses of Iranian borage *Echium amoenum* Fisch & CA Mey. *Journal of Cultivated Plants/Journal für Kulturpflanzen*, 70(12).

Comment [AS104]: Whether italic or not

Comment [AS105]: Pages not given

Ahmed, R.; Li, Y.; Mao, L.; Xu, C.; Lin, W.; Ahmed, S., & Ahmed, W. (2019).Biochar effects on mineral nitrogen leaching, moisture content, and evapotranspiration after 15N urea fertilization for vegetable crop. *Agronomy*, 9(6), 331.

Ali, S.; Rizwan, M.; Qayyum, M.F.; Ok, Y.S.; Ibrahim, M.; Riaz, M.; Arif, M.S.; Hafiz, F.; A-Wabel, M.I., & Shahzad, A. N. (2017).Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environmental Science and Pollution Research*, 24(14), 12700-12712.

AOAC. (1980). Official methods of analysis, 13th edition, Association of Official Analytical Chemists, Washington. DC.

AOAC (1995). Official Methods of Analysis.16th Ed. Association of Official Analytical Chemists, Inc., Virginia, USA. 1080-1344.

Aslani, Z.; Hassani, A.; Mandoulakani, B.A.; Barin, M., & Maleki, R. (2023).Effect of drought stress and inoculation treatments on nutrient uptake, essential oil and expression of genes related to monoterpenes in sage (*Salvia officinalis*). *Scientia Horticulturae*, 309, 111610.

Azizi, A.; Yan, F., and Honermeier, B. (2009). Herbage yield: essential oil content and composition of three oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. *Ind. Crops Prod.* 29:554–561

Bates, L.S.; Waldren, R.P. & Teare, I.D. (1973). Rapid determination of proline for water stress studies. *Plant Soil*, 39:205-207.

- Beiranvandi, M.; Akbari, N.; Ahmadi, A.; Mumivand, H., & Nazarian, F. (2022).** Biochar and super absorbent polymer improved growth, yield, and phytochemical characteristics of *Satureja rechingeri* Jamzad in water-deficiency conditions. *Industrial Crops and Products*, 183, 114959.
- Bodner, G.; Nakhforoosh, A., & Kaul, H.P. (2015).** Management of crop water under drought: a review. *Agro. Sustain. Develop.* 35:401-442.
- Bolan, N.; Hoang, S. A.; Beiyuan, J.; Gupta, S.; Hou, D.; Karakoti, A.; Joseph, S.; Jung, S.; Kim, K.H.; Kirkham, M. B.; Kua, H.W.; Kumar, M.; Kwon, E.E.; Ok, Y.S.; Perera, V.; Rinklebe, J.; Shaheen, S.M.; Sarkar, P.; Sarmah, A. K.& Van Zwieten, L. (2022).** Multifunctional applications of biochar beyond carbon storage. *International Materials Reviews*, 67(2), 150-200.
- Bouyahya, A.; Chamkhi, I.; Benali, T.; Guaouguaou, F.E.; Balahbib, A.; El Omari, N.; Taha, D.; Belmehdi, O.; Gokhan, Z., and El Menyiy, N. (2021).** Traditional use, phytochemistry, toxicology, and pharmacology of *origanum majorana*. *J. Ethnopharmacol.*, 265: 113318.
- British Pharmacopoeia (1963).** Determination of Volatile Oil in Drugs. The Pharmaceutical Press, London, UK, 1210 p.
- Dane, J.H., & Topp, C.G. (Eds.). (2020).** Methods of soil analysis, Part 4: Physical methods (Vol. 20). John Wiley & Sons.
- De Souza, A.; Garcí, D.; Sueiro, L.; Gilart, F.; Porrás, E., & Licea, L. (2006).** Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. *Journal of the Bioelectromagnetics Societ.*, 27(4), 247-257.
- Duletić-Laušević, S.; Aradski, A.A.; Kolarević, S.; Vuković-Gačić, B.; Oalđe, M.; Živković, J.; Šavikin, K., and Marin, P.D. (2018).** Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of *Origanum majorana* L. (Lamiaceae) extracts. *J. Appl. Bot. Food Qual.*, 91, 126-134.

- Edris, A. E.; Shalaby, A., & Fadel, H. M. (2003).** Effect of organic agriculture practices on the volatile aroma components of some essential oil plants growing in Egypt II: sweet marjoram (*Origanum marjorana* L.) essential oil. *Flavour and Fragrance journal*, 18(4): 345-351.
- El-Gamal, S.M.A.; Serag El-Din, W. M.; Farouk, S., & Mokhtar, N.A.Y.O. (2021).** Integrated effects of biochar and potassium silicate on borage plant under different irrigation regimes in sandy soil. *J. Hortic. Sci. Orn. Plants*. 13 (1): 60-76.
- Emami, T. (2022).** Effect of biochar and salicylic acid on physiological traits and yield of echinacea (*Echinacea purpurea* L.) under non-stress and drought stress conditions. *Crop Science Research in Arid Regions*, 4(1), 229-243.
- Farooq, A.; Bukhari, S.A.; Akram, N.A.; Ashraf, M.; Wijaya, L.; Alyemeni, M.N., and Ahmad, P. (2020).** Exogenously applied ascorbic acid-mediated changes in osmoprotection and oxidative defense system enhanced water stress tolerance in different cultivars of safflower (*Carthamus tinctorious* L.). *Plants* 9, 104.
- Farouk, S. and Al-Huqail, A.A. (2020).** Sodium nitroprusside application regulates antioxidant capacity, improves phytopharmaceutical production and essential oil yield of marjoram herb under drought. *Industrial Crops & Products*, 158: 1-11.
- Farouk, S.; Al-Huqail, A. A., & El-Gamal, S. M. (2023).** Potential Role of Biochar and Silicon in Improving Physio-Biochemical and Yield Characteristics of Borage Plants under Different Irrigation Regimes. *Plants*, 12(8), 1605.
- Farouk, S., & Omar, M.M. (2020).** Sweet basil growth, physiological and ultrastructural modification, and oxidative defense system under water deficit and silicon forms treatment. *J. Plant Growth Regul.* 39, 1307-1331.
- García-Caparrós, P.; Romero, M. J.; Llanderal, A.; Cermeño, P.; Lao, M. T., & Segura, M. L. (2019).** Effects of drought stress on biomass, essential oil content, nutritional parameters, and costs of production in six Lamiaceae species. *Water*, 11(3), 573.

- Gomez, K.A., & Gomez, A.A. (1984).** Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons, New York, USA, 680 p.
- Hafez, Y.; Attia, K.; Alamery, S.; Ghazy, A.; Al-Doss, A.; Ibrahim, E.; Rashwan, E.; El-Maghraby, L.; Awad, A., & Abdelaal, K. (2020).** Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agronomy*, 10(5): 630.
- Hameed, M.; Ahmadalipour, A., & Moradkhani, H. (2020).** Drought and food security in the Middle East: An analytical framework. *Agricultural and Forest Meteorology*, 281,107816.
- Ietswaart, J. H. (1980).** A taxonomic revision of the genus *Origanum* (Labiatae). Leiden Botanical series, 4(1): 1-153.
- Javan Gholiloo, M.; Yarnia, M.; Ghorttapeh, A. H.; Farahvash, F., & Daneshian, A. M. (2019).** Evaluating effects of drought stress and bio-fertilizer on quantitative and qualitative traits of valerian (*Valeriana officinalis* L.). *Journal of Plant Nutrition*, 42(13), 1417-1429.
- Khan, Z.; Khan, M. N.; Zhang, K.; Luo, T.; Zhu, K., & Hu, L. (2021).** The application of biochar alleviated the adverse effects of drought on the growth, physiology, yield, and quality of rapeseed through regulation of soil status and nutrient availability. *Industrial Crops and Products*, 171, 113878.
- Kim, Y. J.; Hyun, J.; Yoo, S. Y., & Yoo, G. (2021).** The role of biochar in alleviating soil drought stress in urban roadside greenery. *Geoderma*, 404, 115223.
- Lichtenthaler H.K., and Wellburn A.R. (1983).** Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 603:591-592.
- Ma, D.; Sun, D.; Wang, C.; Qin, H.; Ding, H.; Li, Y., & Guo, T. (2016).** Silicon application alleviates drought stress in wheat through transcriptional regulation of multiple antioxidant defense pathways. *Journal of Plant Growth Regulation*, 35, 1-10.

- Mahmoud, M.A. (2017).** Impact of climate change on the agricultural sector in Egypt. Conventional Water Resources and Agriculture in Egypt. 213-227.
- Maria, A. M.; Gendy, A. A.; Selim, A. H., & Abd El-All, A. M. (2008).** Response of wheat plants grown under water stress in relation to Jasmonic acid. Minufiya Journal of Agricultural Research, 33(6), 1355-1375.
- Meyers, M. (2005).** Oregano and Marjoram: The Herb Society of America. Guide to the genus *Origanum*. Kintland, Ohio: The Herb Society of America.
- Mohamed, M. F.; Abdallah, M. A., and Saad, S. A. H. (2022).** Influence of different rates of irrigation and fertilization on productivity of *Origanum majorana* L. grown in sandy soil. World Journal of Agric. Sci., 18 (4): 185-199.
- Mohammadi, H.; Dizaj, Leila, A.; Aghaee, A., & Ghorbanpour, M. (2021).** Chitosan-mediated changes in dry matter, total phenol content and essential oil constituents of two origanum species under water deficit stress. Gesunde Pflanzen, 73(2): 181-191.
- Mumivand, H.; Izadi, Z.; Amirizadeh, F.; Maggi, F., & Morshedloo, M. R. (2023).** Biochar amendment improves growth and the essential oil quality and quantity of peppermint (*Mentha × piperita* L.) grown under wastewater and reduces environmental contamination from wastewater disposal. Journal of Hazardous Materials, 446, 130674. <https://doi.org/10.1016/j.jhazmat.2022.130674>.
- Naeimi, T.; Fahmideh, L., & Fakheri, A. B. (2018).** The impact of drought stress on antioxidant enzymes activities, containing of proline and carbohydrate in some genotypes of durum wheat at seedling stage. J. Crop Breed, 10(26):22-31.
- Najafian, S., & Zahedifar, M. (2018).** Productivity, essential oil components and herbage yield, of sweet basil as a function of biochar and potassium-nano chelate. Journal of Essential Oil Bearing Plants, 21(4), 886-894.
- Nurzynska-Wierdak, R., & Dzida, K. (2009).** Influence of plant density and term of harvest on yield and chemical composition of sweet marjoram (*Origanum majorana* L.). Acta Sci. Pol., Hortorum Cultus, 8(1): 51-61.

- Osman, A. M.; Rekaby, S. A.; Khalafalla, M. Y., & Awad, M. (2022).** The combined effect of compost and biochar application on carbon sequestration and some soil properties. *Archives of Agriculture Sciences Journal*, 174-191.
- Pandey, V., & Shukla, A. (2015).** Acclimation and tolerance strategies of rice under drought stress. *Rice science*, 22(4), 147-161.
- Sangwan, N.S.; Farooqi, A.H.A.; Shabih, F., and Sangwan, R.S., 2001.** Regulation of essential oil production in plants. *Plant Growth Regul.* 34, 3–21.
- Sattar, A.; Sher, A.; Ijaz, M.; Ul-Allah, S.; Butt, M.; Irfan, M., ... & Cheema, M. A. (2020).** Interactive effect of biochar and silicon on improving morpho-physiological and biochemical attributes of maize by reducing drought hazards. *Journal of Soil Science and Plant Nutrition*, 20, 1819-1826.
- Shao, H. B.; Jiang, S. Y.; Li, F. M.; Chu, L. Y.; Zhao, C. X.; Shao, M. A.; Zhao, X. N., & Li, F. (2007).** Some advances in plant stress physiology and their implications in the systems biology era. *Colloids and surfaces B: Biointerfaces*, 54(1): 33-36.
- Shin, Y. K.; Bhandari, S. R.; Jo, J. S.; Song, J. W., & Lee, J. G. (2021).** Effect of drought stress on chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities in lettuce seedlings. *Horticulturae*, 7(8), 238.
- Sparks, D. L.; Page, A. L.; Helmke, P. A. & Loeppert, R. H. (Eds.) (2020).** Methods of soil analysis, part 3: Chemical methods (Vol. 14). John Wiley & Sons.
- UN, 2015.** Overcoming population vulnerability to water scarcity in the Arab Region: Population and Development Report Issue No. 7. Beirut-United Nations.
- Wang, J., & Wang, S., (2019).** Preparation, modification and environmental application of biochar: A review, *Journal of Cleaner Production*, 227: 1002-1022.
- Wang, L.; Chen, W. & Zhou, W. (2014a).** Assessment of future drought in Southwest China based on CMIP5 multimodel projections. *Adv. Atmos. Sci.*, 31:1035-1050.

Wani, K. I.; Naeem, M.; Castroverde, C. D. M.; Kalaji, H. M.; Albaqami, M., & Aftab, T. (2021).Molecular mechanisms of nitric oxide (NO) signaling and reactive oxygen species (ROS) homeostasis during abiotic stresses in plants. *International Journal of Molecular Sciences*, 22(17), 9656.

Wu, Y.; Wang, X.; Zhang, L.; Zheng, Y.; Liu, X., & Zhang, Y. (2023).The Critical Role of Biochar to Mitigate the Adverse Impacts of Drought and Salinity Stress in Plants. *Frontiers in Plant Science*, 14, 1218.

Yildirim, E.; Ekinci, M., & Turan, M. (2021). Impact of biochar in mitigating the negative effect of drought stress on cabbage seedlings. *Journal of Soil Science and Plant Nutrition*, 21(3), 2297-2309.

Zahedi, H.; Noormohammadi, G.; Rad, A.S.; Habibi, D. & Boojar, M. M. A. (2009). The effects of Zeolite and foliar applications of selenium on growth, yield and yield components of three canola cultivars under drought stress. *World Applied. Sciences journal*, 7(2): 255–262.

Zhou, N.; Chen, H.; Xi, J.; Yao, D.; Zhou, Z.; Tian, Y., & Lu, X. (2017).Biochars with excellent Pb (II) adsorption property produced from fresh and dehydrated banana peels via hydrothermal carbonization. *Bioresource Technology*, 232, 204-210.

No bold author(s) name and check the journal italic or not so follow the pattern of the journal