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Original Article

The Effect of biochar Application and Planting Pattern on the Physiological and Biochemical Traits of Garden Thyme (*Thymus vulgaris* L.) at Different Levels of Irrigation

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ABSTRACT

Water scarcity is a principal limitation for production in arid and semi-arid regions. Biochar increases the water-holding capacity of the soil in drought-stress conditions. To investigate the effect of biochar and planting patterns on the physiological, biochemical, and thyme (*Thymus vulgaris* L.) essential oil traits, an experiment was carried out as a factorial split-plot based on a randomized complete block design for two crop years (2017-2019). The findings revealed that furrow irrigation treatments, both fixed and variable, decreased the flowering branches' yield, relative water content, total chlorophyll, Chlorophyll a, and thymol percentage. Instead the essential oil yield, proline content, soluble sugar content, chlorophyll b, and carvacrol under these treatments increased. The decrease in flowering branches yield and the relative water content in variable alternate furrow irrigation was lower compared to fixed alternate furrow irrigation. Biochar application reduced the effects of drought stress caused by variable alternate furrow irrigation and fixed alternate furrow irrigation treatments but had no significant impact on chlorophyll b, thymol percentage, and total chlorophyll. The highest carvacrol and thymol percentages were obtained in fixed alternate furrow irrigation, variable alternate furrow irrigation treatments, and irrigation of all furrows, respectively. The planting pattern did not affect flowering branch yield, relative water content, soluble sugars content, and proline content. The highest essential oil yield (24.73 kg/ha) was obtained in the treatment combination of variable alternate furrow irrigation, biochar application, and Planting double rows of thyme on the ridge. The study recommended that farmers must observe alternate furrow irrigation methods and biochar application (amount 8 t/ha) as a better option in the limited water environment.

Keyword: Biochar, Fixed Alternate Furrow Irrigation, Variable Alternate Furrow Irrigation, Thyme, Water stress

ABBREVIATION

FI: Full irrigation, FAFI: Fixed Alternate Furrow Irrigation, VAFI: Variable Alternate Furrow Irrigation, POR: planting a single row of thyme on the ridge, PTR: planting double rows of thyme on the ridge, BC: Biochar application, NBC: No application of biochar, FBY: Flowering branch yield, SUGC: Soluble sugars content, RWC: relative leaf water content, Chl a: Chlorophyll a, Chl b: Chlorophyll b, T Chl: Total Chlorophyll, EOY: Essential oil yield, CVR: Carvacrol, and THYM: Thymol.

INTRODUCTION

In recent years, water resources have become limited worldwide for crop production, particularly in response to the over-harvesting of water reservoirs and climate change (1). Therefore, drought stress is one of the most important factors limiting the performance of agricultural plants worldwide (2).

However, in arid and semi-arid regions, irregular rainfall and lack of water cause drought stress in rainfed and irrigated crops (3). Hence, drought is a principal concern delimiting plant growth and crop productivity, which affects leaf photosynthesis, numerous dimensions of plant physiology and biochemistry (4).

Among the proposed solutions to reduce the effect of drought stress on crops, it is possible to mention the modification of the physical conditions of the soil, especially its hydrological characteristics (5). Many water shortage problems can be solved by increasing the moisture-holding capacity in the soil (6).

Researchers have become interested in biochar as a carbon-rich organic amendment material (7) since it is significantly more resistant to microbial breakdown than organic materials and has an aromatic structure (8).

Biochar is created by pyrolyzing organic matter in a low-oxygen environment and has gained popularity as a soil supplement worldwide (9).

Adding biochar as a form of organic matter is a new way to add organic matter to arid and semi-arid soils that have been looked into to make the best use of limited agricultural water resources (10).

The physical characteristics of biochar, such as its porous structure and high specific surface area, increase the porosity of the entire soil and the amount of water that can be used by the plant (11). And it improves the water relations of plants during the dry summer months (12).

According to Lu et al. (13), biochar may enhance the chlorophyll content of plant leaves and improve agricultural productivity. Also, Ahmad et al. (14) found that biochar is a promising approach for alleviating drought-related difficulties. High plant populations can lead to water and nutrient stress in plants (15). Also, choosing the proper density can increase the resistance of plants against some stress factors (16). Adjusting inter-row and intra-row distances is one of the most meaningful agricultural operations to increase crop yield and reduce the competitive power of weeds (17). Plant density manipulation strongly affects growth parameters. Therefore, it is necessary to determine the optimal density of the plant to produce good performance in crops (18).

Adjusting inter-row and intra-row distances is one of the most consequential agricultural operations to increase crop yield and reduce the competitive power of weeds (17).

In Iran, surface furrow irrigation is the most common method used for crop cultivation. This type of irrigation system has a low application efficiency (45-60%) and causes significant water losses, primarily due to excessive deep percolation from irrigated fields.

Accordingly, utilizing the limited water resources necessitates fundamental changes in irrigation methods and water management. A long-term perspective on the depletion of freshwater resources, particularly in arid and semi-arid regions, underscores the urgency of implementing innovative irrigation strategies and agricultural water management (19).

According to Mitchell et al. (20), deficit irrigation has been used as a water-saving method in agricultural production to increase benefits and water use efficiency.

Low irrigation is one of the irrigation management strategies in which the prescribed amount of water is decreased to produce the optimal crop (21).

Another option to increase water productivity through the deficiency level is the intermittent and fixed furrow irrigation system (22).

Alternate furrow irrigation (AFI) is regarded as one of the efficient methods for reducing irrigation and water application costs while increasing crop production (23).

In regions with a scarcity of irrigation water and rainfall, fixed-furrow irrigation is the preferred method of irrigation water management (24).

Thyme, due to its flexibility to a variety of climatic conditions, grows throughout North America, Africa, Asia, Central and Southern Europe (25). It is extensively employed in different industries, including the pharmaceutical, cosmetic, food, and health sectors. At the same time, Thyme yield can be influenced by agricultural operations and environmental factors (26).

According to the above, the purpose of this study was to the effect of biochar application and planting pattern on the essential oil characteristics, Physiological traits, and yield by thyme at different irrigation levels.

MATERIAL AND METHODS

Weather, Soil, and Research Location

The experiment was carried out as a factorial split-plot based on a randomized complete block design with three replications for two crop years (2017-2019) on the research farm of the Faculty of Agriculture and Natural

Resources, Karaj Branch, Islamic Azad University, Mahdasht, Iran (35°43.733′ N, 50°49.721′ E, 1170 meters above sea level).

According to the Koppen climate classification, the region has a mediterranean climate with a hot summer, with an average annual rainfall of 251.2 mm.

The average annual temperature is 12˚C, the average maximum annual temperature is 43˚C and the average minimum annual temperature is -28˚C. Soils in these regions are in the range of alkaline to medium, and their water class is S_1C_1 (They have no restrictions in terms of agriculture).

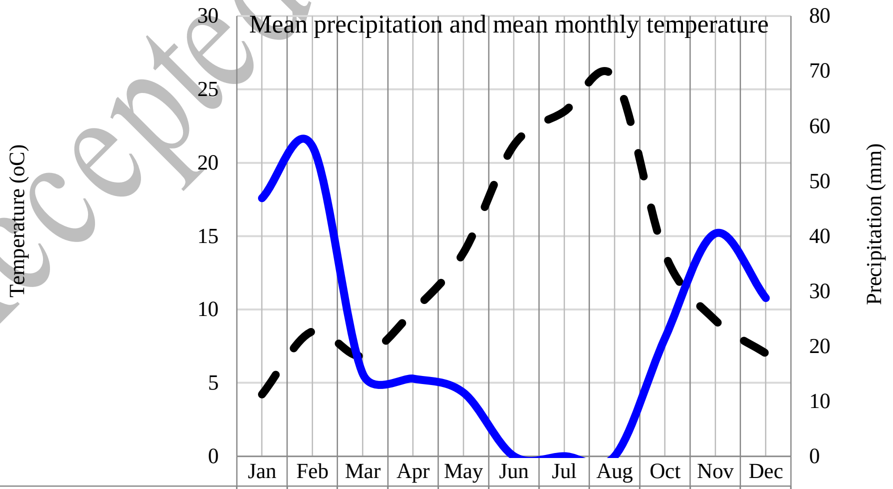
Before planting, soil samples were collected for analysis at a depth of 0-30 cm from the experimental area. The available phosphorus in the soil was measured using the Olsen method (27).

Based on the soil textural triangle, the soil texture was silty clay. Based on fertilizer recommendation, phosphorus, and potassium fertilizers were not used because the amount of absorbable of these elements in the soil was higher than the critical level (Table 1). In addition, other physical characteristics of the soil, such as the field capacity (FC), maximum allowable depletion (MAD) and permanent wilting point (PWP), were measured by random sampling in the soil laboratory. Based on this, FC, MAD, and PWP were determined as 27.4%, 80%, and 13.9%, respectively.

The physicochemical properties of the soil of the experimental location are listed in Table 1. The average monthly rainfall and temperature during the 2017-2019 crop years are presented in Fig 1.

Table 1 Physiochemical features of study site soil (depth 0-30 cm)

Soli properties	Value (2017-2018)	Status	Value (2018-2019)	Status
PH	7.5	Weakly alkaline	7.6	Weakly alkaline
EC (ds m^{-1})	1.02	Salt-free	1.03	Salt-free
Water class	S_1C_1	No restrictions	S_1C_1	No restrictions
Total N (%)	0.077	Deficient	0.080	Deficient
Organic carbon (%)	0.70	Deficient	0.68	Deficient
Olsen's P (mg kg^{-1})	15.2	Sufficient	15.3	Sufficient
Available K (mg kg^{-1})	420	Sufficient	440	Sufficient
Silt (%)	42	-	41	-
Clay (%)	44	-	42	-
Sand (%)	14	-	16	-
Soil texture	Silty Clay		Silty Clay	



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Oct	Nov	Dec
▬ ▬ Mean Monthly Temperature	4.2	8.5	6.8	9.9	13.9	21.2	23.5	25.8	13.8	9.3	7.0
▬ Mean Monthly precipitation	46.9	56.4	15.0	14.1	11.6	0.0	0.0	0.0	21.5	40.5	28.8

Fig. 1 Total monthly precipitations and mean monthly temperature in the years 2017–2019.

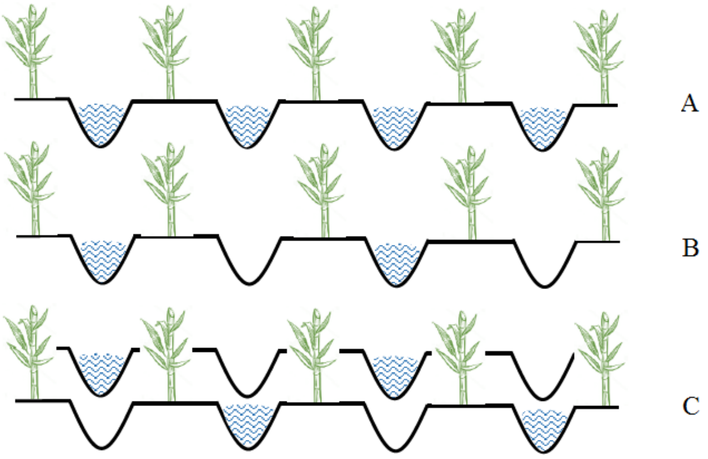


Fig. 2 (A) Conventional, (B) fixed and (C) variable alternate furrow irrigation. Adapted from Adib et al. (28).

Experimental Setup

The main plot included three irrigation regimes ((I_1) Full irrigation (Conventional furrow irrigation) (control), (I_2) Fixed Alternate Furrow Irrigation (FAFI), and (I_3) Variable Alternate Furrow Irrigation (VAFI) (Fig 2). Two factors, biochar and cultivation pattern, were factorially placed in the subplots. Biochar factor includes (1) No application of biochar (control) (NBC), (2) biochar application (8 t/ha^{-1}; Before planting mixed with soil) (BC), and planting pattern factor includes (1) Planting a single row of thyme on the ridge (POR), and (2) Planting double rows of thyme on the ridge (PTR).

For two years preparing the seedbed was done. The ground was late spring plowed, and then bedded and ridged with the disk and leveler.

The dimensions of each experimental plot were 2.4 × 4 m^{-2}. A space of 2 m was left between the blocks to avoid margin effects. A distance of 1.8 m between the main plots and 0.6 meters between the subplots was set in all the blocks. The arrangement of the plants inside each plot was crosswise (rhombus).

Before planting, biochar prepared by Soil and Water Research Institute, Iran to a depth of 15 cm was mixed with the soil (At the rate of 8 t/ha). Garden Thyme seeds (*Thymus vulgaris* L.), supplied by the Isfahan Pakan-Bazr company, Iran, were selected as the experimental plant material (Table 2). Following land preparation, the seed-planting operation was performed manually in the autumn season (October 1) during the 2017-2019 both crop years, Similarly.

The plots in double-row cultivation included three planting lines, with a row spacing of 30 cm, and in one-row cultivation, there were five planting lines, with a row spacing of 60 cm. The distance between the ridges in both planting patterns was 60 cm.

Three weeks after planting, thinning and replanting operations were done. The plants were watered weekly from planting until the establishment of seedlings. Both manual weed management and hoeing of the inter-rows were used as plant protection measures; no pesticides or herbicides were used.

After the establishment of plants and after the first stage of weed weeding, according to the soil test data (Table 1), the amount of fertilizer required for each experimental plot was based on 60 kg/ha^{-1} urea fertilizer was used.

Potassium and phosphorus fertilizers were not used because the absorbable amount of these elements was higher than the critical level in the soil (Table 1).

Table 2 Characteristics of the studied cultivar

Characteristics	
Botanical Name	Thymus vulgaris L.
Soil Preference	Dry Soil
Growth Rate	Moderate
Country Or Region Of Origin	Mediterranean region, Southwestern Europe and Southeastern Italy
Type	Hybrid
Plant Leaf Characteristics	Broadleaf Evergreen

Season:	Both Spring & Fall
Pollination	Primarily entomophilous, Rarity self-pollination
Primary pollinators	Bees, Butterflies
Bloom Time	Late spring, Summer
Physiological maturity	Early season
Plant Type	Herbaceous Perennial
Life Cycle	Perennial
pH	Neutral (6.0-8.0 (to alkaline ((٨٫٠<
Fruit Type	Schizocarp
Flower Inflorescence	Raceme
Leaf Type	Simple
Leaf Arrangement	Opposite
Resistance To Challenges	Drought

The time and amount of irrigation treatments were applied in the spring according to the standard irrigation requirements of crops (prepared by Soil and Water Research Institute, Iran) (Tables 3 and 4).

In this tables (3 and 4), the amount of ETc (Crop Evapotranspiration), and SIR (Standard Irrigation Requirement) or Net irrigation requirement were calculated from the following formula:

$ETc=Kc \times ET_o$

Where ET_o is Reference Crop Evapotranspiration (mm/day), Kc is Crop Coefficient (dimensionless), and ETc is Crop evapotranspiration or crop water use (mm/day) (29).

$SIR=ETc-Er$

Where SIR is the Standard irrigation requirement (mm/day), ETc is Crop evapotranspiration or crop water use (mm/day), and Er is effective rainfall (mm/day) (30).

Table 3 Standard irrigation requirement (2017-2018)

Date		Plant profile		Evapotranspiration (mm/dec)		Effective rainfall (mm/dec)	Standard Irrigation requirement (mm/dec)
Month	Decade	Growth stage	Kc	ET_o	ETc		
April	2	Ini	0.29	34.62	10.04	1.5	8.54
April	3	Ini	0.32	42.48	13.59	16.24	0
May	1	Dev	0.45	53.56	24.10	3.63	20.47
May	2	Dev	0.62	50.13	31.08	5.7	25.38
May	3	Dev	0.8	69.30	55.44	0	55.44
June	1	Dev	0.98	72.18	70.74	0	70.74
June	2	Dev	1.13	78.92	89.18	0	89.18
June	3	Mid	1.12	85.79	96.08	0	96.08
July	1	Mid	1.12	78.94	88.41	0	88.41
July	2	Mid	1.13	74.56	84.25	0	84.25
July	3	End	0.94	73.84	69.41	8.8	60.61
August	1	End	0.73	52.59	38.39	0	38.39
Total				766.91	670.71	35.87	637.49

ER (Effective rainfall), ETc (Crop Evapotranspiration), ET_o (Reference evapotranspiration), Kc (Crop coefficient), Ini (Initial stage of growth), Dev (Development stage), Mid (Mid-season of growth), End (End of growth), Dec (Decade), Crop Evapotranspiration (ETc).

Table 4 Standard irrigation requirement (2018-2019)

Date		Plant profile		Evapotranspiration (mm/dec)		Effective rainfall (mm/dec)	Standard Irrigation requirement (mm/dec)
Month	Decade	Growth stage	Kc	ETo	ETc		
April	2	Ini	0.3	41.24	12.37	2.88	9.49
April	3	Ini	0.31	47.19	14.63	7.2	7.43
May	1	Dev	0.45	50.19	22.59	6.36	16.23
May	2	Dev	0.62	50.32	31.20	10.63	20.57
May	3	Dev	0.8	69.47	55.58	4.41	51.17

June	1	Dev	0.98	72.31	70.86	2.07	68.79
June	2	Dev	1.13	79.01	89.28	1.29	87.99
June	3	Mid	1.11	92.21	102.35	0	102.35
July	1	Mid	1.12	82.32	92.20	0	92.20
July	2	Mid	1.11	81.48	90.44	0	90.44
July	3	End	0.92	82.58	75.97	8.8	67.17
August	1	End	0.73	51.80	37.81	0	37.81
Total				800.12	695.28	43.64	651.64

ER (Effective rainfall), ETc (Crop Evapotranspiration), ETo (Reference evapotranspiration), Kc (Crop coefficient), Ini (Initial stage of growth), Dev (Development stage), Mid (Mid-season of growth), End (End of growth), Dec (Decade), Crop Evapotranspiration (ETc).

The irrigation depth was calculated from the following formula:

$$dn = \frac{(Fc - PWP)}{100} \times Z \times MAD$$

Where dn is irrigation depth (mm), Fc is field capacity (%), PWP is permanent wilting point (%), Z is root depth (Cm), and MAD is Maximum Allowable Depletion (dimensionless). The values of MAD, Fc, and PWP are fixed numbers for each plant and soil type. But because the depth of root development is constantly changing, maximum Z is considered (31).

Irrigation interval was calculated from the following formula:

$$F = \frac{dn}{SIR}$$

Where F is the Irrigation interval (day), dn is irrigation depth (mm), and SIR Standard irrigation requirement (mm/day) (32).

Irrigation volume was calculated from the following formula:

V=dn×A

Where V is Irrigation volume (m^{-3}), dn is irrigation depth (mm), and A is Plot area (m^{-2}) (31).

Plant Sampling and Analysis

Chlorophylls a, b and Total

The upper young leaves in the vegetative growth stage were selected and placed in aluminum foil immediately following collection. Leaves samples were washed with water to remove soil and dried in the shade. The dried leaves were powdered by using the dry grinder and then passed through the sieve, and 0.5 g of fresh leaf powder was weighed with a digital scale and poured into closed tubes, 0.5 g of fresh leaf powder was weighed with a digital scale and poured into closed tubes, and 3 ml of 99.5% methanol was added to it and placed in the dark for 2 hours. To homogenize the solution, the tubes were placed in a shaker for a few seconds and then centrifuged for 10 minutes at a speed of 13,000 rpm. The samples' level of light absorption was read at 650 and 665 nm using an ELISA device (BioTek-Power WaveXS2 model). (33).

The amount of chlorophyll a, b, and total were calculated using the equation the following equation:

Chlorophyll a (µg/mL) = 16.5 × A665 – 8.3 × A650

Chlorophyll b (µg/mL) = 33.8 × A650 – 12.5 × A665

Total Chlorophyll (µg/mL) = 25.8 × A650 + 4.0 × A665

Relative Water Content (RWC)

The fresh weight and turgid weight of leaf samples were measured after they had been in the water for six hours, and then they were dried in an oven until they had a consistent weight. The RWC was determined using the following formula:

RWC=((FW-DW)/(TW-DW)) ×100

Where FW is the fresh weight, TW is the turgid weight of hydrating samples in an envelope at about 25°C for six hours, and DW is the dry weight of leaves after oven-drying the samples at 85°C for four hours (34).

Proline Content (PC)

The proline content was extracted from 0.5 g leaf samples in 3% (w/v) aqueous sulpho-salicylic acid and estimated using ninhydrin reagent according to the method described by Bates et al. (35). The absorbance of the fraction with toluene obtained from the liquid phase was read at 520 nm. Proline concentration was determined using a calibration curve and expressed as μmol proline g^{-1} FW.

Soluble Sugar Content (SSC)

Using the method of Hizukuri et al. (36), the soluble sugar content was measured when the perfect flowering. A fresh sample (1 g) of each organ was ground with 10 ml of distilled water to homogenate, and then the homogenate was boiled in a water bath for 10 min. After cooling, the supernatant (0.2 ml) was pipetted, and 5 ml of anthrone-sulfuric acid reagent was added, followed by boiling it in a water bath for 10 min. After cooling to room temperature, a standard curve was prepared with glucose solution, and the absorbance was measured at 620 nm.

Essential Oil Yield (EOY)

In order to determine the amount of essential oil, after separating, washing, and drying leaves samples in the shade (for 2 days, 25°C), finally were powdered with an electric mill (Waring model) (37).

Extraction of essential oils was carried out using a Clevenger machine (Laborota 4003 model, Heidolph Co.) and the Water distillation method. To this end, 30 grams of the powdered plant sample, were transferred to the Clevenger machine. The samples were heated for 3 hours until the formation of essential oils (38).

To calculate the performance of the essential oil, essential oil weight was determined with a scale (accuracy 0.0001 gr, model), then the performance of the essential oil was calculated using the following relations (39).

(1) Essential oil%= Essential oil weight (g)/ Initial dry weight (g)

(2) Essential oil yield= Essential oil percentage×Biomass yield

Thymol and Carvacrol

To extract the essential oil, 100 grams of the plant's dry branches were picked and processed at 100% flowering. The next stage included obtaining the essential oil using water distillation for 2 hours and then calculating its percentage. The percentage of thymol and carvacrol was calculated using Gas Chromatograph (GC) (Shimadzu model) and Gas Chromatography/Mass Spectrometry (GC/MS) (3400-V) methods (40).

Flowering Branch Yield (FBY)

In the flowering stage, to measure the flowering branches' yield, the bushes were selected simultaneously and randomly, taking into account the marginal effects, gently separated from the plant, then after drying in the oven (*Memmert, Gmbh+Catky, Germany*) (48 h, 75°C), were weighed with a precise laboratory balance (*Sartoriouwith, Germany, TE313S*) (Accuracy=0.001 g).

Statistical Analyses

All of the data from the measurements were statistically evaluated using SAS 9.2 software. Following Bartlett's test to examine the uniformity of data variance and test the variance analysis's presumptions, a composite analysis of variance was conducted, which included normalizing the data, testing the uniformity of the errors, and the error uniformity test (p=0.05). For mean comparisons, the LSD (least significant difference) test was applied. Diagrams were drawn using Excel software.

Results and Discussion

The results of the two-year combined variance analysis revealed that the irrigation regimes had significant effects on the, EOY, SUGC, RWC, Chl a, Chl b, T Chl, CVR, THYM ($p<0.01$), and FBY ($p=0.05$). Also, the biochar application had significant effects on FBY, EO, EOY, SUGC, RWC, Chl a, and T Chl ($p<0.01$) (Table 5).

The interaction of irrigation regimes and biochar application, except for carvacrol and thymol, significantly affected other studied traits ($p<0.01$ and $p=0.05$). Also, the biochar application had significant effects on FBY, EO, EOY, SUGC, RWC, Chl a, and T Chl ($p<0.01$) (Table 5).

The interaction of irrigation regimes and biochar application, except for carvacrol and thymol, significantly affected other studied traits ($p<0.01$ and $p=0.05$) (Table 5).

The effect of planting patterns on studied traits was not significant. Also, the interaction of irrigation regimes and planting patterns, except for SUGC and T Chl ($p=0.05$), had no significant effect on other studied traits (Table 5).

The interaction of biochar application and planting patterns significantly affected the EOY, Chl a, T Chl, CVR, and THYM traits ($p<0.01$ and $p=0.05$) (Table 5).

Also, the interaction of irrigation regimes, biochar application and planting patterns significantly affected the Chl b, and T Chl traits ($p<0.01$ and $p=0.05$) (Table 5).

However, the year's interaction effect with other treatments on studied traits was not significant. The interaction of biochar application, planting patterns and irrigation regimes significantly affected the EOY, Chl b, and T Chl traits ($p<0.01$ and $p=0.05$) (Table 5).

Flowering Branch Yield (FBY)

The highest FBY (1701 kg/ha^{-1}) was obtained in FI+BC, while the lowest FBY (979.6 kg/ha^{-1}) belonged to FAFI+NBC (Table 6).

By decreasing irrigation water use, FBY diminished in both treatments— application and no-application of biochar (Table 6).

The reason for that is that drought stress leads to a decrease in the water potential of the leaves, diminished turgescence, Stomatal closure, decrease in cell enlargement and growth, decreased photosynthesis, disruption of metabolism, and as a result, a decrease in plant biomass (41).

In NBC and BC conditions, the comparison of irrigation regimes showed that the highest yield (1520 and 1710 kg/ha, respectively) belonged to the FI treatment. Restricted irrigation (VAFI and FAFI) reduced FBY under both NBC and BC conditions, with the difference that yields reduction in VAFI compared to FAFI was lower by 12 and 25.1%, respectively (Table 6).

Root growth was significantly increased using VAFI (Results not shown). This illustrates that the VAFI system results in better root growth than the other systems and a lesser drop in root development when irrigation is substantially reduced. Our results contradict those of Tilaye et al. (42).

Uniform water distribution between ridges in the alternative furrow irrigation technique promoted root development and nutrient absorption of crops, increasing yield in the fixed furrow irrigation system (42).

In such a way that variable alternate furrow irrigation (VAFI) and fixed alternate furrow irrigation (FAFI) treatments in the absence of biochar application showed a 27.8% and 35.6% decrease in flowering branch yield, respectively, compared to full irrigation (Table 6). but when biochar was applied, this reduction was 13.6% and 30.9%, respectively.

In this sense, under the conditions of no biochar application, with the reduction of irrigation, the decrease in flowering branch yield was more than under the conditions of biochar application (Table 6). Similar results were obtained by using the biochar in basil by Abdipour et al. (43).

Increasing the absorption of nutrients with the application of biochar is one of the most important reasons for the positive effect of this compound. Nutrient elements are absorbed on the surface of biochar particles, resulting in less leaching in the soil (44). The BC treatment improves soil structure, which benefits plant growth and development (45).

RWC (Relative Water Content)

The lowest RWC belonged to combined FAFI+NBC (44.38%) and VAFI+NBC (44.41%) treatments, which had no significant difference. Also, the highest amount was observed in IF+NBC (84.07%) and IF+BC (81.72%) (Table 6).

Drought stress highly diminished the RWC of thyme. Similar results were obtained in Basil (*Ocimum gratissimum* L.) by Hazzoumi et al. (46). In drought-stressed conditions, decreased water potential brought on by stomata closing lowers the RWC (47).

In the NBC condition, especially under FAFI and VAFI treatments, RWC decreased by 45.69% and 45.65%, respectively, compared to the FI regime (Table 6). The reduction of RWC is due to diminished leaf water potential and water absorption decreasing from the roots in water deficit conditions in FAFI and VAFI treatments.

Only in the IF regime, biochar application and no application of biochar treatments have no significant effect on RWC (Table 6).

It can be concluded that biochar application had a more positive effect under drought stress conditions. So in FAFI and VAFI treatments, biochar application increased RWC by 25% and 31.7%, respectively, compared to not using it (Table 6).

Chlorophyll a and b Content

The highest and lowest chlorophyll a was obtained in FI+BC (2.8 mg/g^{-1} FW) and FAFI+NBC (1.4 mg/g^{-1} FW) treatments, respectively (Fig 3). Drought stress reduced the quantity of chlorophyll, which is the primary cause of reactive oxygen species (ROS) formation in thylakoids (48).

In the FI regime, BC caused a 17.15% increase in the amount of Chl a compared to NBC. Also, BC in other irrigation regimes caused a significant increase in the Chl a compared to NBC (Fig 3). Similar results were obtained in Wheat (*Triticum aestivum* L.) by Zulfiqar et al. (49).

Biochar lessened the effects of drought by trapping moisture in the soil's pores and slowly releasing it when moisture levels dropped (50).

Drought stress caused a significant decrease in Chl a content in thyme leaves. Similar results were obtained in Corn (*Zea mays* L.) by Khayatnezhad et al. (51).

The most Chl a (2.1 mg/g^{-1} FW) was obtained under BC and PTR combination treatment. This treatment resulted in a 5.26% increase in Chl a compared to the NBC+PTR combination. The lowest amount was obtained in the NBC+PTR treatment (1.70%), which led to a 6.98% decrease in chlorophyll compared to the NBC+POR treatment (Fig 4).

Similar results were obtained in chickpea (*Cicer arietinum* L.) by Jat and Mali (52). They stated that the amount of chlorophyll increases with the increase in density. The reason for this decrease is the competition of plants to absorb nutrients from the soil (53).

Combining the FI+BC or NBC+PTR treatments produced the lowest chlorophyll b. Its highest rate (1.26 µg/ml) was observed in VAFI+NBC+PTR treatment. These treatments were not significantly different from each other (Table 7).

The only significant difference was observed in VAFI+NBC+PTR or POR combined treatments. Other treatments were not significantly different from each other (Table 7).

VAFI+NBC+PTR treatment compared to VAFI+NBC+POR reduced chlorophyll b by 10.11% (Table 7).

Drought stress induced a considerable rise in chlorophyll b content in this research, which contradicts the findings of the impact of dehydration on chlorophyll a content (Table 7). Similar results were obtained in sunflower (*Helianthus annus* L.) by Manivannan et al. (54).

Total Chlorophyll Content

The highest T Chl a was obtained in FI+BC+PTR (3.61 mg/g^{-1} FW) and FI+BC+POR (3.47 mg/g^{-1} FW) treatments, respectively. These treatments had no significant difference from each other (Table 7).

The lowest T Chl was also obtained in the FAFI+NBC+PTR (2.41 mg/g^{-1} FW) which was not significantly different from FAFI+NBC+POR (2.49 mg/g^{-1} FW) and VAFI+NBC+ POR (2.55 mg/g^{-1} FW) treatments (Table 7).

Drought stress by producing reactive oxygen species (ROS) and destroying existing chlorophylls caused the reduction of total chlorophyll content (55).

The obtained results are in agreement with the results of Setaish-Mehr and Ganjali (56) on a dill plant (*Anethum graveolens* L.).

In this study, BC treatment increased the amount of total chlorophyll compared to NBC treatment (Table 7).
In agreement with these results, Nurul-Azallia and Wan-Zaliha (57) showed that biochar application increases the total chlorophyll content in Kaempferia parviflora.
These researchers reported the increased nutrient absorption needed for chlorophyll production as the most important reason for this increase.
Planting a single row or double rows of thyme on the ridge, apart from the FI+NBC+POR and FI+BC+PTR treatments, had no significant effect on the total chlorophyll in other treatments.
In the mentioned treatments, FI+NBC+POR increased the amount of total chlorophyll by 10.4% compared to FI+BC+PTR (Table 7). It seems that with the increase in plant density, the light penetration in the canopy decreases (58).

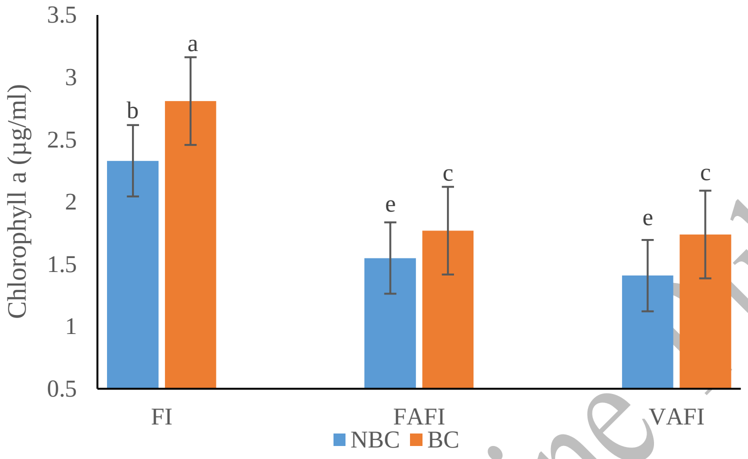


Fig. 3 The mean comparison results of the Chlorophyll a affected by the interaction of irrigation regimes and biochar application (Means with similar letters have no significant difference at the probability level of 5 percent). The black and silver bar, Indicates biochar application and No application of biochar, respectively. FI: Full irrigation, FAFI: Fixed Alternate Furrow Irrigation, VAFI: Variable Alternate Furrow Irrigation.

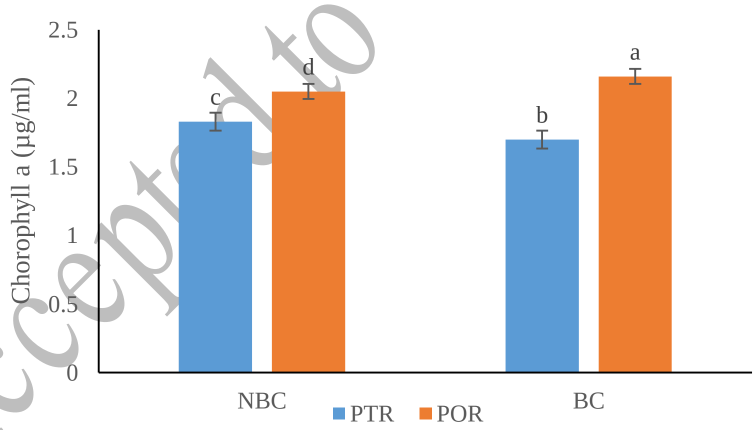


Fig. 4 The mean comparison results of the Chlorophyll a affected by the interaction of biochar application and planting pattern (Means with similar letters have no significant difference at the probability level of 5 percent). The black and silver bar, Indicates Planting double rows of thyme on the ridge and Planting a single row of thyme on the ridge, respectively. NBC: No application of biochar, and BC: biochar application.

Soluble Sugar Content (SUGC)

In NBC conditions, FAFI and VAFI treatments increased the soluble sugar content. The highest SUGC was obtained in FAFI+NBC (8.23%) and VAFI+NBC (8.01%) treatments, respectively. These treatments had no significant difference from each other (Table 6).

Biochar application in combination with FAFI and VAFI treatments led to a 13.78% and 12.54% reduction of SUGC, respectively (Table 6).

BC improves plant growth mainly by ameliorating the characteristics of the root environment, such as nutrient status, pH, and soil cation exchange capacity (59).

Therefore, plant growth improvement by biochar application requires the application of soluble sugar content and, thus, the reduction of these compounds.

Drought stress caused a significant increase in the SUGC of thyme leaves (Table 6). The obtained results are in agreement with the results of Hassan et al. (60) on a Rosemary plant (*Rosmarinus officinalis* L.).

Increasing the soluble sugar concentration is a popular response to drought stress conditions (61).

Osmotic potential is regulated by converting insoluble polysaccharides into soluble sugars such as oligosaccharides, sucrose, and glucose (61). Plants reduce their water potential by accumulating soluble sugars (62).

Biochar application improves plant growth mainly by improving the characteristics of the root environment, such as the nutrient status, pH, and cation exchange capacity of the soil (59).

Therefore, plant growth improvement by biochar application requires the application of soluble sugar content and, thus, the reduction of these compounds.

Proline Content

The highest proline content was obtained in the combined VAFI+NBC (3.37 mg g^{-1} FW) and FAFI+NBC (3.36 mg g^{-1} FW) treatments, respectively. The lowest value (1.67 mg g^{-1} FW) belonged to the combined FI+NBC treatment (Table 6).

The difference between the highest amount of proline obtained in the FAFI+NBC treatment and the lowest amount it (FI+NBC treatment) was 50.28% (Table 6).

Drought stress increased the proline content significantly, while with improved water conditions (FI treatment), the proline content decreased (Table 6). Due to proline instability in situations of high moisture availability, proline content is reduced with full irrigation (63).

Proline buildup is the first reaction of drying tissues to dehydration, and its function is to protect cells from being damaged (64).

With biochar application, proline content increased in all investigated treatments, but this increase was higher in drought stress conditions (VAFI and FAFI treatments) (Table 6).

Lehmann and Joseph (65) claimed that biochar has significantly improved the water-holding capacity in soil. Therefore, it can be said that this reduction is due to the improvement of the moisture conditions of the plant.

Essential Oil Yield (EOY)

The highest and lowest yield of EOY was obtained with 24.7 and 11.6 kg/ha in FAFI+BC+TR and VAFI+NBC+PTR treatments, respectively (Table 7).

FAFI and VAFI treatments caused the highest increase in the yield of thyme essential oil by 52.2 and 26% in the NBC+PTR treatment combination, respectively (Table 7). It means that, under the BC+PTR treatment combination, drought stress causes a higher increase in EOY.

In agreement with these results, studies have shown that drought stress can increase essential oil yield by stimulating the production of secondary compounds (66).

FAFI treatment causes a higher decrease in EOY compared to VAFI treatment (Table 7). It seems that in FAFI treatment, the plants have faced more severe stress, which causes a higher increase in EOY.

In the present study, biochar application in often of the irrigation treatment combinations and planting patterns caused a significant increase in EOY (Table 7).

The use of biochar prevents the destructive effects of drought stress (67). This can be due to the effect of biochar in the transfer of proteins that are located in membranes and play a role in the development of the cell wall and cell elongation (68).

Table 5 Combined ANOVA of the effect of irrigation regimes and biochar application on some traits of thyme at different irrigation levels.

S.O.V	DF	FBY	EOY	CVR %	THM (%)	SUGC	Proline	RWC	Chl a	Chl b	T Chl
Y	1	118804.58 **	0.063 ^{ns}	0.038 ^{ns}	1.471 ^{ns}	5.986 **	0.091*	201.4 ^{ns}	0.12 ^{ns}	0.033 *	0.029 ^{ns}
R (Y)	4	1581.62 ^{ns}	1.125	0.025 ^{ns}	2.404	0.364	0.089 *	92.2 ^{ns}	0.205*	0.003 ^{ns}	0.192 ^{ns}
A	2	1732476.42 *	314.871**	0.137**	769 **	58.7 **	14.2 **	7082.5 **	7.283**	0.989 **	3.121 **
Y×A	2	61332.15 **	0.487 ^{ns}	0.041 ^{ns}	1.163 ^{ns}	0.053 ^{ns}	0.038 ^{ns}	85.8 ^{ns}	0.015 ^{ns}	0.004 ^{ns}	0.036 ^{ns}
Error	8	2695.95	0.468	0.014	9.784	0.348	0.016	57.1	0.047	0.005	0.074
B	1	1119155.05 *	309.21**	0.420 ^{ns}	0.498 ^{ns}	11.5 **	11.5**	2854.2 **	2.108 **	0.001 ^{ns}	2.023 **
Y×B	1	22873.2 *	2.844 ^{ns}	0.184 ^{ns}	2.876 ^{ns}	0.24 ^{ns}	0.021 ^{ns}	8.4 ^{ns}	0.024 ^{ns}	0.002 ^{ns}	0.041 ^{ns}
A×B	2	67800.85**	40.04 **	0.044 ^{ns}	4.274 ^{ns}	1.32 *	1.095 **	522.3 **	0.109 *	0.170 **	0.237 **
Y×A×B	2	317.28 ^{ns}	2.15 ^{ns}	0.009 ^{ns}	2.104 ^{ns}	0.003 ^{ns}	0.013 ^{ns}	0.18 ^{ns}	0.01 ^{ns}	0.001 ^{ns}	0.009 ^{ns}
C	1	0.459 ^{ns}	6.37 ^{ns}	0 ^{ns}	7.914 ^{ns}	0.24 ^{ns}	0.043 ^{ns}	32.6 ^{ns}	0.001 ^{ns}	0.007 ^{ns}	0.002 ^{ns}
Y×C	1	161.25 ^{ns}	0.105 ^{ns}	0.007 ^{ns}	1.802 ^{ns}	0.112 ^{ns}	0.006 ^{ns}	0.5 ^{ns}	0.021 ^{ns}	0.002 ^{ns}	0.013 ^{ns}
A×C	2	1456.45 ^{ns}	1.05 ^{ns}	0.012 ^{ns}	0.601 ^{ns}	1.29 *	0.038 ^{ns}	8.2 ^{ns}	0.055 ^{ns}	0.006 ^{ns}	0.091 *
Y×A×C	2	1566.12 ^{ns}	0.516 ^{ns}	0.023 ^{ns}	5.496 ^{ns}	0.455 ^{ns}	0.005 ^{ns}	4.4 ^{ns}	0.066 ^{ns}	0.002 ^{ns}	0.066 ^{ns}
B×C	1	347.73 ^{ns}	39.176 **	0.304 *	25.4 *	1.248 ^{ns}	0.052 ^{ns}	5.5 ^{ns}	0.262 **	0.004 ^{ns}	0.200 **
Y×B×C	1	2.941 ^{ns}	0.043 ^{ns}	0.159 ^{ns}	7.252 ^{ns}	0.085 ^{ns}	0.008 ^{ns}	1.3 ^{ns}	0.064 ^{ns}	0 ^{ns}	0.072 ^{ns}
A×B×C	2	36763.88 ^{ns}	11.373 **	0.068 ^{ns}	8.573 ^{ns}	0.549 ^{ns}	0.081 ^{ns}	40.1 ^{ns}	0.047 ^{ns}	0.019 **	0.079 *
Y×A×B×C	2	34881.14 ^{ns}	0.125 ^{ns}	0.129 ^{ns}	6.61 ^{ns}	0.675 ^{ns}	0.037 ^{ns}	14.5 ^{ns}	0.038 ^{ns}	0.002 ^{ns}	0.033 ^{ns}
Error	36	15311.24	1.614	0.051	4.816	0.357	0.031	27.6	0.022	0.003	0.024
CV(%)		7.94	8.93	7.37	8.33	7.59	6.05	5.4	14.85	13.4	9.65

^{ns}: Non-significant, * and **: Significant at α=0.05 and α=0.01, respectively. S.O.V: Source of Variation, Y: Year, R: Replication, A: Irrigation regimes, B: Biochar, C: Planting pattern, C.V: Coefficient of variation, DF: Degree of Freedom, FBY: Flowering branch yield, EOY: Essential oil yield, CVR: Carvacrol, THYM: Thymol, SUGC: Sugars content, RWC: Relative Water Content, Chl a: Chlorophyll a, Chl b: Chlorophyll b, T Chl: Total Chlorophyll.

Table 6 Mean comparison results of the interaction effect of irrigation regimes and biochar application on some of the studied traits of thyme during two cropping years.

Treatment		FBY (Kg/ha^{-1})	Proline (mg g^{-1} FW)	RWC (%)	SUGC (%)
Irrigation Regimes	Biochar Application				
A_1	B_1	1520 ^{b}	1.672 ^{d}	81.72 ^{a}	5.013 ^{c}
	B_2	1701 ^{a}	1.356 ^{e}	84.07 ^{a}	4.75 ^{c}
A_2	B_1	1098 ^{c}	3.363 ^{a}	44.38 ^{d}	8.233 ^{a}
	B_2	1470 ^{b}	2.407 ^{b}	59.18 ^{c}	7.098 ^{b}
A_3	B_1	979.6 ^{d}	3.371 ^{a}	44.81 ^{d}	8.016 ^{a}
	B_2	1175 ^{c}	2.245 ^{c}	65.03 ^{b}	7.01 ^{b}

In each column, those with similar letters are not significantly different at the 5% level of probability.

A: Irrigation regimes, A_1: Full irrigation, A_2: Variable Alternate Furrow Irrigation, A_3: Fixed Alternate Furrow Irrigation, B_1: No application of biochar, B_2: biochar application, FBY: Flowering branch yield, RWC: Relative Water Content, SUGC: Sugars content.

Table 7 Mean comparison results of the interaction effect of irrigation regimes, biochar application, and planting pattern on some of the studied traits of thyme during two cropping years.

Treatment			EOY (Kg/ha^{-1})	Chl b (µg/ml)	T Chl (µg/ml)
Irrigation Regimes	Biochar Application	Planting Pattern			
A_1	B_1	C_1	13.30 ^{f}	0.693 ^{e}	3.173 ^{b}
		C_2	13.55 ^{f}	0.661 ^{e}	2.843 ^{c}
	B_2	C_1	15.97 ^{e}	0.726 ^{e}	3.47 ^{a}
		C_2	16.25 ^{e}	0.726 ^{e}	3.61 ^{a}
A_2	B_1	C_1	21.04 ^{b}	0.888 ^{d}	2.552 ^{de}
		C_2	19.36 ^{cd}	0.896 ^{d}	2.68 ^{cd}
	B_2	C_1	20.92 ^{b}	0.996 ^{c}	2.668 ^{cd}
		C_2	24.73 ^{a}	1.042 ^{c}	2.832 ^{c}
A_3	B_1	C_1	12.89 ^{fg}	1.137 ^{b}	2.492 ^{de}
		C_2	11.67 ^{g}	1.265 ^{a}	2.41 ^{e}
	B_2	C_1	18.35 ^{d}	1.022 ^{c}	2.678 ^{c}
		C_2	20.47 ^{bc}	0.988 ^{c}	2.813 ^{c}

In each column, those with similar letters are not significantly different at the 5% level of probability.

A: Irrigation regimes, A_1: Full irrigation, A_2: Variable Alternate Furrow Irrigation, A_3: Fixed Alternate Furrow Irrigation, B_1: No application of biochar, B_2: biochar application, C_1: Planting a single row of thyme on the ridge, C_2: Planting double rows of thyme on the ridge, EOY: Essential oil yield, Chl b: Chlorophyll b, T Chl: Total Chlorophyll.

Carvacrol Percentage (CVR%)

The highest amount of carvacrol was obtained in VAFI and FAFI treatments by 2.91% and 2.84%, respectively. FAFI and VAFI treatments were not significantly different from each other in terms of increasing carvacrol percentage (Fig 5).

Drought stress led to an increase in carvacrol percentage (Fig 5). The obtained results are in agreement with the results of Gholinezhad (69) on a pot marigold (*Calendula officinalis* L.).

In this condition, the plant uses most photosynthetic materials available to produce osmotic regulating compounds such as proline, glycine betaine, and sugar compounds such as sucrose, fructose, and fructan, which can reduce the water potential and essential oil percentage (70).

On the other hand, irrigation caused a decrease in carvacrol percentage compared to low irrigation treatments (VAFI and FAFI) by 5.15% and 2.81%, respectively (Fig 5).

The obtained results are in agreement with the results of Tátrai et al. (71) on a thyme plant (*Thymus citriodorus* L.).

In planting double rows of thyme on the ridges (PTR), BC or NBC had no significant effect on carvacrol percentage (Fig 6). Only planting one row of thyme on the ridges with no application of biochar (NBC+POR), a significant increase in carvacrol percentage was observed compared to planting two rows of thyme on the ridges in combination with biochar application (BC+PR) (Fig 6).

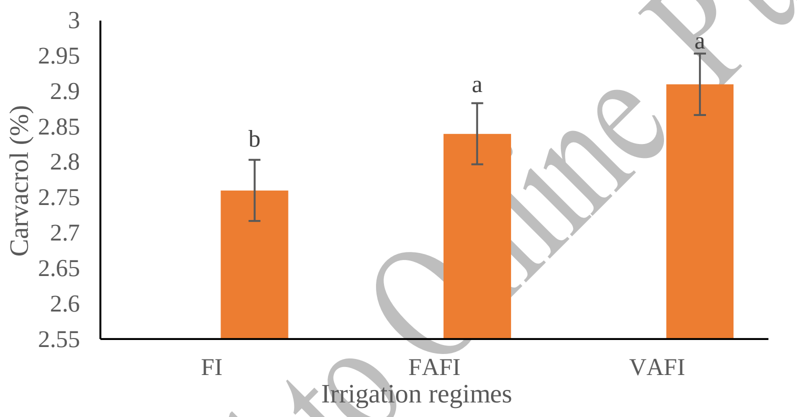


Fig. 5 The mean comparison results of the Carvacrol affected by the irrigation regimes (Means with similar letters have no significant difference at the probability level of 5 percent). FI: Full irrigation, FAFI: Fixed Alternate Furrow Irrigation, VAFI: Variable Alternate Furrow Irrigation.

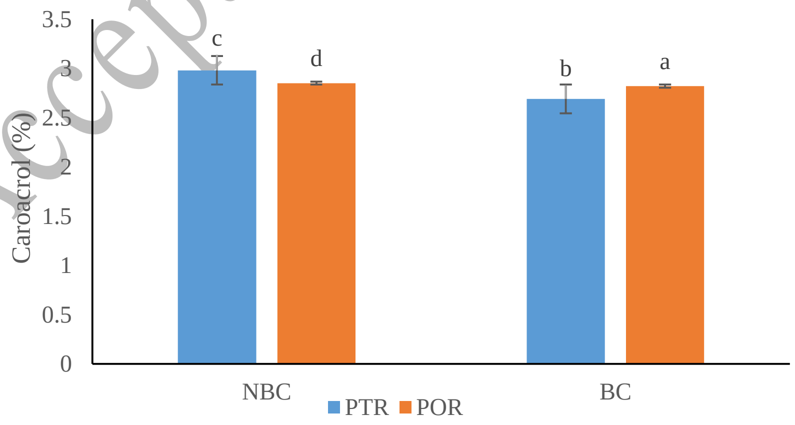


Fig. 6 The mean comparison results of the Carvacrol affected by the irrigation regimes (Means with similar letters have no significant difference at the probability level of 5 percent). The black and silver bar, Indicates Planting double rows of thyme on the ridge and Planting a single row of thyme on the ridge, respectively. NBC: No application of biochar, and BC: biochar application.

Thymol Percentage (THM%)

The highest thymol percentage was obtained in the FI treatment (38.43%) and the lowest in the FAFI (29%) and VAFI (28.28%) treatments (Fig 6).

The reduction of available water decreased the thymol percentage. At the same time, there was no significant difference between the irrigation regimes (FAFI and VAFI) in terms of thymol percentage (Fig 6).

Similar results were obtained in thyme (*Thymus daenensis* L.) by Alavi-Samani et al. (72). These researchers reported that the reason for this decrease is the change in the biosynthesis pathways of these compounds under the influence of dehydration conditions.

In this study, BC or NBC conditions, did not have a significant effect on the thymol percentage. But the planting pattern had a significant effect on thymol percentage (Fig 7).

Under BC conditions, no significant difference was observed between planting patterns in terms of thymol percentage (Fig 7).

In NBC conditions, the thymol percentage in the PTR (32.91%) was lower by 5.62% compared to the POR (31.06%) (Fig 7).

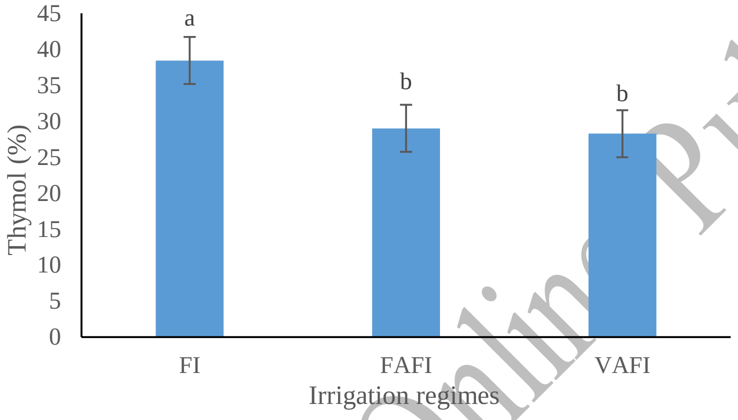


Fig. 7 The mean comparison results of the Thymol affected by the irrigation regimes (Means with similar letters have no significant difference at the probability level of 5 percent). FI: Full irrigation, FAFI: Fixed Alternate Furrow Irrigation, VAFI: Variable Alternate Furrow Irrigation.

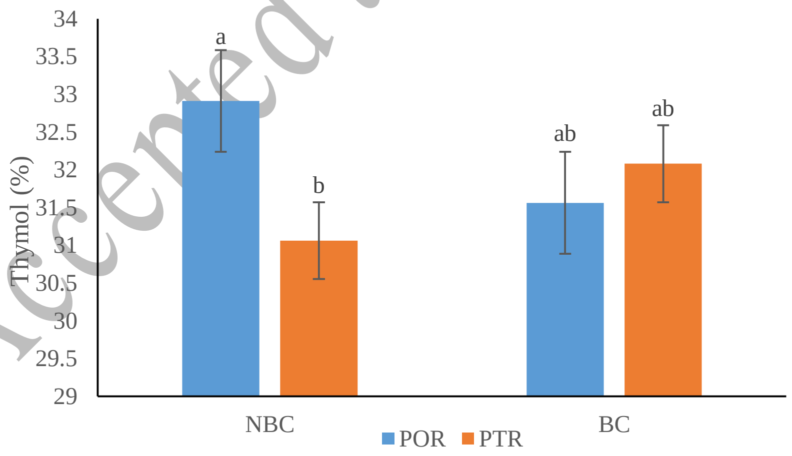


Fig. 8 The mean comparison results of the Thymol percentage affected by the interaction of biochar application and planting pattern (Means with similar letters have no significant difference at the probability level of 5 percent). The black and silver bar, Indicates Planting double rows of thyme on the ridge and Planting a single row of thyme on the ridge, respectively. NBC: No application of biochar, and BC: biochar application.

CONCLUSION

Crop production in arid and semi-arid regions is strongly affected by water scarcity. Hence, there is a binding need to investigate water-saving approaches and to design more efficient irrigation systems in agriculture. In the meantime, Alternate furrow irrigation and Fixed furrow irrigation save water. In the alternative furrow irrigation method, with a biochar application combination, the minimum mean Flowering branch yield reduction has happened. Even though the highest yield was obtained at Conventional furrow irrigation at complete irrigation application, it consumes a considerable water amount. Using alternate furrow irrigation can solve the water shortage problem and improve water productivity without a meaningful reduction in yield. An alternate furrow irrigation system along with biochar application is a promising technology for the utilization of deficit irrigation with negligible diminishing in flowering branch yield and essential oil yield of thyme in semi-arid conditions.

Conflict of Interest

The authors declare no conflict of interest.

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