



# Effect of irrigation intervals and humic acid on physiological and biochemical characteristic on medicinal plant of *Thymus vulgaris*

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## Abstract

To investigate the effects of irrigation intervals and humic acid application on some physiological and biochemical of thyme (*Thymus vulgaris*), the experimental in the form of factorial in the design complete randomized block in four replications at Miandoab University Research Field. Experimental treatments were at four levels of irrigation intervals (once every three days, once every six days, once every nine days and every day as control) and three levels of humic acid solution (zero, 200 and 400 mg l<sup>-1</sup>). The results showed that increasing irrigation intervals, significantly increased total phenol, antioxidant activity, proline and soluble sugars. Irrigation interval at highest level (once every nine days) compared to control increased proline, phenolic compounds, antioxidant activity, soluble sugars, essential oil and thymol by 58.79%, 47.27%, 21.25%, 62.57%, 66.67% and 19.24%, respectively. Increasing irrigation intervals, significantly reduced plant height, fresh and dry weight of plant. Application of humic acid had a significant positive effect on all studied traits. Also, interaction of irrigation intervals and humic acid application had significant effect on all traits except essential oil percentage, phenolic compounds and thymol. According to the results of this experiment, the application of 400 mg l<sup>-1</sup> humic acid at irrigation interval of once every nine days is considered to be the best economically treatment because with less water consumption with humic acid application, this plant can achieve the same functions as consuming more water, especially this treatment caused a significant increase in effective substance of *Thymus vulgaris*.

**Keywords:** Antioxidant; deficit stress; essential oil; physiology; proline

**Sorkhi, F.** 2020. 'Effect of irrigation intervals and humic acid on physiological and biochemical characteristic on medicinal plant of *Thymus vulgaris*'. *Iranian Journal of Plant Physiology* 10(4), 3367-3378.

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## Introduction

Water deficit is one of the most important environmental factors to reduce growth and yield of many crops, horticultural and medicinal plants, especially in arid and semi-arid regions of the world (Silva et al., 2013).

Water deficit stress for a long interval affects all metabolic processes of the plant and reduces plant production (Li et al., 2019). In a pot experiment, drought stress on growth indices decreased yield and essential oil of Thyme (*Thymus vulgaris* L.) (Mohammadpour et al., 2015). The results of the study showed that drought stress increased the levels of proline and glucose in *Chenopodium quinoa* cultivars (Gamez et al., 2019) and in three chickpea cultivars increased the activity of antioxidant enzymes (Esfahani, 2013). Recently, the use of organic acids

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Received: January, 2020

Accepted: July, 2020

has been widely used to improve the quality and quantity of crops and horticultural products. Very low levels of organic acids due to the presence of hormone compounds have significant effects on improving soil physical, chemical and biological properties and increasing production and quality of agricultural products (Dong et al., 2004). Thus, using a variety of natural fertilizers, including humic acid, without adverse environmental damage, can improve yield of plant.

Humic acid is a natural organic polymer compound formed from the decay of soil organic matter that can increase plant productivity and quality (Eshwar et al., 2017). The important benefits of humic acid is the presence of various nutrients such as sodium, potassium, magnesium, zinc, calcium, iron, copper and other elements (Ihsanullah and Bakhshwain, 2013). Humic acid with physical correction and improving soil aggregation, more space creates for water penetration (Ahmed et al., 2013) and by increasing the activity of the rubisco enzyme, it increased the photosynthetic activity of the plant (El-Razek et al., 2012). Evaluation of the humic acid effect on medicinal plant of *Nigella sativa* under different levels of water stress showed the concentration of soluble proteins decreased and increased production of antioxidant enzymes and proline with increasing water deficit stress (Soltan and Mansourifar, 2017). Also, the effect of drought stress and humic acid on *Cucumis melo* showed that with increasing drought stress under humic acid treatment, the content of carotenoids and relative humidity decreased and proline concentration increased, indicating that humic acid application reduced severely the negative effects of drought stress on the plant (Kiran et al., 2019).

Thyme (*Thymus vulgaris* L.) is a woody, perennial aromatic plant belonging to the lamiaceae family. That grows in different regions of the world (Kulisicet et al., 2005). Essential oils are present in all parts of the plant but most commonly found in flowering shoots and the most important composition of the essential oil is thymol (Ballester-Costa et al., 2016). Attention to drought in Iran has always one of the most important agricultural problems. Due to the importance of using sustainable farming systems

and management methods, the present study was conducted to evaluate the use of humic acid for achieving sustainable agricultural goals and determining of the water requirement of thyme in irrigation regimes in the field to use optimal and appropriate water.

## Materials and Methods

In order to investigate the effect of irrigation intervals and humic acid on some physiological and biochemical characteristics of Thyme (*Thymus vulgaris*), an experiment was conducted in the research field of Miandoab Azad University in 2019. In this research, the effects of two factors of irrigation intervals and humic acid were evaluated by factorial experiments based on a randomized complete block design with four replications. Irrigation intervals at four levels included every day as control and once every three days, once every six days and once every nine days and humic acid at three concentrations of 0, 200 and 400 mg L<sup>-1</sup>. Seedlings were prepared in greenhouse conditions. Plowing, levelling and land preparation were carried out in the second half of April 2019. Inside each of the test plots, 9 produced transplants were cultivated in three rows of 1.6 m with a spacing of 40 cm on a row and 40 cm in a row (Lebaschi et al., 2018). Irrigation was performed immediately after cultivation. Irrigation was performed for two weeks after planting daily and then irrigation treatments were applied until the end of growth period. To determine the qualitative and quantitative characteristics of the soil at the site of the experiment, the soil sample was transferred to a laboratory for chemical and physical analysis and the results of soil decomposition are presented in Table 1.

Eight weeks after the start of irrigation treatments (when 50% of the plants were in flowering stage), they began to take morphological and physiological characteristics such as plant height, fresh weight and dry weight, relative water content of leaf, proline and soluble sugars and biochemical characteristics including antioxidant activity, total phenol, thymol and essential oil percent. Three samples were selected from each treatment and after removing the root and drying the shoots at room temperature (about

Table 1  
Soil properties before plant sowing

Soil Texture	Silt (%)	Clay (%)	Sand (%)	Total N (%)	K (mg/kg)	P (mg/kg)	OC (%)	EC (dS/m)	pH
Silty loam	54.6	25.7	19.7	0.09	368.34	14.79	0.82	1.25	7.18

Table 2  
Analysis of variance of irrigation intervals and humic acid application on thyme properties

S.V	df	Plant height	Fresh weight	Dry weight	Essential oil percentage	Proline
Repeat	3	19.58 <sup>ns</sup>	73.55 <sup>ns</sup>	22.88 <sup>ns</sup>	0.34 <sup>ns</sup>	1.96 <sup>ns</sup>
Irrigation intervals	3	35.39 <sup>**</sup>	320.54 <sup>**</sup>	173.13 <sup>**</sup>	1.19 <sup>**</sup>	6.80 <sup>**</sup>
Humic acid	2	20.42 <sup>*</sup>	127.61 <sup>*</sup>	40.01 <sup>*</sup>	1.08 <sup>*</sup>	7.12 <sup>**</sup>
Irrigation intervals × Humic acid	6	64.59 <sup>**</sup>	289.41 <sup>**</sup>	26.04 <sup>*</sup>	0.43 <sup>ns</sup>	5.07 <sup>**</sup>
Error	33	7.39	31.05	8.87	0.21	0.95
C.V		9.26	11.17	5.96	2.43	3.27

\*, \*\* and ns, significant at the 5% and 1% probability levels and non-significant, respectively.

Table 3  
Analysis of variance of irrigation intervals and humic acid application on thyme properties

S.V	df	Soluble Sugar	Relative water content	Antioxidant activity	Total phenol	Thymol
Repeat	3	10.58 <sup>ns</sup>	20.16 <sup>ns</sup>	6.45 <sup>ns</sup>	0.12 <sup>ns</sup>	4.72 <sup>ns</sup>
Irrigation intervals	3	106.09 <sup>**</sup>	81.41 <sup>**</sup>	44.75 <sup>**</sup>	0.58 <sup>**</sup>	31.28 <sup>**</sup>
Humic acid	2	41.38 <sup>*</sup>	47.88 <sup>*</sup>	30.38 <sup>*</sup>	0.24 <sup>**</sup>	60.21 <sup>**</sup>
Irrigation intervals × Humic acid	6	59.84 <sup>**</sup>	35.51 <sup>*</sup>	23.04 <sup>*</sup>	0.069 <sup>ns</sup>	11.75 <sup>ns</sup>
Error	33	10.38	13.01	8.91	0.044	6.52
C.V		6.72	9.71	3.32	2.05	4.36

\*, \*\* and ns, significant at the 5% and 1% probability levels and non-significant, respectively.

26 °C) and in the shade was measured the dry weight of the plant, methanol extract, and antioxidant activity, total phenol, Proline, soluble sugar for each plot. The amount of total phenol in the leaf was measured by the Folin–Ciocalteu method. First, 20 ml of the extract was extracted with 1.16 ml of distilled water and 100 µL of Folin–Ciocalteu and after 5-8 minutes' rest, 300 microliters of sodium carbonate was dissolved in a molar solution for 30 minutes in a 40 °C steam bath in the dark. Pure methanol was replaced with methanol extract in the control, then samples were read at a wavelength of 760 nm by a spectrophotometer (UNICO Model 2800). Different concentrations of gallic acid (50, 100, 150, 200, 250 mg L<sup>-1</sup>) in 80% methanol were used to draw the calibration curve. This value was calculated for one milligram per liter, and total phenol was obtained in milligrams of gallic acid per

100 grams of dry matter (McDonald and Ho, 2002).

For determine antioxidant activity was used DPPH (diphenyl-1-picrylhydrazyl) method. At first one ml of the methanolic extract (80% methanol) was mixed with one ml DPPH at a concentration of 0.1 mM. For the control, 1 ml of pure methanol was replaced with 1 ml of methanol extract and for blanc was used pure methanol. After 30 min of darkness, the samples were read at 517 nm by the spectrophotometer instrument of the Cecil model made in UK. Finally, the percentage of free radical scavenging by the extract was calculated by this formula (Gedikoglu et al., 2019).

Relation (1)

Free radical scavenging =  $(A_C - A_S) / A_C \times 100$   
In this form  $A_C$  and  $A_S$  are the sample absorption and control absorption, respectively.

Extraction of soluble sugars was carried out using the Qayyum method. According to this method, 40 mg of fresh leaf tissue was mixed with 5 ml of 80% ethanol and incubated in a water bath at 70 °C for 10 minutes. Then obtained alcoholic extract was centrifuged for 10 minutes at 3000 rpm. The obtained clear solution containing soluble sugars was transferred to a dish and this procedure was repeated 4 times on the remaining tissues. Finally, the alcoholic extract was condensed by heat to a volume of about one-fifth the original volume. For remove chlorophyll, the extract was mixed with chloroform at a ratio of 1:5 and allowed to rest for 5 minutes after stirring. This action causes the chlorophyll to be separated from the aqueous phase. The aqueous phase was centrifuged for 10 minutes at 3000 rpm. The transparent upper phase was separated and used to measure soluble sugars by spectrophotometer (Qayyum et al., 2011).

For measure proline, 0.5 g of fresh plant leaf was chopped with mortar and poured into a tube, then 10 ml of 3% sulfosalicylic acid was added to it and the sample was put into the ice. The tube was centrifuged at 15,000 rpm for 10 minutes at 4 °C until extract the surplus materials from the solution. 2 ml of the filtered extract was poured into a new tube and 2 ml of ninhydrin acid and 2 ml of acetic acid were added and then mixed well. At the same time, 2 ml of the standard solution of 0, 4, 8, 12, 16 and 20 mg per liter of proline were poured into new tubes and 2 ml of acid anhydrous and 2 ml of acetic acid were added and mixed well. Samples were put for 1 hour in the spa bath and then in the ice bath. 4 ml of toluene was added to the solution and stirred for 20 seconds. Then the soluble proline standards in toluene phase were transferred to a spectrophotometer and the proline content was read at 520 nm and plotted as a standard curve. Then the amount of adsorption was read in the plant samples and by putting it in the linear equation was obtained the amount of proline (Bates et al., 1973).

For determine the relative water content of leaf, three extended leaves were cut from the end of the stem and one cm pieces were prepared and their fresh weight was measured with digital scales. To determine their saturation weight, they were transferred to distilled water containers for

24 hours in the dark at 4 °C. After removing the pieces of distilled water, to remove the excess moisture, the leaf pieces were dried in two layers of filter paper and then their saturation weight was measured. Then, the dry weight was determined by placing the same plant samples in the oven at 70 °C for 24 hours. Finally, relative water content (RWC) of leaf was calculated using the relation 2 (Pirzad et al., 2011).

Relation (2)

$$RWC_{(\%)} = (FW - DW) / (TW - DW) \times 100$$

In this form FW, DW and TW are the fresh weight, dry weight and turgid weight, respectively.

For extraction and measurement of essential oils, head of flowering shoots was cut at full flowering stage and dried at room temperature (about 25 °C) and in shadow. Then 100 grams of each dried sample were milled and by the distillation method with water (100 cc), essential oil was obtained with cleverger apparatus (Gedikoglu et al., 2019). Analysis of variance was performed by SAS software and mean comparison was done by Duncan multiple range test method.

## Results

According to the analysis of variance (Table 2), the treatments applied had a significant effect on all measured traits. Irrigation intervals had a significant effect on the level of one percent for all treatments. Also, humid acid effect on all plant characteristics was significant at 5% level except for proline, total phenol and thymol which was significant at 1% level. Interaction of irrigation intervals and humic acid had significant effect on all traits except essential oil percentage, total phenol and thymol and significant effect was observed on dry weight, Antioxidant activity and relative water content of leaf at 5% and on the other traits at 1% level.

### Plant height, fresh weight and dry weight

Plant height, fresh and dry weight of *Thymus vulgaris* were significantly (1% level) affected by different irrigation intervals (Table 2). The highest values for these traits were obtained from the first level of irrigation (Once for every day) (Table 4). Also, humid acid application was

significant at the 5% probability level for these traits (Table 2).

The highest plant height, fresh and dry weight of plant were 26.15 cm, 68.53g and 25.41.g, respectively, in the application of 400 mg L<sup>-1</sup> humic acid (Table 6). In combination treatments, the above mentioned traits decreased with increasing irrigation interval, such as the lowest plant height (16.35 cm), fresh plant weight (31.50 g) and dry weight (5.39 g) of the irrigation once every nine days and non humic acid application. In general, application 400 mg L<sup>-1</sup> of humic acid in daily irrigation obtained the highest amount of studied traits (Table 8).

### Total phenol and antioxidant activity

Total phenol and antioxidant activity were significantly (1% level) affected by irrigation intervals and humic acid treatments but the interaction treatments on antioxidant activity was significant at 5% level. (Table 2). Comparison of the mean showed that the highest amount of total phenol averaged 0.081 mg g<sup>-1</sup>DW for irrigation interval of once every nine days and the minimum value is 0.055 mg g<sup>-1</sup>DW corresponds to the first level of irrigation interval (every day of irrigation) (Table 5). Application of 400 mg L<sup>-1</sup> humic acid significantly increased this trait compared to control. However, there were statistically similar levels of humic acid at concentrations of 200 and 400 mg L<sup>-1</sup> (Table 7).

Antioxidant activity was significantly increased by increasing irrigation intervals. Irrigation intervals of once every three, six and nine days, with mean values of 70.66, 73.13 and 82.90 increased 11.50%, 15.40% and 30.81% antioxidant activity compared to control, respectively (Table 5). Under long-distance irrigation, the humic acid application increased the antioxidant content, so that the highest amount of antioxidant activity (85.78%) was obtained from application of 400 mg L<sup>-1</sup> of humic acid treatment with irrigation interval of once every nine days (Table 9).

### Soluble Sugar and Proline

Results of analysis of variance in Table 2 indicate that the irrigation regime, humid acid and their

interaction had a significant effect on the amount of soluble sugar (Table 2). The highest amount of soluble sugar (41.23 mg g<sup>-1</sup>DW) was obtained from the irrigation interval of once every nine days' treatment and the lowest with 25.36 mg g<sup>-1</sup>DW from the first level of irrigation intervals (daily irrigation) (Table 5). Application of 400 mg L<sup>-1</sup> of humic acid with mean of 38.07 mg g<sup>-1</sup>DW increased 30.11% of this trait compared to control (Table 7). Interaction of irrigation intervals and humic acid content (Table 9) was observed that the highest amount of soluble sugar was related to irrigation interaction once in nine days and 400 mg L<sup>-1</sup> humic acid with 49.06a mg g<sup>-1</sup>DW.

According to Table 4, irrigation intervals of nine, six and three days with mean of 2.89, 2.12 and 1.91 mg g<sup>-1</sup>DW, increased proline content compared to control (1.82 mg g<sup>-1</sup>DW). Results of mean comparison showed that the highest proline content was obtained from the interaction of irrigation treatment once every nine days and application of 200 and 400 mg L<sup>-1</sup> of humic acid with 3.08 and 3.16 mg g<sup>-1</sup>DW, respectively (Table 8).

### Relative water content

The relative water content of leaf was significantly affected by irrigation intervals at 1% level, humic acid and their interactions at 5% level (Table 3). Mean comparison showed that irrigation treatments including once every nine days, once every six days, once every three days and irrigation every day, the relative water content of leaf was 45.90, 56.21, 69.46 and 74.76 percent respectively, indicating that relative water content decreased with increasing irrigation intervals (Table 5). The levels of humic acid significantly increased this trait. Humic acid application at both levels of 200 and 400 mg L<sup>-1</sup> increased 12.40 and 20.37 percent relative water content of leaf respectively, compared to non-humic acid application (Table 7).

According to the comparison table (Table 9), the interaction effect of once daily irrigation and 400 mg L<sup>-1</sup> humic acid had the highest relative water content of leaf (78.53%) and the irrigation treatment of once every nine days and non humic

Table 4

Comparison of the average effects of simple irrigation intervals on thyme properties.

Irrigation interval	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Essential oil (%)	Proline (mg 100g <sup>-1</sup> DW)
Every day (control)	26.14 a	66.37 a	23.32 a	0.21 d	1.82 c
Once every three days	24.10 b	66.25 b	20.99 b	0.29 c	1.91c
Once every six days	22.11 c	55.87 c	18.07 bc	0.35 a	2.12 b
Once every nine days	21.23 d	51.91 d	16.22 c	0.37 b	2.89 a

Means with similar letters in each column are not significantly different.

Table 5

Comparison of the average effects of simple irrigation intervals on thyme properties.

Irrigation interval	Soluble Sugar (mg g <sup>-1</sup> DW)	Relative water content (%)	Antioxidant activity (%)	Total phenol (mg g <sup>-1</sup> DW)	Thymol (%)
every day (control)	25.36 d	74.76 a	68.37 d	0.055 c	8.99 d
once every three days	30.03 c	69.46 b	70.66 c	0.057 c	9.24 c
once every six days	36.29 b	56.21 c	73.13 b	0.067 b	9.70 b
once every nine days	41.23 a	45.90 d	82.9 a	0.081 a	10.72 a

Means with similar letters in each column are not significantly different.

Table 6

Comparison of average simple humic acid effects on thyme properties.

Humic acid (mg l <sup>-1</sup> )	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Essential oil (%)	Proline (mg 100g <sup>-1</sup> DW)
Non humic acid	19.31 c	46.33 c	11.38 c	0.29 b	1.89 c
200	24.68 b	62.45 b	21.92 b	0.31 b	2.25 b
400	26.15 a	68.53 a	25.41 a	0.34 a	2.32 a

Means with similar letters in each column are not significantly different.

Table 7

Comparison of average simple humic acid effects on thyme properties.

Humic acid (mg l <sup>-1</sup> )	Soluble Sugar (mg g <sup>-1</sup> DW)	Relative water content (%)	Antioxidant activity (%)	Total phenol (mg g <sup>-1</sup> DW)	Thymol (%)
Non humic acid	29.26 c	55.52 c	70.57 b	0.063 c	9.29 b
200	32.35 b	62.41 b	75.01 a	0.065 b	9.87 a
400	38.07 a	66.83 a	75.72 a	0.068 a	9.89 a

Means with similar letters in each column are not significantly different.

acid application had the lowest moisture content (39.17%). Overall, relative water content of leaf decreased with increasing irrigation intervals.

### Essential oil percentage

The percentage of essential oil was significantly affected by irrigation intervals at 1% probability level and humic acid at 5% probability level (Table 3). The highest essential oil percent (0.37%) was obtained in irrigation treatment of once every nine

days and the lowest essential oil percentage (0.21%) was obtained in daily irrigation. (Table 4).

Application of 400 mg L<sup>-1</sup> significantly increased the essential oil percentage compared to non-humic acid application. However, also application of 200 mg L<sup>-1</sup> improved this trait compared to non-humic acid but did not increase significantly (Table 6).

### Thymol

Table 8  
Comparison of the average interaction effects of irrigation intervals and humic acid application on thyme properties.

Irrigation interval	Humic acid (mg L <sup>-1</sup> )	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Proline (mg 100g <sup>-1</sup> DW)
Every day (control)	Non humic acid	22.16 f	56.68 ef	17.54 e	1.52 f
	200	26.69 b	65.85 bc	25.18 ab	1.89 d
	400	29.57 a	76.59 a	27.26 a	2.05 c
Once every three days	Non humic acid	20.38 g	53.69 f	13.15 f	1.72 e
	200	25.12 c	63.15 cd	22.80 c	1.94 d
	400	26.82 b	69.93 b	26.03 ab	2.07 c
Once every six days	Non humic acid	18.38 h	43.47 g	9.45 g	1.88 d
	200	23.53 e	60.52 de	20.61 d	2.11 c
	400	24.42 cd	63.65 cd	24.16 bc	2.35 b
Once every nine days	Non humic acid	16.35 i	31.5 h	5.39 h	2.43 b
	200	23.41 e	60.29 de	19.08 e	3.08 a
	400	23.82 de	63.95 cd	24.19 bc	3.16 a

Means with similar letters in each column are not significantly different.

Table 9  
Comparison of the average interaction effects of irrigation intervals and humic acid application on thyme properties.

Irrigation interval	Humic acid (mg L <sup>-1</sup> )	Soluble Sugar (mg g <sup>-1</sup> DW)	Relative water content (%)	Antioxidant activity (%)
Every day (control)	Non humic acid	22.30 i	71.32 b	62.15 g
	200	24.55 h	74.44 ab	70.05 e
	400	29.33 fg	78.53 a	71.40 de
Once every three days	Non humic acid	28.26 g	64.37 c	67.72 f
	200	29.33 fg	70.68 b	70.94 e
	400	32.5 e	73.35 ab	73.36 d
Once every six days	Non humic acid	31.18 ef	47.22 e	71.45 de
	200	36.19 d	58.29 cd	72.53 de
	400	41.5 b	63.14 c	75.41 c
Once every nine days	Non humic acid	35.28 d	39.17 f	80.20 b
	200	39.36 c	46.25 e	84.72 a
	400	49.06 a	52.30 d	85.78 a

Means with similar letters in each column are not significantly different.

Thymol percentage was significantly affected by irrigation intervals and humic acid at 1% probability level (Table 3). The mean comparison showed that the highest amount of thymol (10.72%) was related to irrigation treatment once every 9 days and the lowest was with the mean of 8.99% irrigation per day (Table 5). Application of 200 and 400 mg L<sup>-1</sup> humic acid significantly increased thymol compared to no humic acid. However, treatments of 200 and 400 mg L<sup>-1</sup> humic acid were at the same statistical level. (Table 7).

## Discussion

Increasing irrigation interval decreased plant height, fresh and dry weight of plant and relative leaf water content, but humic acid application could compensation the negative effects of water deficit and increase plant biomass, on the other hand, increasing the irrigation interval increased proline content, phenolic compounds, essential oil percentage and antioxidant activity, thus increasing plant quality. Plant height decreased under water deficit stress condition due to

reduced turgor pressure and consequently reduced cell division and growth compared to optimal irrigation conditions. (Bahreinnejad et al., 2013).

In relation to the increase in antioxidant activity of water deficit stress, one of the biochemical changes that occur in plants under drought conditions is the accumulation of reaction oxygen species (ROS). Numerous reports have stated that drought stress increases reaction oxygen species (ROS) production. Plant cells can inhibit oxidative stress under stress through antioxidant defence systems (Gedikoglu et al., 2019). The resistance of the plant to various environmental stresses may be related to the level of activity of the enzymes responsible for the trapping of oxygen free radicals (Boldaji et al., 2012). Increased levels of phenolic compounds are one of the antioxidant mechanisms of plants under drought stress because they act as refining oxygen free radicals, therefore they stabilize the cell membranes and prevent lipid peroxidation (Tohidi et al., 2017). Under drought stress increased phenolic compounds in *Eucalyptus camaldulensis* (Babaian et al., 2008), *Linum usitatissimum* (Ghorbanli et al., 2011) and *solanum tuberosum* (Andre et al., 2009).

A study reported that drought stress significantly increased stomata resistance and antioxidant enzyme activity in all genotypes of wheat (Khazaie and Borzooei, 2006). Some researchers believe that the increase in the levels of antioxidants of catalase, superoxide dismutase and peroxidase increase plant tolerance to environmental stresses (Ballester-Costa et al., 2016). Antioxidant activity increased with increasing acidic level. Soil type and amount of humic compounds in soil can have significant effects on the plant under stress so that the higher the soil humic compounds, the greater antioxidant activity (Kiran et al., 2019). The amount of phenolic compounds increased with increasing irrigation intervals. In this regard, the researchers investigated the effects of different concentrations of vermicompost and humic acid on the quantitative and qualitative characteristic of *Calendula officinalis* that 500 mg L<sup>-1</sup> humic acid could increase phenolic compounds (Abedini et al., 2015). Under drought conditions, due to the weakening of the immune system of the plant

*Arachis hypogea*, phenolic substances, along with other defense enzymes, increase in resistance to microorganisms (Latha et al., 2007). In the study of wheat, it was concluded that the phenolic compounds in wheat under drought stress were increased due to the increased activity and the amount of biosynthetic enzyme in phenols. (Sallam et al., 2019).

In an experiment that investigated the effect of humic acid on antioxidant activity and pepper fruit quality, the phenolic compounds did not increase by humic acid effect but flavonoid and antioxidant activity increased (Aminifard et al., 2012). In this study, the amount of phenolic compounds was not affected by the combination treatment of irrigation interval and the humic acid application but significantly increased the antioxidant activity. With increasing irrigation intervals, the amount of proline increased in Thyme, in addition humic acid application increased proline accumulation and thus increased plant resistance to stress conditions. In many plants, changes in proline content are related to their ability to tolerate or adapt to drought stress conditions and can be used as an indicator for selection of drought tolerant plants (Oliveira et al., 2018). When the plant is exposed to drought stress, the proteins break down, resulting in an increase in amino acids, which is one of these amino acids, proline (Qayyum et al., 2011). As plants are affected by stresses, they increase the concentration of their osmolytes to continue water absorption under stress conditions. Among organic osmolytes, proline is one of the most commonly that accumulate under stress conditions (Pirzad et al., 2011). Proline increased during drought stress in Thyme (*Thymus vulgaris*) (Bahreinnejad et al., 2013). In a study on the effects of drought and humic acid stress on some physiological characteristics of (*Phaseolus vulgaris*), it was found that drought stress increases the accumulation of proline (Sani et al., 2018), which is in agreement with the results of the present study.

Humic acid improves yield by providing suitable conditions for increasing plant nitrogen content and increasing the production rate of nitrogenous organic compounds such as protein and amino acids increases the growth rate and biomass production in *Cucumis melo* (Kiran et al.,



2019). Proline is a type of amino acid stored in the cytoplasm and, plays a role in protecting the structure of macromolecules inside the cell during drought (Lebaschi et al., 2008). Proline is considered as an indicator in determination of sensitivity to salinity and drought stress in plants. High levels of proline in plant tissues are somehow indicative of an osmotic regulation mechanism that optimizes plant conditions for the uptake of water and solutes from the root environment (Oliveira et al., 2018). In the present study, according to the results of increasing irrigation intervals, the amount of soluble sugars increased in Thyme (*Thymus vulgaris*) and the other hand, humic acid application increases the soluble sugar content and consequently increases plant resistance to stress conditions. Carbohydrates are very important in physiological processes such as photosynthesis, transpiration and respiration, so change in their amounts are very important. In plants, sugars are reproduced during the photosynthesis process (Ciereszko et al., 2018). The role of sugars is known as a precursor to the production of carbon materials and energy. Protecting the plant from dehydration depends on increasing sugars (Qayyum et al., 2011). Studies have shown that the drought stress converted hexoses and other carbohydrates such as sucrose and starch to simple sugars (Pirzad et al., 2011).

The distribution of hydrocarbon materials is directly affected by the effects of water scarcity and indirectly by the influence of plant hormones. Humic acid application increases plant tolerance to stress conditions due to increased photosynthesis and production of carbohydrates (Ahmed et al., 2013). On the other hand, humic acid has quasi-hormonal activity and increases the uptake of minerals such as phosphorus and potassium in plants, which in turn improves photosynthesis and increase in the amount of sugar produced (Eshwar, 2017). humic acid in complete irrigation treatments in *Acacia Saligna* produces the highest amount of leaf carbohydrates (El-khateeb et al., 2011) which corresponds to the results of the present test. The results showed that the percentage of essential oil was inversely related to the amount of water content, as the percentage of essential oil in plant organs decreased with increasing amount of available water and the least was observed in

treatments that were irrigated daily. Carbon is used to grow when conditions allow for cell growth and division. With the drought stress, growth is stopped and the plant is allocated carbon to the product of drug efficacy (Soltan and Mansourifar., 2017). Any deficiency that restricts growth beyond photosynthesis, increases the production of secondary metabolites (Soltaninejad et al., 2013). Investigation of the effect of water deficit stress and fertilizer on the essential oil of *Salvia officinalis* was reported to increase the percentages of essential oil by increasing drought stress (Govahi et al., 2015), which is consistent with the results of the present study. Research on *Hibiscus sabdariffa* under drought stress showed that the highest amount of essential oil was obtained from irrigation treatment with 40% field capacity (Mijani et al., 2015). The researchers reported that humic acid application of up to 3 kg / ha increased the essential oil content in the medicinal plant of *Carum copticum* (Barghamadi et al, 2015).

The results of this study showed that relative water content of Thyme (*Thymus Vulgaris*) decreased with increasing water deficit stress, but the humic acid application could partially offset this decrease and thereby increase plant resistance to water deficit conditions. The relative water content of leaf is a good indicator for the water stress of the plant (Lebaschi et al., 2008). Drought stress decreases plant growth by decreasing relative water content (RWC) and total water potential (Kiran et al.,2019). The osmotic adjustment mechanism in drought tolerant plants maintains the relative water content and prevents its reduction (Surendar et al., 2013). In an experiment on the plant of *Lycopersicon esculentum*, researchers showed that relative water content decreased with increasing water deficit stress (Barzegar et al.2019). Also, the results of the study on *Carthamus tinctorius* showed that the mean relative water content decreased significantly with decreasing irrigation (Pasban Eslam et al.,2011). Cunhua et al. (2011) reported that the highest and lowest relative water content in *Amaranthus retroflexus* plant was under normal irrigation (94.07%) and severe drought stress (64.03%), respectively.

In one study, drought stress reduced the relative water content of *Melissa officinalis*

(Lebaschi et al., 2008), which is in agreement with the results of the present study. In another study, the application of vermicompost biofertilizer in drought stress conditions on *Lactuca sativa* showed that increasing stress level (moderate and severe) decreased the relative water content of leaf and grain yield compared to normal irrigation (Kiran, 2019). The results of this study showed that humic acid application reduced the effects of drought stress because humic acid improved soil physical properties, providing more space for soil water infiltration with soil texture and on the other hand, by bonding with water molecules to prevent water evaporation, thereby increases the relative water content of leaf under drought conditions (Mijani et al., 2015; Eshwar et al., 2017). Humic acid promote the plant's growth by absorbing water and nutrients by expanding the roots (Kiran et al., 2019).

According to the results obtained from the study, many of the effects of drought stress on Thyme could be modified by the application of humic acid, thereby reducing the use of chemical fertilizers and achieving the sustainable agriculture goals. The results also showed that with increasing irrigation interval, plant height, fresh and dry weight of plant and relative water content were decreased and in contrast to proline content, phenolic compounds, essential oil percentage and antioxidant activity increased. This is a form of adaptation to drought stress conditions, but the dependence of plants on compounds such as proline in these conditions is costly for the plant. In general, the protective and modulating role of humic acid application on drought stress can be attributed to its positive effect on improving plant nutritional conditions and osmotic adjustment under irrigation regime and more importantly, its use instead of chemical fertilizers that can promote sustainable agriculture and reduce environmental pollution in the future.

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