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Research Article

**Effects of Vermicompost, Compost and Animal Manure on Vegetative Growth, Physiological and Antioxidant Activity Characteristics of *Thymus vulgaris* L. under Water Stress**

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**Abstract:** This study investigated the effect of organic fertilizers on vegetative growth and the physiological and antioxidant activity characteristics of thyme plants grown under stress. A factorial experiment was conducted according to randomized complete block design with 12 combinations and 3 replications in the 2018 growing season. The experiment factors were the implementation of organic fertilizers at 4 levels (vermicompost, manure compost, animal manure, and control) and irrigation regime at 3 levels (Irr1, Irr2, and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan). The results showed that With delayed irrigation, the chlorophyll a and b contents, total chlorophyll, and carotenoid decreased, while the application of low water stress enhanced the amount of oil and the oil yield with the respective highest values of 2.61% and 3.68 g/m under mild stress conditions. Nonetheless, higher values for the aforementioned properties were noted with the application of vermicompost. Water deficit decreased nutrient uptake (K, P, and N) and relative water content, biological yield, and seed yield of thyme, indicating that thyme was sensitive to drought, and organic fertilizers application improved nutrient uptake (K, P, and N) and relative water content, biological yield and seed yield of the plant within irrigation levels. The activities of catalase, superoxide dismutase and ascorbate peroxidase were reduced under organic fertilizers such as vermicompost and manure compost as compared with control under drought stress. The plants of thyme showed a good response to organic fertilizers under water deficit circumstances, with vermicompost being the most effective.

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**1. Introduction**

The thymus genus, consists of 215 species, belongs to the Lamiaceae (Labiatae) family and is one of the most prominent medicinal herbs that are commercially grown in the Mediterranean region

(Azadi *et al.*, 2021). *Thymus vulgaris* L. is a perennial shrub commercially cultivated in several northern countries of the Western Mediterranean region (Kosakowska *et al.*, 2021). Its volatile components are often used as flavors, herbal tea, as well as several medicinal purposes. Also, its blossoms, stems, and leaves are known as digestive, anti-inflammatory, carminative, anti-spasmodic, and expectorant remedies (Hossain *et al.*, 2022).

Thyme production is significantly affected by water stress conditions. The water stress (drought stress) normally decreases the water supply in the soil by evapotranspiration. As water stress is a key factor in agricultural productivity, improving water efficiency by increasing crop yield per unit area is very important in water deficit conditions (Abd Elbar *et al.*, 2019; Raza *et al.*, 2021). Water stress can adversely affect host plants at the molecular, physiological, biochemical, and morphological levels (Arpanahi *et al.*, 2020). A study on biochemical traits in stress conditions would be useful to understand the adaptation mechanisms (Mohammadzadeh and Pirzad, 2020). Reduction in leaf senescence, as well as the increase in photosynthetic capacity, are the key indices of drought stress, which adversely impacts crop growth (Mohammadzadeh and Pirzad, 2020; Nasar *et al.*, 2021). Photosynthesis, particularly under water stress conditions, leads to increase electron leakage by producing several types of reactive oxygen species (ROS) including hydroxyl radicals, superoxide, hydrogen peroxide as well as oxygen radicals (Heydarzadeh *et al.*, 2022).

Intensive use of chemical fertilizers affected non-target organisms, altered biological ecosystems, and influenced soil microorganisms (Maddahi *et al.*, 2021). Organic farming, which involves the cultivation with preservation of soil health could be considered as an alternative to the current farming systems which are mainly dependent on chemical application (Heydarzadeh *et al.*, 2022). Organic fertilizers have been considered as eco-friendly approaches to improving soil fertility, and improving production, resulting in minimizing chemical fertilizers application (Khosravi Shakib *et al.*, 2019; Maddahi *et al.*, 2021).

Vermicompost (VC) is a nutrient-rich organic matter, which is a microbiologically active compound produced by the interaction of microorganisms with earthworms (Celikcan *et al.*, 2021). It could be used in sustainable agriculture for improving soil porosity, thus increasing nutrient availability (Ievinsh *et al.*, 2020). Vermicompost is rich in microorganisms that release several organic acids, including oxalic acid, and increase the solubility of elements, especially potassium and phosphorus (Celikcan *et al.*, 2021).

Manure compost (MC) is also important in the sustainable farming system by improving soil porosity, increasing water consumption efficiency as well as nutrient availability (Ievinsh *et al.*, 2020). Composting is widely regarded as a possible alternative for untreated manure and is used for the production of high-quality organic fertilizer due to stabilizing organic matter and destroying pathogens in raw manure in high temperature conditions (50-60 °C) (Jiang *et al.*, 2021).

Animal manures (AM) are organic sources of nutrients for plants' sustainable production. Also, the increase of organic matter/root improves fertility, structure, and water and air permeability of soils, so, improves host plant growth and yield (Rahimi *et al.*, 2019). In Iran, rainfall fluctuations, as well as pollution with industrial fertilizers and pesticides are the two major challenges in the production of medicinal and aromatic plants, which have resulted in a significant reduction in farm products. Introducing such organic fertilizers, as well as providing the protocols of their application, can be useful for farming under water stress conditions. Thus, the current study aims to study the effect of organic fertilizers on the physiological, antioxidant, and yield parameters of *T. vulgaris* L. under water stress conditions.

## 2. Material and Methods

### 2.1. Field experiment

Field experiments were conducted in 2018 at Urmia University, Iran, which is located 45°10' E, 37°44' N, at an altitude of 1338 m. The study aimed to assess the effect of organic fertilization on the physiological, antioxidant, and yield characteristics of Thyme Garden (*T. vulgaris* L.). The physiochemical characteristics of the soil used in this study are presented in Table 1.

Table 1. Average physicochemical properties of the soil sample used in the study

| ppH   | EEC    | Organic carbon | Total N | Olsen-P | Available K | CaCO <sub>3</sub> | Sand | SSilt | Clay |
|-------|--------|----------------|---------|---------|-------------|-------------------|------|-------|------|
|       | (dS/m) | (%)            | (%)     | Mg/kg)  | (mg/kg)     | (%)               | (%)  | (%)   | (%)  |
| 77.33 | 00.06  | 1.14           | 0.03    | 9.02    | 282         | 115.71            | 444  | 332   | 224  |

The study adopted a factorial experiment with 12 treatments and three replications based on a Randomized Complete Block Design. The first factor was assigned to the irrigation regime at three levels, including irrigation after 60, 90, and 120 mm evaporation from A pan. The second factor was assigned to the application of organic fertilizers at sowing time at four levels: vermicompost (VC), manure compost (MC), animal manure (AM), and control, in which no fertilizer was applied. Experimental treatments included cow manure at 30 ton/ha, compost at 20 ton/ha and vermicompost at 10 ton/ha, and control (no fertilizer application). Physicochemical characteristics of vermicompost, manure compost, and animal farm manure used in this study are presented in Table 2. The water required during watering to resupply the soil moisture deficit and restore field capacity is known as irrigation water needed before watering (VN). According to Walker (1984), the value of VN was calculated (Eq 1).

$$VN=[(FC-WP)\times BD\times D\times(1-ASM)\times A]/100 \tag{1}$$

Table 2. Physiochemical characteristics of vermicompost, compost and animal manure

| Parameter          | Vermicompost | Compost | Animal manure |
|--------------------|--------------|---------|---------------|
| EC (dS/m)          | 3.81         | 7       | 4.21          |
| pH                 | 7.2          | 7.6     | 7.4           |
| Organic carbon (%) | 30           | 29      | 35            |
| Available K (%)    | 1.85         | 0.75    | 1.25          |
| Available P (%)    | 2.3          | 1.1     | 0.94          |
| Total N (%)        | 1.45         | 1.25    | 2.27          |

Where FC is field capacity (%), VN is the irrigation water in m<sup>3</sup> needed before watering, WP is wilting point (%), Dis root zone depth (m), BD is bulk density (g/cm<sup>3</sup>), A is the field area (m<sup>2</sup>), and ASM is available soil moisture before watering (a fraction). The respective amounts of irrigation for 60, 90, and 120 mm evaporation from A pan water were 2150, 1850, and 1550 m<sup>3</sup>/ha. The data on rainfall and temperature of the research area is given in Figure 1.

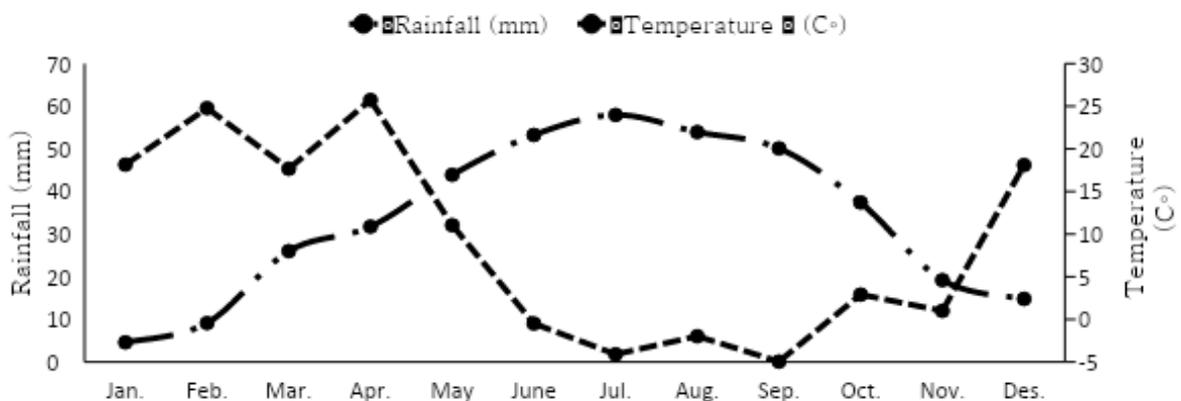


Figure 1. Monthly average air temperature and total precipitation for the years 2018-2019.

In January 2018, the seeds were sown in seedling trays containing a mixture of perlite: soil (1:2, v: v). After about 90days, the seedlings were relocated to the farm's experimental site. Each experimental

unit comprised eight rows of planting with a length of four meters and 15 cm tall thyme seedlings planted at a spacing of 50 × 25cm (Figure 2). Seedlings of thyme were irrigated immediately after planting; the subsequent irrigations were done on a weekly basis. Weeds were controlled manually when required. The treatments were sampled at full flower state. They were individually stored in nitrogen tanks and stored in a -80°C freezer. All cultivation methods were carried out uniformly for all experimental treatments.



Figure 2. The cultivated *T. vulgaris* L. in the experimental field.

## 2.2. Measurement

### 2.2.1. Plant growth characteristics

All experimental treatments were harvested individually after reaching full maturity of growth, paying attention to grain yield and biological yield from 10 plants per plot. The plant samples were oven-dried to a constant weight at 40°C.

### 2.2.2. Plant pigment contents

About 0.5 g of fresh leaves was milled in liquid nitrogen, blended with 10 mL of 80% acetone, and homogenized by centrifuge at 4000 rpm for 15 minutes. The chlorophyll a and b contents and carotenoid were measured by using a spectrophotometer at respective wavelengths of 645, 662, and 470 nm according to the following equations 2,3,4,5 (Lichtenthaler and Wellburn, 1987) where A is the absorbance of light at 662, 645, and 470 nm.

$$\text{Chlophyll } a = 11.24 \times A_{662} - 2.04 \times A_{645} \quad (2)$$

$$\text{Chlophyll } b = 20.13 \times A_{645} - 4.19 \times A_{662} \quad (3)$$

$$\text{Total chlophyll} = 7.05 \times A_{662} + 18.09 \times A_{645} \quad (4)$$

$$\text{Carotenoid} = \frac{1000 \times A_{470} - 1.90 \times \text{chlorophyll } a - 63.14 \times \text{chlorophyll } b}{214} \quad (5)$$

### 2.2.3. Relative water content

Relative water content on leaf samples was measured by the method of [(fresh weight – dry weight) / (turgid weight – dry weight)] × 100 (Khosravi Shakib et al., 2019).

### 2.2.4. Nutrients of (N, P, and K)

To determine the nutrient content of leaves' samples, dried leaves samples were milled, digested, and analyzed with combustion (4 h at 500 °C) of the leaf sample. On the 5 mg ash samples were added 1 ml of 2 N HCl, and the extracts obtained were filtered. After the samples were then filtered, the phosphorus (P) content of samples was detected calorimetrically by the vanado-molybdate method based on the yellow color of the unreduced vanado-molybdo-phosphoric heteropoly acid suspended in an HNO<sub>3</sub> medium. The amount of potassium (K) was measured by a flame photometer (Edward et al., 1999). The total concentration of nitrogen (N) in the plant leaves was measured by the Kjeldahl method (Schuman et al., 1973).

### 2.2.5. Antioxidant enzyme activity

For quantification of antioxidant enzyme activity, fresh plant material (100 mg) was ground in 2 mL of 0.1 M potassium phosphate with containing 5% polyvinylpyrrolidone (PVP) and buffered at a pH of 6. Then the extracts were centrifuged at 3°C for half an hour at 15.000 rpm, and the activity of the enzymes was estimated from the clear supernatant (Tejera et al., 2004). Catalase activity was determined at 240 nm based on the variation in concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The reaction mixture contained 1.9 mL of 50 mM K<sub>3</sub>PO<sub>4</sub>, which was buffered at a pH of 7.10, mM H<sub>2</sub>O<sub>2</sub>, and 0.2 mL of enzyme extract. Enzymatic activity was then determined in 60 seconds per mg of protein based on absorption variations (Aebi, 1984). Superoxide dismutase activity was assessed at 560 to minimize the loss of nitroblue tetrazolium (NBT) photochemical (Beyer and Fridovich, 1987). In this study, one unit of Superoxide dismutase (SOD) was taken as the quantity of enzyme that inhibits a 50% decrease in NBT. By employing the Nakano and Asada (1987) method, ascorbate peroxidase activity (APX) was measured with a reaction mixture containing 1mL of 0.5 mM ascorbic acid, 1 mL 100 mM potassium phosphate-buffered at a pH of 7, 100 µL enzyme extract, and 0.1 mL H<sub>2</sub>O<sub>2</sub> 0.1 mM. The absorption was then determined at 290 nm.

### 2.2.6. Essential oil and essential oil yield

Essential oil extraction was carried out by distillation using a Clevenger apparatus. For extraction (which was done for 3 hours), 10 g of dried leaves were transferred into a 1000 ml balloon, followed by the addition of 100 ml of distilled water. By use of a Clevenger machine (Adams, 2007), the volatile compounds were extracted with water vapor as characterized by the formation of a distinct layer on the surface of the water in the graduated tube after cooling. Essential oil yield was estimated using Eq. 6.

$$\text{Essential oil yield} = \text{dry matter yield of the plant} \times \text{the percentage of essential oil} \quad (6)$$

## 2.3. Statistical analysis

The results were expressed as mean  $\pm$  SE then analysis of variance was accomplished by ANOVA procedure, after which significant differences were computed using SAS (version 9.1.3) software as per Duncan's multiple range tests ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Characteristics of thyme

ANOVA data showed that the content of chlorophyll a, b, a+b and carotenoid, essential oil, and essential oil yield of thyme were significantly affected by the effect of irrigation and fertilizer treatments. While RWC, the content of nutrients (N, P, K), the activity of the CAT, APX, and SOD enzymes, the biological yield, and seed yield of thyme were significantly affected by the interaction of irrigation and fertilizer treatments (Table 3).

Table 3. Analysis of variance of some traits of *T. vulgaris* L. medicinal plant under the influence of irrigation regime and organic fertilizers

| Source of variation | df            | Chl a                 | Chl b                | Chl a+b              | Car                   | RWC      | N      | P        | K      | CAT    | SOD       | APX     | EEO                  | EEOY               | BY         | SY        |
|---------------------|---------------|-----------------------|----------------------|----------------------|-----------------------|----------|--------|----------|--------|--------|-----------|---------|----------------------|--------------------|------------|-----------|
| Repetition          | 2             | 0.00007               | 0.006                | 0.005                | 0.0007                | 3.27     | 6.0006 | 0.00001  | 0.002  | 0.007  | 5.65      | 0.00008 | 0.008                | 0.01               | 328.57     | 2.04      |
| Irrigation (Irr)    | 2             | 0.42**                | 0.04**               | 0.76**               | 0.03**                | 663.80** | 0.36** | 0.02**   | 0.15** | 7.55** | 4300.39** | 0.72**  | 1.22**               | 4.64**             | 53787.84** | 1729.47** |
| Fertilizer (F)      | 3             | 0.74**                | 0.24**               | 1.67**               | 0.02**                | 143.77** | 0.37** | 0.009**  | 0.03** | 0.29** | 51.52**   | 0.08**  | 2.67**               | 10.44**            | 27980.43** | 1754.13** |
| Irr × F             | 6             | 0.00001 <sup>ns</sup> | 0.0001 <sup>ns</sup> | 0.0001 <sup>ns</sup> | 0.00001 <sup>ns</sup> | 6.35*    | 0.01*  | 0.0005** | 0.002* | 0.04** | 16.98**   | 0.009** | 0.0001 <sup>ns</sup> | 0.11 <sup>ns</sup> | 2947.29**  | 68.78*    |
| Error               | $\frac{2}{2}$ | 0.02                  | 0.002                | 0.01                 | 0.0009                | 1.80     | 0.005  | 0.00001  | 0.0007 | 0.0005 | 0.59      | 0.0001  | 0.017                | 0.06               | 37.50      | 23.93     |
| CV (%)              |               | 12.59                 | 0.73                 | 0.73                 | 8.90                  | 2.13     | 3.53   | 1.32     | 2.09   | 0.93   | 1.36      | 1.21    | 5.75                 | 7.75               | 1.51       | 3.53      |

\*, \*\* and ns signify significant at 5% and 1% levels of probability and non-significant, respectively. Chl a and b, chlorophyll a and b content; Car, carotenoid; RWC, relative water content; N, nitrogen; P, phosphorus; K, potassium; CAT, catalase activity; SOD, superoxide dismutase activity; APX, ascorbate peroxidase activity; EO, essential oil; EOY, essential oil yield; BY, biological yield; SY, seed yield; ns, non-significant.

### 3.2. Photosynthesizing pigments

The average comparison revealed that the chlorophyll a and b contents, total chlorophyll, and carotenoid decreased significantly with the delay in irrigation (Table 4). On the other hand, based on the plants that had been subjected to organic fertilizer, these properties were significantly higher compared to the control plants (Table 4). So that the highest chlorophyll a and b contents, total chlorophyll and carotenoid were 1.47, 0.86, 2.33, and 0.42 mg/g FW, respectively, obtained from plants that were treated with VC fertilizer. While the lowest chlorophyll a and b contents, total chlorophyll and carotenoid were 0.86, 0.49, 1.35, and 0.29 mg/g FW, respectively, observed in the control treatment (Table 4). The content of chlorophyll in living plants is a key factor in maintaining photosynthetic efficiency. Increased degradation or loss of synthesis of these pigments (chlorophyll and carotenoids), in addition to the deterioration of enzyme activity that is in charge of the synthesis of photosynthesizing pigments, cause a photosynthetic deficiency in plants exposed to drought stress, resulting in reduced assimilation and production declines (Ashrafi et al., 2018; Khosravi Shakib et al. 2019). In peanuts, salt stress decreased chlorophyll content compared to the control (Yolci et al., 2021). Carotenoid and chlorophyll content increased with the application of AM, VC, and MC to the substrate (Table 4). Organic fertilizers secure the N requirement of photosynthesizing pigments and plant amino acids, and at the same time, N wastage (by leaching, sublimation, or fixation) is reduced (Rahimi et al., 2019), thereby affecting the amount of these pigments in the crops. In addition, the plants exhibited not only higher sunlight absorption capacity and photosynthate synthesis but also improved growth and yield (Khosravi Shakib et al., 2019). This combination of traits increases plant productivity.

### 3.3. Relative water content

The highest RWC (75.85%) was obtained in the application of VC fertilizer and irrigation after 60 mm of evaporation from A pan. While the lowest one (50.55%) was obtained in severely stressed plants and without the application of organic fertilizer (Figure 3). It has been reported that RWC of Marigold and melons decreases with increasing water stress (Kusvuran et al., 2011; Khosravi Shakib et al., 2019). The first impacts of water deficit can be a reduction in relative water content in leaves and a reduction of turgor in plant tissues, which can naturally impact cell growth and final size (Amirnia et al. 2019). Organic fertilizers increase water absorption and enhance water connections in the host plant, most likely by altering root morphology and improving the host plant's root system, as well as by increasing the absorption region (Ashrafi et al., 2018; Bistgani et al., 2018).

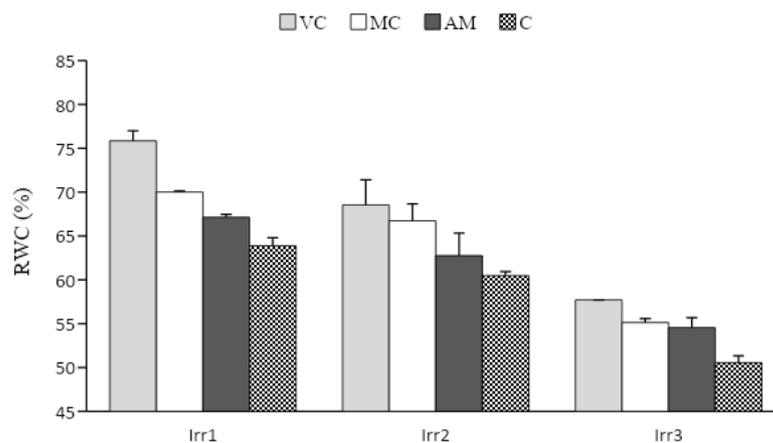


Figure 3. Comparing of the impact of irrigation regime and organic fertilizers implementation on RWC, relative water content ( $P \leq 0.05$  by LSD test). VC, vermicompost; MC, manure compost; AM, animal manure; C, Control; Irr1, Irr2, and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan.

### 3.4. Nutrients of P, K, and N

According to means comparison results, the amount of P, K, and N was increased under the use of organic fertilizers compared to the control treatment (no fertilizer application) in all irrigation levels. The highest P, K, and N were 0.36, 1.48, and 2.48% respectively (Figure 4), which were surveyed in plants under well-watered circumstances (Irr1) conditions and the application of VC. Whereas the lowest ones 0.20, 1.11, and 1.67%, respectively, were obtained under the most restrictive irrigation regime and without the application of fertilizer. However, the amount of P, K, and N in well-watered plants were considerably higher than in plants in deficit irrigation appearances (Figure 4). When the water supply in the soil is decreased, its uptake is limited. Furthermore, from a physiological perspective, the reduced water uptake results in not only reduced photosynthesis but also transpiration (Nyawade et al., 2021; Alavi-Samani et al., 2015). Under such conditions, active mobilization systems are equally disrupted for the purpose of saving biological energy consumption. These combine to cause a substantial loss of root absorbability hence reducing the nutrient uptake capacity (Bistgani et al. 2018). Under water deficit conditions, plants are exposed to both water deficit and osmotic stress due to a decrease in the soil matrix potential resulting in ionic imbalance and nutrient deficiency (Rahimi et al., 2022). In these conditions, organic fertilizers extend the root network and improve nutrition uptake; it also provides improved conditions for water uptake by plants and, so, better thriving conditions for the crop (Khosravi Shakib et al. 2019; Ievinsh et al., 2020). Besides, organic fertilizers enrich the nutrition of the plants, improving their vegetative growth (Maddahi et al., 2021).

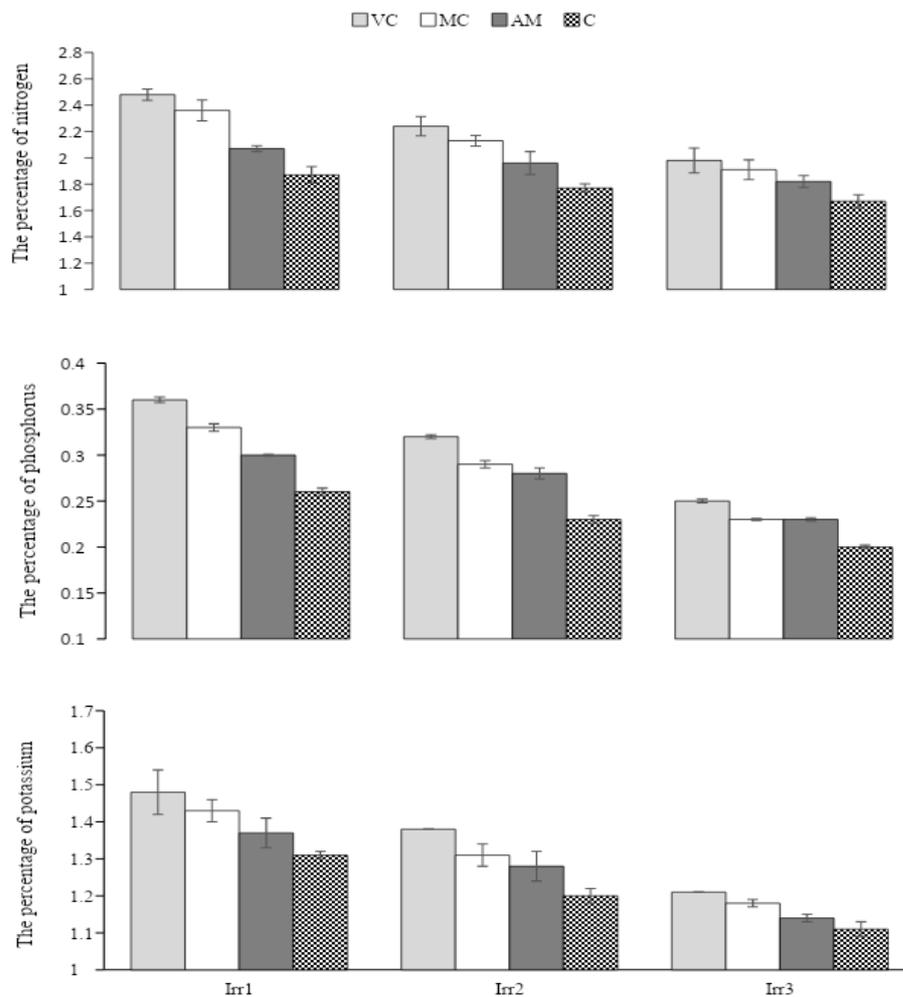


Figure 4. Comparing for impact of irrigation regime and organic fertilizers implementation on the percentage of nitrogen (a), phosphorus (b) and potassium (a). ( $P \leq 0.05$  by LSD test). VC, vermicompost; MC, manure compost; AM, animal manure; C, Control; Irr1, Irr2 and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan.

Table 4. Means a comparison of the effect of irrigation regime and organic fertilizers on some traits of *T. vulgaris* L.

|                            | Chlorophyll (mg/g FW) | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Chlorophyll a+b (mg/g FW) | Carotenoid (mg/g FW) | Essential oils (%) | Essential oils yield (g/m) |
|----------------------------|-----------------------|-------------------------|-------------------------|---------------------------|----------------------|--------------------|----------------------------|
| <b>Irr1</b>                | 1.37±0.12a            | 0.69±0.03a              | 2.06±0.11a              | 0.40±0.02a                | 2.29±0.13b           | 3.51±0.20a         |                            |
| <b>Irr2</b>                | 1.18±0.12b            | 0.62±0.04b              | 1.80±0.09b              | 0.34±0.02b                | 2.61±0.11 a          | 3.68±0.22a         |                            |
| <b>Irr3</b>                | 0.99±0.12c            | 0.56±0.04c              | 1.56±0.09c              | 0.29±0.02c                | 1.97±0.11c           | 2.53±0.19b         |                            |
| <b>Organic fertilizers</b> |                       |                         |                         |                           |                      |                    |                            |
| <b>VC</b>                  | 1.47±0.25a            | 0.86±0.10a              | 2.33±0.15a              | 0.42±0.03a                | 2.96±0.19a           | 4.45±0.33a         |                            |
| <b>MC</b>                  | 1.35±0.06a            | 0.58±0.02b              | 1.94±0.08b              | 0.34±0.00b                | 2.48±0.10b           | 3.64±0.22b         |                            |
| <b>AM</b>                  | 1.02±0.09b            | 0.56±0.04b              | 1.59±0.07c              | 0.33±0.01b                | 2.01±0.09c           | 2.70±0.15c         |                            |
| <b>C</b>                   | 0.86±0.09c            | 0.49±0.00c              | 1.35±0.08d              | 0.29±0.05c                | 1.71±0.11d           | 2.08±0.11d         |                            |

The same letters are shown statistically non-significant at  $P \leq 0.05$  by LSD test. VC, vermicompost; MC, manure compost; AM, animal manure; C, Control; Irr1, Irr2, and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan, irrigation after evaporation from Class A pan.

### 3.5. Antioxidant enzymes activity

According to the results, the SOD, CAT, and APX in water-deficit stress conditions were higher than in well-watered plants (Figure 5). The utmost SOD, CAT, and APX of 80.04, 3.48, and 1.32  $\mu\text{mol/g}$ , respectively (Figure 5), were observed in plants under water impairment stress and without the implementation of fertilizer, whereas the lowest ones (33.82, 1.5, and 0.75  $\mu\text{mol/g}$ ) was obtained from plants with well-watered and treated with VC (Figure 5). Water stress increased CAT, APX, and SOD activity. In some plants, damage emanating from water stress causes oxidative stress resulting in the production of toxic oxygen species and their accumulation preventing respiration and photosynthesis, hence negatively affecting plant growth (Keshavarz Mirzamohammadi et al., 2021).

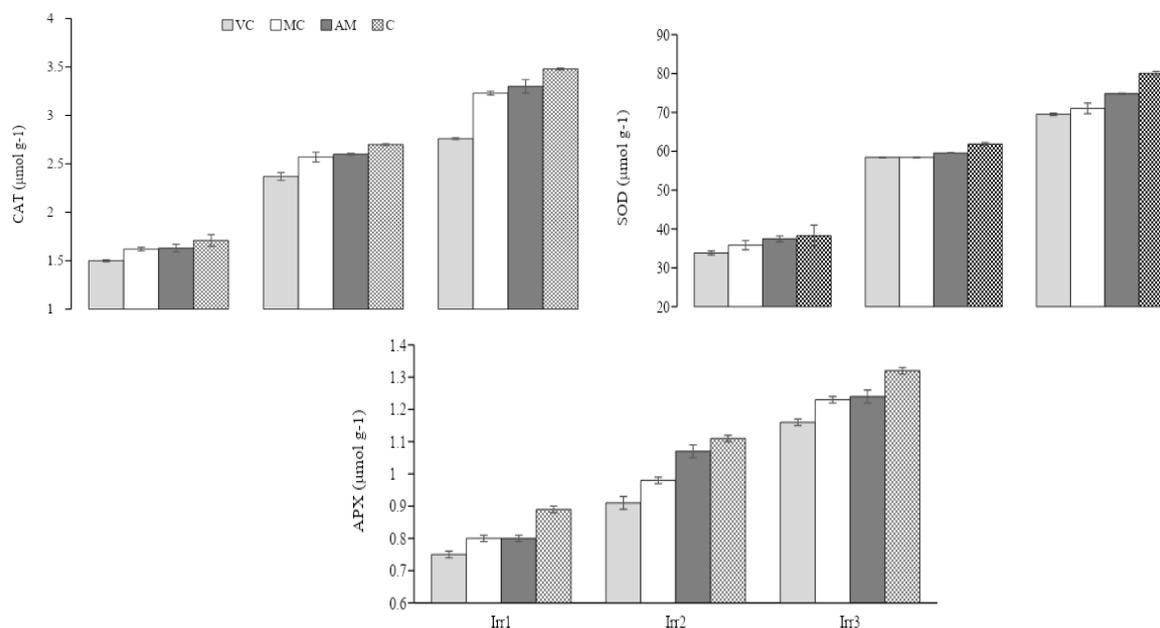


Figure 5. Comparing the interactive impact of irrigation regime and organic fertilizers implementation on CAT, catalase activity (a); SOD, superoxide dismutase activity (b) and APX, ascorbate peroxidase activity (c). ( $P \leq 0.05$  by LSD test). VC, vermicompost; MC, manure compost; AM, animal manure; C, Control; Irr1, Irr2, and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan.

### 3.6. Essential oil and essential oil yield

The results of mean comparisons showed that the highest essential oil (2.61%) and essential oil yield (3.68 g/m) were obtained under mild stress conditions (irrigated plants after 80 mm of evaporation from A pan). Also, there was no significant effect on essential oil yield in mild stress conditions

compared to the well-watered plants (Table 4). The highest essential oil (2.96%) and essential oil yield (4.45 g/m) were obtained from plants treated with VC, while the lowest ones (1.71% and 2.08 g/m) were obtained from plants without the use of fertilizers (control) (Table 4). It has been reported that the application of organic fertilizers can enhance the essential oils of canola by increasing nutrient absorption and the resulting improvement in CO<sub>2</sub> absorption and photosynthetic efficiency (Bistgani et al., 2017b; Bistgani et al., 2017a). Likewise, the application of vermicompost, compost, and animal manure increased the essential oil percentage and yield of Marigold (Khosravi Shakib et al., 2019). The results established that thymus' essential oil increases under slight drought stress conditions, with severe drought stress reducing the percentage of essential oil, and it is ascribed to essential oil storage, reduction in leaf area, and antioxidant power, which results in higher oil glands density. As drought level increases, the percentage of essential oil decreases, leading to not only protein degradation but also cell and plant death (Rahimi et al., 2022). Organic fertilizers stimulate the metabolite process and plant growth hence enhancing the production of medicinal plants (Saki et al., 2019). Nitrogen is fundamental in the promotion of cell division, suggesting that such organics can improve essential oil synthesis in plants and especially medicinal ones (Amooaghaie and Golmohammadi, 2017; Heidarzadeh et al., 2022). A decrease in essential oil content and biological yield in the plant can reduce the essential oil yield (Rahimi et al., 2022). Nonetheless, essential oil yield improved with the application of VC, MC, and AM. This might be attributed to improvement in soil cation exchange capacity (CEC) besides an increase in the availability of some elements such as nitrogen, which together could have decreased nutrient leaching, leading to not only higher total dry matter but also increased phytochemical concentration of plants (Saki et al., 2019; Heidarpour et al., 2019).

### 3.7. Biomass yield and seed yield

The biomass yield and seed yield of the plants that had been subjected to severe stress (irrigated after 120 mm of evaporation from A pan) were significantly lower compared to the irrigated plants after 60 mm of evaporation from A pan (Irr1). The highest biomass and seed yields were 570.14 and 169.36 g/m, respectively, were found to be related to well-watered plants and the application of VC, whereas the lowest ones (291.17 and 113.92 g/m) were obtained in severely stressed plants and without the use of fertilizers (control) (Figure 6). Cell growth is among the most key processes that are affected by water deficit due to lower turgor pressure (Das et al., 2017). When leaf size is reduced, the capacity to intercept light decreases, which subsequently decreases the total photosynthesis capacity (Das et al., 2017). Additionally, drought stress resulted in smaller stomatal apertures, leading to a significant decrease in the CO<sub>2</sub> exchange rate. Such reduction, therefore, resulted in lower photosynthesis and, consequently, reduced plant growth and performance (Amirnia et al., 2019; Nasar et al., 2021). Organic fertilizers (vermicompost and compost) can enhance plant growth and photosynthesis assimilation by increasing leaf area and photosynthetic activity capacity during the pre-flowering phase. As a consequence, increased re-mobilization of this organic matter from source to sink enhances growth regulators' characteristics during the post-flowering cycle (Khosravi Shakib et al., 2019). Organic fertilizers can have a direct effect on plant growth by increasing nitrogen absorption, phytohormone synthesis, and mineral solubility, all of which can have a significant impact on plant production (Goswami et al., 2017; Khosravi Shakib et al., 2019). Manure application may improve growth, biomass production, and seed yield by increasing mineral uptake, as well as reduce the negative effects of water scarcity on plants by improving leaf water efficiency, photosynthetic efficiency, transpiration rate, and water uptake of host plant roots (Askary et al., 2018). As a result, it leads to high leaf and stem dry weight as well as high total dry weight per plant.

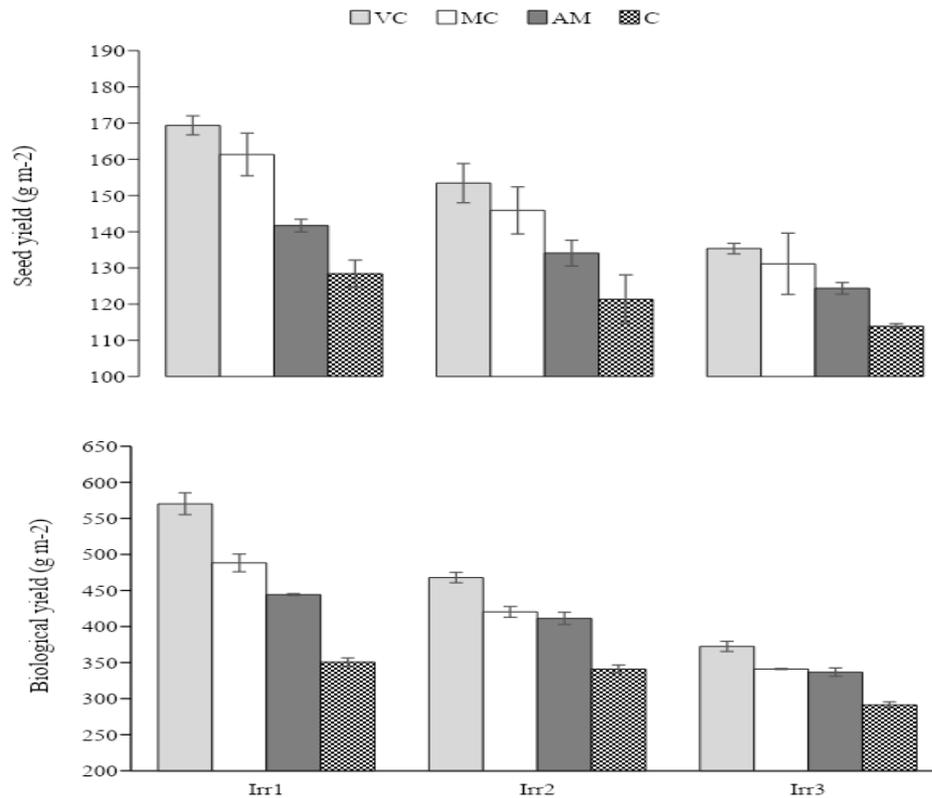


Figure 6. Comparing irrigation regime and organic fertilizers implementation on seed yield (a) and biological yield (b). ( $P \leq 0.05$  by LSD test). VC, vermicompost; MC, manure compost; AM, animal manure; C, Control; Irr1, Irr2, and Irr3, respectively, irrigation after 60, 90, and 120 mm evaporation from A pan.

#### 4. Conclusions

The results showed maximum contents of carotenoid chlorophyll a and b, and total chlorophyll of thyme were attained in well-watered conditions, while essential oil and essential oil yield were maximized under mild stress conditions. The highest chlorophyll a and b contents, total chlorophyll, carotenoids, essential oil, and essential oil yield were achieved in the application of VC. Under each irrigation regime, the application of VC fertilizer is more effective in improving nutrient uptake (K, P, and N) and RWC, biological yield, and seed yield of thyme. Water stress increases antioxidant activity by activation of enzymes (SOD, APX, and CAT), which enhances the protection of plants against MDA (lipid peroxidation). Hence, the application of organic fertilizers through increased water efficiency under water deficit conditions could improve antioxidant activity and thyme production in order to move towards sustainable agriculture.

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