

## EFFECT OF NITROGEN AND TEMPERATURE ON PHYTOMASS PRODUCTION AND ESSENTIAL OIL YIELD OF THYME<sup>1</sup>

### EFEITO DO NITROGÊNIO E DA TEMPERATURA NA PRODUÇÃO DE FITOMASSA E RENDIMENTO DE ÓLEO ESSENCIAL DE TOMILHO

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#### ABSTRACT

Thyme (*Thymus vulgaris*) is extensive used in the food and cosmetics industries, as well as for its medicinal purposes. The objective was to evaluate the phytomass production of thyme plants grown in different seasons and with different nitrogen concentrations in the nutrient solution, as well as to calculate the essential oil yield. The experiments were conducted in a greenhouse between the years 2019 and 2020. Fresh mass was measured to calculate phytomass production, and essential oil production was analyzed for yield. The design used in the greenhouse experiment was entirely randomized, conducted in a factorial scheme (2X5), in two seasons of the year (winter and summer) and 5 nitrogen concentrations in the nutrient solution (5, 7, 9, 11 e 13 mmol.L<sup>-1</sup>). Data on fresh mass production and essential oil yield were compared by the Scott-Knott test and analyzed by polynomial regression, at 5% probability of error. The treatment with the highest nitrogen concentration in summer promoted the highest average dry mass per plant (34.95 g) and the highest essential oil yield (10.27%). Thus, the nitrogen, added to the nutrient solution, promoted an increase in the vegetative growth of thyme plants only when the temperature was high. The increase in phytomass of the plants promoted a higher essential oil yield.

**Keywords:** Nutritional solution; Summer; *Thymus vulgaris*; Vegetative growth; Winter.

#### RESUMO

O tomilho (*Thymus vulgaris*) é amplamente utilizado nas indústrias alimentícia e cosmética, bem como para fins medicinais. O objetivo foi avaliar a produção de fitomassa de plantas de tomilho cultivadas em diferentes estações do ano e com diferentes concentrações de nitrogênio na solução nutritiva, bem como calcular o rendimento do óleo essencial. Os experimentos foram conduzidos em casa de vegetação entre os anos de 2019 e 2020. A massa fresca foi mensurada para cálculo da produção de fitomassa, e a produção de óleo essencial foi analisada para rendimento. O delineamento utilizado no experimento em casa de vegetação foi inteiramente casualizado, conduzido em esquema fatorial (2x5), em duas estações do ano (inverno e verão) e 5 concentrações de nitrogênio na solução nutritiva (5, 7, 9, 11 e 13 mmol.L<sup>-1</sup>). Os dados de produção de massa fresca e rendimento de óleo essencial foram comparados pelo teste de Scott-Knott e analisados por regressão polinomial, a 5% de probabilidade de erro. O tratamento com maior concentração de nitrogênio no verão promoveu a maior massa seca média por planta (34,95 g) e o maior rendimento de óleo essencial (10,27%). Assim, o nitrogênio, adicionado à solução nutritiva, promoveu aumento no crescimento vegetativo

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das plantas de tomilho somente quando a temperatura estava elevada. O aumento da fitomassa das plantas promoveu maior rendimento de óleo essencial.

**Palavras-chave:** Solução nutritiva; Verão; *Thymus vulgaris*; Crescimento vegetativo; Inverno.

## INTRODUÇÃO

Bioactive plants (aromatic, spice and medicinal) are used worldwide, contributing to the health and wellness condition of the population in general (Silva and Miranda, 2019) and their secondary metabolites are mainly responsible for their perfume and for their richness of applications (Mavandi *et al.*, 2021). The production chain of bioactive plants offers many opportunities, especially for family farmers, as it extends from production to packaging, essential oil extraction, and marketing for the food and beverage industry, cosmetics, phytotherapeutics, phytopharmaceuticals, dyes, handicrafts, insect repellents, or agricultural use (SENAR, 2017).

Thyme (*Thymus vulgaris* L.) is an example of a bioactive plant. In addition to being used as a spice in cooking, it is also used in folk medicine. The essential oil (EO) of thyme has been used as an expectorant (Heinzmann *et al.*, 2017), and in aromatherapy it is used to treat fatigue, anxiety, headache and rheumatic pain (Azambuja, 2019). In the cosmetic industry, thyme EO is used in products such as: disinfectants, mouthwashes and toothpastes due to its antimicrobial activity (Grossman, 2005). In addition, in terms of EO yield, each species has its own peculiarities and some have higher yield when the oil is extracted from fresh material and others from dry material. Regarding thyme, according to Usai *et al.* (2011), freezing was the best method to preserve the composition of EO and also showed the best thymol retention.

Although it is not possible to control all the abiotic factors that can have an impact on the plantations of aromatic, spice and medicinal plants, the use of greenhouses can minimize the negative effects that can reduce the quality and still allow to maintain a constancy over the years, a very important characteristic to maintain the therapeutic properties. The application of nitrogen fertilizers is also a strategy to improve the vegetative growth of plants. Since nitrogen (N) is one of the nutrients most required by plants (Vieira, 2017; Batista *et al.*, 2018), changes in its availability can alter the production of secondary metabolites (Verma and Shukla, 2015; Alami *et al.*, 2024). This is because nitrogen plays a fundamental role in essential oil production, acting as a crucial component of key cellular elements in plants, including chlorophyll, amino acids, and nucleic acids (Honorato *et al.*, 2024). For producers of EO-producing plants, it is important to obtain sufficient quantities of good quality biomass with high EO content.

Considering the extensive use of *T. vulgaris* in folk medicine, cooking and also in industry, further studies are needed to fill the gaps in knowledge about some of the production factors of this important species, such as how variables such as seasons (winter and summer) and different N concentrations influence the phytomass production of thyme. Assuming there is a difference in the

growth and essential oil production of *T. vulgaris* under different climatic seasons and with varying concentrations of nitrogen (N) in the nutrient solution, this study aimed to analyze the influence of winter and summer, as well as different nitrogen availabilities, on the phytomass production and essential oil yield of *T. vulgaris*.

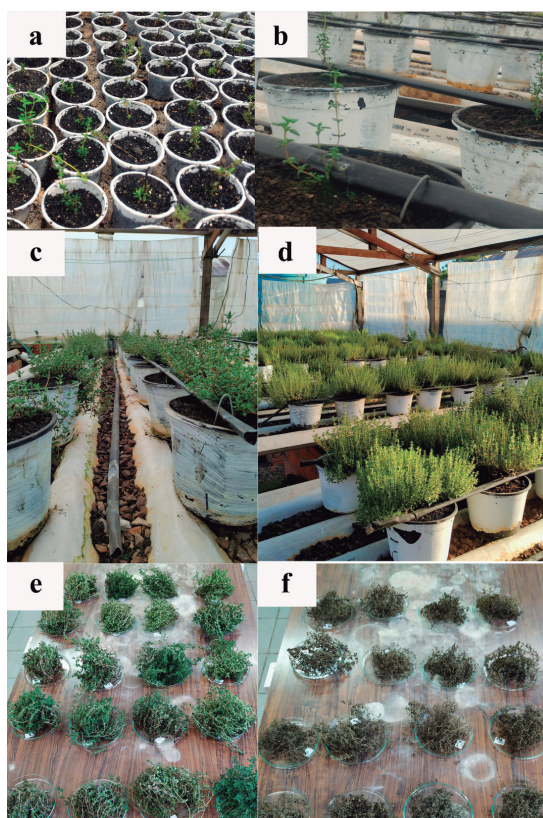
## MATERIAL AND METHODS

### GROWING THYME

The experiment was installed in a low-density polyethylene greenhouse with a thickness of 100  $\mu\text{m}$ , adapted with a fertirrigation system, at the Federal University of Santa Maria (UFSM). Santa Maria is a city with Cfa climate (humid subtropical, with hot summer and sometimes drought), according to Köppen, located in the center of the State of Rio Grande do Sul (S: 29 ° 42' 23" ; W: 53 ° 43' 15" ; 95 meters above sea level), with a clear division between the seasons. The experiment was carried out in two different climatic seasons, winter (June to September 2019) and summer (December 2019 to March 2020).

*T. vulgaris* seedlings of homogeneous size were transplanted into 4 dm<sup>3</sup> polypropylene pots filled with Maxfertil® substrate (Figure 1, a and b). The pots were placed on benches with a 500 L reservoir installed in each to store the nutrient solution.

**Figure 1** - *Thymus vulgaris* grown in greenhouse. a - *T. vulgaris* young seedlings; b - seedlings of homogeneous size transplanted; c - plants in greenhouse during the winter; d - plants in greenhouse during the summer; e - plants collected to fresh mass (FM) determined; f - dry mass (DM) determined.



Both experiments consisted of five replicates of 20 plants each, for a total of 100 plants per experiment. In each of the pots, the plants received nutrient solutions with different N concentrations.

The nutrient solution was delivered to the plants by a drip tape connected to an underwater pump in each tank. A timer was used to program the delivery schedule of the nutrient solution, three times per day. In each fertirrigation, excess nutrient solution was drained through the benches and the drained solution was collected through pipes that returned to the original reservoir in a closed system.

The nutrient solution for the cultivation of thyme was based on that proposed by Frescura *et al.* (2018), which was used for the cultivation of *Rosmarinus officinalis* (rosemary), also belonging to the Lamiaceae family, with minor modifications so that the concentrations of N were increased between the benches (treatments), totaling: 5, 7, 9, 11 and 13 mmol.L<sup>-1</sup>, supplied in the form of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>. The other nutrients had the same composition (in mmol.L<sup>-1</sup>) in all treatments: 1.4 of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>; 4 de K<sup>+</sup>; 3.06 of Ca<sup>+2</sup>; 2 de Mg<sup>+2</sup> and 6 de SO<sub>4</sub><sup>-2</sup>. The micronutrients were supplied by a solution composed of (mmol.L<sup>-1</sup>): 0.03 Mo; 0.26 B; 0.06 Cu; 0.50 Mn; 0.22 Zn by a stock solution and, the iron chelate, separately (1 mg.L<sup>-1</sup>).

The electrical conductivity (EC) of the standard solution was maintained at 1.5 dS.m<sup>-1</sup>. A maximum deviation of 20% in EC was tolerated, and for possible corrections, water or aliquots of new nutrient solution were used as needed. A pH between 5.5 and 6.5 was maintained, allowing a deviation of 0.2 units. Whenever the final volume was equal to or less than 50% of the initial volume, the nutrient solution was replenished.

The plants were placed in the greenhouse on June 13, 2019, and remained for 58 days (Figure 1, c). To measure the effect of seasonality, the material was collected on the day that marked the middle of the winter season. The heat sum was calculated for the days that the plants remained in the greenhouse during the winter. For the summer experiment, the winter temperature sum was used to define the day for collecting the plants, which was when the summer temperature sum reached a value close to the winter temperature sum (Figure 1, d).

The calculation of the daily temperature sum (STd°C day) followed the method proposed by Arnold (1960) and Gilmore and Rogers (1958), where:

$$STd = Tmed - Tb. 1 day$$

If  $Tmed < Tb$ , then  $Tmed = Tb$ . Considering that  $Tb$  = basal temperature (°C) for the species, and that there are no reports in the literature about the basal temperature for *T. vulgaris*, we agreed to use 0 °C as basal temperature;  $Tmed$  = mean daily air temperature (°C), calculated by the arithmetic mean between the minimum and maximum daily air temperatures, according to the conventional station of INMET/8° DISME, located approximately 500 meters from the experimental area.

## ANALYSIS OF THE COLLECTED MATERIALS

### Determination of phytomass

The thyme plants were collected in the early morning. In the first experiment, the plants were collected on August 12, 2019, and the thermal sum was calculated for those days. In the other experiments, when the thermal sum approached the value of the first, the plants were collected.

Immediately after collection, the fresh mass (FM) was determined by weighing four whole plants per treatment on analytical scales (Figure 1, e and f).

### Essential oil extraction

The extraction of the essential oil of *T. vulgaris* was performed from fresh frozen whole plants (leaves and stems) and also from dried plants. The process used to extract the essential oil was hydro distillation (Durazzini *et al.*, 2019). In a round bottom flask with a capacity of one liter, 50 g of fresh frozen material was placed, adding 700 mL of distilled water and boiled for two hours in a Clevenger apparatus. For the extraction of oil from dried material, a round bottom flask with a capacity of two liters was used and 25 g of dried material was placed in it, adding one liter of distilled water and boiled for two hours.

After extraction, the essential oil was collected in the form of supernatant, placed in an amber flask, weighed on an analytical balance, identified and stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  until use.

### Calculation of the essential oil yield

The essential oil yield (EOY), expressed as percent grams per fresh mass weight (%g.FM<sup>-1</sup>), was determined by the following equation:

$$\text{EOY} = \frac{\text{average treatment fresh mass X weight (grams) of extracted oil}}{\text{sample weight (g)}}$$

The EO content was obtained by the ratio between the weight (g) of extracted oil and the weight of the sample (g), multiplied by 100 to obtain the value of the content in percentage.

These calculations were performed in three repetitions in order to verify if extrinsic factors, such as different seasons (winter and summer) and different N inputs affect the production of essential oil from *T. vulgaris*.

## Statistical Analysis

The experimental design used in the greenhouse was entirely randomized, conducted in a bifactorial scheme (2X5), with a qualitative factor, two seasons of the year, and a quantitative factor, five nitrogen concentrations in the nutrient solution.

All data of phytomass production, essential oil yield and content were submitted to analysis of variance (ANOVA), were compared by the Scott-Knott test and evaluated by polynomial regression, at 5% probability of error, using the statistical program SISVAR 5.6 (Ferreira, 2014).

## RESULTS AND DISCUSSION

In the winter 2019 experiment, the plants were collected after 58 days, on the day that marked the middle of the season (August 12, 2019), and the thermal sum was calculated as STd = 837.45. During this period, the average temperature was 14.9°C and the average daily total insulation - the total time interval (between sunrise and sunset) when the sun was not hidden - was 4.93 hours.

In the summer experiment (2019/20), the plants stayed in the greenhouse for 33 days (STd = 839.10), receiving nutrients by fertirrigation, and the average temperature during this period was 25.4°C. During this period, the average total daily irradiation was 8.5 hours.

The analysis of variance showed the presence of a significant interaction between the two factors analyzed: growing season (winter and summer) versus treatments (different concentrations of N in the nutrient solution).

The fresh phytomass of the greenhouse experiments is shown in Table 1.

**Table 1** - Mean values of plant fresh mass and yield of essential oil extracted from fresh frozen plants of *Thymus vulgaris* (thyme) grown under different nitrogen (N) concentrations in winter and summer seasons in the years 2019 and 2020.

	Winter 2019	Summer 2019/20	Winter 2019	Summer 2019/20
Treatment	FM (g)	FM (g)	EOY (%g.FM <sup>-1</sup> )	EOY (%g.FM <sup>-1</sup> )
1	12,79 <sup>aA</sup>	17,50 <sup>aA</sup>	2,23 <sup>aA</sup>	3,40 <sup>aA*</sup>
2	17,69 <sup>aB</sup>	24,43 <sup>aB</sup>	2,33 <sup>aA</sup>	5,70 <sup>bB</sup>
3	17,99 <sup>aA</sup>	24,86 <sup>aB</sup>	3,10 <sup>aA</sup>	5,83 <sup>bB</sup>
4	20,09 <sup>aB</sup>	23,26 <sup>aB</sup>	5,07 <sup>bA</sup>	6,67 <sup>bB</sup>
5	19,30 <sup>aB</sup>	34,95 <sup>bD</sup>	5,80 <sup>bA</sup>	10,27 <sup>cC</sup>

T1 = N concentration in the nutrient solution of 5 mmol.L<sup>-1</sup>, T2 = 7 mmol.L<sup>-1</sup>, T3 = 9 mmol.L<sup>-1</sup>, T4 = 11 mmol.L<sup>-1</sup> and T5 = 13 mmol.L<sup>-1</sup>. FM = fresh mass. EOY = essential oil yield.

\*Averages followed by equal letters do not differ statistically using the Scott-Knott test (p<0.05).

Lower case letters compare treatments and upper case letters compare seasons.

Source: Authors.

The FM in the winter 2019 period was not statistically different between treatments. In the summer 2019/20 period, the FM of treatment 5 (highest dose of N = 13 mmol.L<sup>-1</sup>), 34.95 g, was

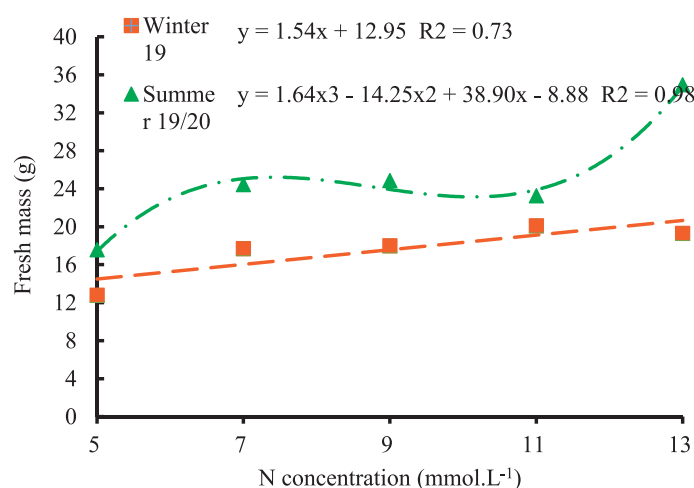
statistically different from the other treatments, with mean fresh mass 50.2% higher than treatment 4 (23.26 g) in summer 2019/2020 and 81% higher than treatment 5 (19.30 g) in winter 2019.

This proves that, in addition to the increased N concentration in the nutrient solution, the higher temperature observed in the summer season (12°C higher on average) played an important role in increasing the vegetative growth of the thyme plants.

In a study by Mortensen (2014), a 6°C increase in temperature from 18°C to 24°C increased the weight of thyme by 58%. In addition to temperature stimulating the development of thyme plants, increased N availability is also expected to positively affect growth, as the thermal sum was calculated over the time the plants remained in the greenhouse. From treatment 1 (N = 5 mmol.L<sup>-1</sup>) to treatment 5 (N = 13 mmol.L<sup>-1</sup>), there was a 99% increase of FM in summer (Dec2019/Jan20), from 17.50 g to 34.95 g.

Figure 2 shows the evolution of the phytomass with the increase of the N input available in the nutrient solution and also between winter 2019 and summer 2019/2020, where the higher temperatures positively influenced the growth of the thyme plants.

**Figure 2** - Increase in phytomass of thyme (*Thymus vulgaris*) plants in relation to different nitrogen (N) concentrations in the nutrient solution and growing seasons (winter 2019; 2019/20).

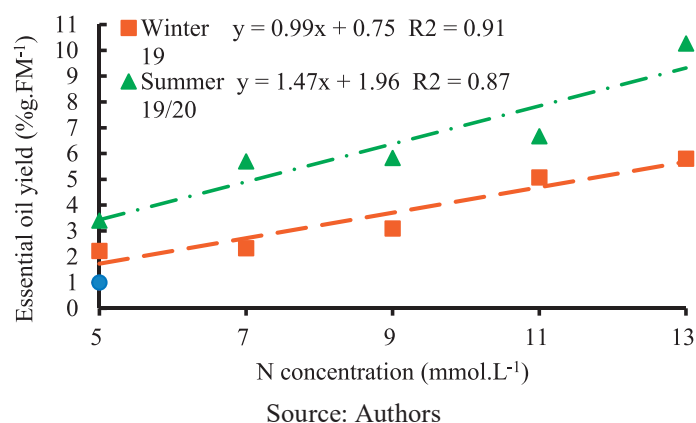


Source: Authors

In an experiment conducted by Baranauskienė *et al.* (2003), different doses of nitrogen fertilizer in thyme plantation had no significant effect on the content and composition of essential oil but resulted in higher biomass production and consequently higher yield of essential oil per area of cultivation, with doses of 45-90 kg.ha<sup>-1</sup> being the most effective.

Figure 3 shows the polynomial regression of essential oil yield for the two growing seasons different N concentrations.

**Figure 3** - Essential oil yield of fresh frozen thyme (*Thymus vulgaris*) plants grown under different nitrogen (N) concentrations in the nutrient solution and growing seasons (winter and summer).



By calculating the yield of thyme essential oil, expressed as a percentage of grams per weight of fresh mass (%g.FM<sup>-1</sup>), it was observed that the increase of N and the higher temperature provided a greater vegetative growth of thyme plants and consequently increased the yield of essential oil.

It can be said that the higher N input caused an increase in yield, most markedly in summer. In winter (Jun/Aug 2019), treatments 4 and 5 (N doses = 11 and 13 mmol.L<sup>-1</sup>, respectively) differed from the others, with higher EOY. In summer (Dec 2019/Feb 20) treatments 2, 3 and 4 did not differ from each other, but differed from treatment 1 and also from treatment 5, which again had the highest yield. The EOY of treatment 5 (10.27 %g.FM<sup>-1</sup>) in summer 2019/20 was 77 % higher than treatment 5 (5.80 %g.FM<sup>-1</sup>) in winter 2019. From treatment 1 (N = 5 mmol.L<sup>-1</sup>) to treatment 5 (N = 13 mmol.L<sup>-1</sup>), there was a 202% increase in oil yield in summer (2019/20), from 3.40 to 10.27 %g.FM<sup>-1</sup>.

Other authors obtained lower essential oil yields of thyme, 1.86% (Jakiemiu *et al.*, 2010) and 1.57% (Ozcan and Chalchat, 2004), lower than those found in this study, probably because they used dried plant material.

Authors such as Sifola and Barbieri (2006) also obtained an increase in phytomass production and essential oil yield of *Ocimum basilicum* (basil) with nitrogen fertilization. Studies by Honorato *et al.*, (2024) showed that thyme showed higher dry weight gains after the application of cattle and quail manure, while cattle manure also led to higher essential oil yield compared to the control (no organic fertilizer application). Furthermore, thymol, the main component of thyme EO, increased with higher doses of all three organic fertilizers (cattle, quail and goat) tested.

Deschamps *et al.* (2012) concluded that nitrogen sources and doses did not significantly affect the biomass of leaves and branches of *Mentha x piperita* L., as well as productivity and essential oil content; however, according to the authors, inorganic nitrogen applied in the form of urea and ammonium sulfate can significantly alter the composition of the oil.

To compare the amount of thyme essential oil in fresh and dry plants, oil was also extracted from dry plants, regardless of the N doses in the nutrient solution. The yield in grams per weight of

dry matter (%g.DM<sup>-1</sup>) was calculated from the extraction of 25 g of dry material. These data are presented in Table 2.

**Table 2** - Mean essential oil yield values of dried thyme (*Thymus vulgaris*) plants grown in the winter (Jun/Aug 2019) and summer (Dec 2019/Jan 2020) seasons.

Winter 2019	Summer 2019/20
EOY (%g.DM <sup>-1</sup> )	EOY (%g.DM <sup>-1</sup> )
4,0 <sup>a</sup>	7,5 <sup>b*</sup>

EOY = essential oil yield. DM = dry mass.

\*Averages followed by equal letters do not differ statistically using the Scott-Knott test (p<0.05).

Source: Authors

It can be seen that the average yield of EO from dried plants grown in summer (7.5 %g.DM<sup>-1</sup>) was 87.5 % higher than from plants grown in winter (4.0 %g.DM<sup>-1</sup>), which is related to a higher vegetative growth of plants when exposed to higher temperatures.

The average EO yield from fresh *T. vulgaris* plants during winter was 3,70 (%g.FM<sup>-1</sup>), while in summer it reached 6,37 % (as shown in Table 1). While the average EO yield from fresh plants was 72,16 % higher in summer compared to winter, the average EO yield from dried plants cultivated in summer (7,5 %g.DM<sup>-1</sup>) was 87,5 % higher than that of plants grown in winter (4,0 %g.DM<sup>-1</sup>). This comparison clearly demonstrates the influence of seasonality on the average EO yield, with summer positively affecting and increasing the EO content.

According to Taiz *et al.* (2017), seasonal variations in the concentration, content, and composition of essential oils are expected for most plant species. A study by Maldaner *et al.* (2025) also clearly shows the influence of seasonality on different species, for example: *Artemisia camphorata* Vill. and *Baccharis trimera* (Less.) DC. showed increased EO yields in spring; *Cupressus sempervirens* L. had higher EO yields in winter; *Ocimum basilicum* L. and *Melaleuca alternifolia* Cheel. exhibited increased yields in autumn and *Mentha* sp. had a higher EO yield in summer. In contrast, species such as *Corymbia citriodora* Hill & Johnson, *Foeniculum vulgare* Mill., *Pinus elliottii* Engelm. and *Schinus terebinthifolius* Raddi. did not show significant EO yield variation throughout the year. In our experiment, thyme also presented a higher EO yield during the summer, which is highly relevant, as it allows for the optimization of EO extraction and the production of higher-quality oils.

Moreover, drying *T. vulgaris* plants also increased EO yield when compared to fresh plant material. In this case, the increase in EO yield from dried plants was due to water loss during the drying process, which concentrates the volatile components of the essential oil in the plant biomass. In summer, dried thyme plants yielded 17.74% more EO than fresh plants harvested in the same season. *Origanum vulgare* L. also showed higher EO yields when dried at room temperature compared to fresh material (Caputo *et al.*, 2022), a trend also observed in *Zanthoxylum rhetsa* (Roxb.) (Theeramunkong & Utsintong, 2018) and *Thymus daenensis* Celak. (Mashkani *et al.*, 2018).

Thus, the cultivation systems aimed at producing high-value medicinal and aromatic plants, especially in a context of climate change that directly affects growth patterns and secondary metabolism in plant species.

## CONCLUSION

Nitrogen, added to the nutrient solution, promotes an increase in the vegetative growth of thyme plants only when the temperature is high. The higher amount of fresh mass of thyme plants during the summer resulted in higher EO yields compared to winter. Furthermore, the yield of EO from dried plants was higher than from fresh frozen plants.

Obviously, understanding how abiotic factors - such as temperature, nutrient availability, and humidity - influence the production of medicinal plants is of great importance in the search for optimal growing conditions in the field, along with maximum quality in terms of bioactive compounds of medicinal interest.

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