UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, e89744, v. 47, 2025 • https://doi.org/10.5902/2179460X89744
Submitted: 11/11/2023 • Approved: 09/25/2025 • Published: 11/07/2025

Biology-Botany

Chromatographic profile and cytogenotoxic activity of thyme (*Thymus vulgaris* L.) essential oil cultivated in two seasons

Perfil cromatográfico e atividade citogenotóxica do óleo essencial de tomilho (*Thymus vulgaris* L.) cultivado em duas estações

Carmine Aparecida Lenz Hister[I], Nadia Helena Bianchini[I], Berta Maria Heinzmann[I], André Schoffel[II], Sidinei José Lopes[I], Jerônimo Luiz Andriolo[I], Solange Bosio Tedesco[I]

[I] Universidade Federal de Santa Maria, Santa Maria, RS, Brasil
[II] Universidade Federal de Cruz Alta, Cruz Alta, RS, Brasil

ABSTRACT

Thyme (*Thymus vulgaris* L.) besides its wide use in the food and cosmetic industries, is also widely used for medicinal purposes, being used as an antimicrobial, fungicide and for the treatment of respiratory diseases, thanks to its antioxidant property. This work aimed to evaluate the chemical composition of the essential oil of thyme cultivated in a greenhouse in different seasons (winter and summer), as well as to evaluate its cytogenotoxicity potential. Experiments were conducted in a greenhouse with a nutrient solution supplied to the plants through a drip tape connected to a submerged pump inside a reservoir. The essential oil of thyme was extracted by the hydrodistillation method. Gas chromatography (GC) was used to profile the essential oil chemicals, and the *in vivo Allium cepa* L. test was employed to analyze the cytotoxic and genotoxic potential. The data were subjected to analysis of variance (ANOVA) and compared by Skott-Knott test. The GC data were subjected to cluster analysis and showed thymol as the majority substance in the oil in both winter (52.41%) and summer (45.94%). The essential oil showed antiproliferative activity at the highest concentration tested (0.25%) and genotoxic effect, by *A. cepa* test.

Keywords: *Allium cepa*; Antiproliferativo; Estações climáticas; Genotoxicidade

RESUMO

O tomilho (*Thymus vulgaris* L.), além de sua ampla utilização nas indústrias alimentícia e cosmética, também é amplamente utilizado para fins medicinais, sendo utilizado como antimicrobiano, fungicida e também no tratamento de doenças respiratórias, graças a sua propriedade antioxidante. Este trabalho teve como objetivo avaliar a composição química do óleo essencial de tomilho cultivado em casa de

vegetação em diferentes estações (inverno e verão), bem como avaliar seu potencial citogenotóxico. Foram conduzidos experimentos em casa de vegetação com solução nutritiva fornecida às plantas por meio de uma fita gotejadora conectada a uma bomba submersa dentro de um reservatório. O óleo essencial de tomilho foi extraído pelo método de hidrodestilação. A cromatografia gasosa (CG) foi usada para traçar o perfil dos produtos químicos do óleo essencial e o teste *in vivo* de *Allium cepa* L. foi empregado para analisar o potencial citotóxico e genotóxico. Os dados foram submetidos à análise de variância (ANOVA) e comparados pelo teste de Scott-Knott. Os dados de CG foram submetidos à análise de cluster e mostraram o timol como a substância majoritária no óleo tanto no inverno (52,41%) quanto no verão (45,94%). O óleo essencial apresentou atividade antiproliferativa na maior concentração testada (0,25%) e efeito genotóxico, pelo teste de *A. cepa*.

Palavras-chave: *Allium cepa*; Antirpoliferativo; Estações climáticas; Genotoxicidade

1 INTRODUCTION

Through the generations, ancient civilizations passed on their empirical knowledge, which resulted in the spread of the use of plants for healing purposes. On a global scale, so-called alternative or traditional medicine, which uses plants to treat and cure ailments, is used by about 88% of the world's population (Who, 2019). In Brazil, there is no data on the number of people who make use of medicinal plants, but presumably it follows this global trend, both in the consumption of plants *in natura* and in preparations such as teas and herbal medicines (Carvalho et al., 2007). The low-income population is the main user of plants for therapeutic purposes, often as the only alternative (Planta et al., 2000; Martelli et al., 2019).

Besides their use in the treatment of diseases, several other uses are attributed to plants and their derivatives, which has been arousing interest mainly in the food and cosmetics industries. An element originating from the secondary plant metabolism that is being widely used is the essential or volatile oil, and some of its medicinal uses can be cited as: aromatherapy, antitumor, antidepressant, antimicrobial, insecticide, acaricide (Raut & Karuppayil, 2014; Teixeira et al., 2013); as herbicides, in the alternative control of phytopathogens, as allelopathic (Sharifi-Rad et al., 2017) and postharvest disease control (Combrinck et al., 2011; Lopes et al., 2023).

A species widely used both in cooking and in folk medicine that deserves attention is *Thymus vulgaris* L. (Lamiaceae), popularly known as thyme. Usually, thyme is used for the treatment of respiratory diseases (cough, bronchitis and asthma) in the form of tea, ointment, syrup or vapor inhalation (Javed et al., 2013), and it also has antiseptic, antimicrobial, carminative and disinfectant actions (Hosseinzadeh et al., 2015). Thyme essential oil is widely used by the cosmetic and also pharmaceutical industry, mainly for its antioxidant properties (Asbaghian et al., 2011).

The cultivation of medicinal plants for various purposes, including the production of herbal medicines, is a practice that should be increasingly improved to solve possible quality problems that may occur with plants from extractivism, for example. In this perspective, the production of bioactive plants with control of abiotic factors, is important for the generation of standardized vegetable by-products, with quality control, aiming at the constancy of chemical components, which meet the most varied needs of both producers and consumers (Morais, 2009). In this scenario, protected cultivation systems and soilless cultivation allow for adequate production planning, which consequently leads to higher productivity and improved final product quality (Andriolo, 2017).

In addition, to guarantee the population's safe use of certain medicinal plants, preliminary tests using bio indicators should be performed, especially regarding toxicity. Among the toxicogenetic biotests, the *Allium cepa* (onion) test is widely used to identify possible harmful effects of numerous substances on onion root meristematic cells. In the natural environment, the first part of the plant to be exposed to toxic agents dispersed in soil and water is the root, so its analysis represents a rapid method for monitoring toxicity (Vieira & Silveira, 2018).

In this sense, this biotest is considered ideal for a preliminary verification of genotoxic and antiproliferative effects, because it presents high sensitivity and correlation with tests using animal cells (Carmo et al., 2020; Tedesco et al., 2017). In addition to its long history of use in cytotoxicological tests, other advantages of using *A.*

cepa include its large chromosomes that are easily observable under a microscope; the ability to reveal effects (positive or negative) even at relatively low levels of interaction between the tested substance and the genetic material; and, as an in vivo test, the results obtained can be applied to the evaluation of genotoxicity in plants as well as in other eukaryotes, including man (Bonciu et al., 2018).

In view of the above, the objective of the present article was to determine the chemical composition of the essential oil of *T. vulgaris* grown in two seasons (winter and summer), and evaluate the potential genotoxic and antiproliferative effects by *A. cepa* test.

2 MATERIAL AND METHODS

2.1 Thyme cultivation

The experiment was installed in an adapted greenhouse with fertigation system, located in the Department of Plant Science, Federal University of Santa Maria (UFSM). Santa Maria is a city with Cfa climate (humid subtropical, with hot summer, and sometimes drought), according to Köeppen (Alvares et al., 2013), located in the center of the state of Rio Grande do Sul, 95 meters above sea level, having as characteristic the clear division between seasons.

One hundred *Thymus vulgaris* L. seedlings, produced by cuttings, were grown in pots filled with commercial Maxiplant® substrate, in two seasons, winter 2018 (june-august) and summer 2018/2019 (december-january). The pots were on benches, inside a low-density polyethylene greenhouse with a thickness of 100 µm. Nutrient solution was supplied to the plants through a drip tape connected to a submerged pump inside a reservoir. A timer was used to schedule the supply time of the nutrient solution (three times a day). The drained solution was collected and then returned to the original reservoir in a closed system.

After 60 days in the greenhouse during the winter, the plants were collected. In order to measure the effects of seasonality, the material was collected on the day that marked the mid-season (August 06, 2018). The heat sum for the 60 days that the plants remained in the greenhouse during the winter was calculated. For the summer experiment, the sum temperature of the winter was used to define the day of collection of the plants, which was when the sum temperature of the summer reached a value close to that of winter (January 9, 2019).

2.2 Essential oil extraction and dilution

The essential oil of thyme was extracted in the Laboratory of Plant Cytogenetics and Genotoxicity (LABCITOGEN) of the Department of Biology. Immediately after collection, the fresh mass (FM) was determined by weighing 20 whole plants on analytical scales. Next, 50 grams (g) samples of fresh plants were weighed and frozen for later extraction of essential oil.

The hydrodistillation method was performed using a Clevenger apparatus for two hours, with 50 g of fresh-frozen thyme. After extraction, the EO was collected in the form of supernatant, deposited in an amber flask, weighed on an analytical balance, identified and stored at 4°C ± 2°C until its use. Dilution of the EO was performed with absolute ethanol, obtaining concentrations of 0.10% and 0.25%.

Oil samples were analyzed by gas chromatography to determine the phytochemical composition.

2.3 *Allium cepa* test

Cytogenotoxicity analysis was performed using the *A. cepa* test, which consists of examining meristematic cells from onion roots to determine mitotic indexes and to look for chromosomal changes.

For the test, seven groups of four onion bulbs (repetitions) were placed in distilled water until they emitted roots. After root emergence, each group of four onion

bulbs was transferred to one of the following treatments: controls (negative control = distilled water; negative control relative to oil dilution = ethanol; and positive control = glyphosate) and diluted thyme EOs, where they remained for 24 hours. The detailed description of the treatments and controls used in this experiment is shown in Table 1.

Table 1 – Treatments used in the evaluation of the cytotoxicity and genotoxicity of the essential oil of *Thymus vulgaris*, by the *Allium cepa* test

Treatment	Description
T1	Negative control in distilled water (NC)
T2	Negative control in ethanol (NCE)
T3	Positive control in 2% glyphosate (PC)
T4	Essential oil (0.10%) - extracted from fresh-frozen thyme plants cultivated in winter (EO010W)
T5	Essential oil (0.10%) - extracted from fresh-frozen thyme plants cultivated in summer (EO010S)
T6	Essential oil (0.25%) - extracted from fresh-frozen thyme plants cultivated in winter (EO025W)
T7	Essential oil (0,25%) - extracted from fresh-frozen thyme plants cultivated in summer (EO025S)

Source: Organized by the authors

After the treatment period, all roots were collected, fixed in ethanol: acetic acid (3:1 ratio) for 24 hours, and stored in 70% ethanol under refrigeration. The slides for the analysis of meristematic cells of *A. cepa* roots were prepared according to the crushing method (Guerra & De Souza, 2002), with minor modifications (Pereira et al., 2022). For each treatment, two slides were prepared (repetitions). Under a Leica optical microscope, the slides were examined by the scanning method at 40X magnification. In each slide, 500 cells were analyzed, totaling 1000 cells per bulb and 4000 cells per treatment.

The meristematic cells were classified according to the stage of cell division in which they were found (interphase, prophase, metaphase, anaphase and telophase), for subsequent calculation of the mitotic index (MI). The MI was calculated by the ratio

of the number of dividing cells by the total number of cells analyzed and multiplied by 100. Any chromosomal irregularities were also counted and photographed. The genotoxic index (GI) was calculated by the ratio of the number of cells with alterations by the total number of cells analyzed and multiplied by 100.

2.4 Chemical analysis of the essential oils

The chromatographic analysis was performed in the Laboratory of Plant Extractives (LABEVE) of the Department of Forest Sciences at UFSM. For the analysis, an aliquot of 2 microliter (µL) of essential oil was diluted in 1 mL of hexane (HPLC grade) in duplicate.

The chemical composition of the EO was determined using an Agilent 7890A gas chromatograph coupled to a 5075C mass spectrometer (GC-MS) with a non-polar DB5-MS capillary column (Hewlett Packard, 5% phenylmethyl siloxane, 30 m × 0.25 mm i.d. × 0.25 µm film thickness), electron ionization mode at 70 eV. Helium was the carrier gas (1.0 mL·min-1), injector and detector temperatures of 150°C and 280°C were used, respectively. The samples were injected in split inlet mode (ratio 1:100). The oven was heated at 40 °C for 4 min, and then the temperature was increased to 320 °C at a rate of 4 °C·min^{-1}.

The identification of compounds was based on comparison of retention indices, calculated by linear interpolation, to the retention times of an n-alkane series, and a comparison of their mass spectra with authentic samples and with literature data (Adams, 2009; NIST, 2009). The relative amounts of the components were calculated based on GC peak areas, using gas chromatography with a flame ionization detector (GC-FID). The analysis parameters were the same as those presented above, except for the split ratio (1:50) and the injector and detector temperatures (300ºC).

2.5 Statistical analysis

The corresponding data from the *A. cepa* test were compared by the Scott-Knott test ($p < 0.05$), using the statistical software SISVAR 5.6 (Ferreira, 2014). The CG data were subjected to cluster analysis in the GENES software (Cruz, 2013), using the Mahalanobis distance as a dissimilarity measure, and the clustering was performed using the Tocher method.

3 RESULTS AND DISCUSSION

The temperature sum corresponding to the 60 days of the winter experiment (June-August) was calculated as TS = 827.15, with an average temperature during this period of 13.3°C. In the summer experiment (December - January), the plants were collected when the temperature sum reached a value close to that of winter (TS = 836.85), completing 33 days in the greenhouse, and on these days the average temperature was 25.4 ºC. The average fresh mass per plant was 17.57 g in winter and 25 g in summer. The average essential oil yield per thyme plant was 3.70% $g.MF^{-1}$ in winter (percentage in grams by weight of fresh mass), and 6.37% $g.MF^{-1}$ in summer (Hister et al., 2025). Taking into account a spacing of 30 cm between plants and 45 cm between rows, in 1 hectare it would be possible to plant approximately 74 thousand thyme plants per hectare, each hectare could yield on average 372.59 kg of essential oil.

The analysis of meristematic cells of *A. cepa* root tips subjected to thyme essential oil (EO) treatments are listed in Table 2.

It is observed that only the treatment with 0.25% diluted EO from winter-grown plants (OE025W) showed antiproliferative cytotoxic activity, differing statistically from the negative controls (NC and NCE). Although the other treatments reduced the number of cells in cell division, this reduction in proliferation did not differ significantly from the negative controls. In this sense, only the treatment with the highest concentration of essential oil had cytotoxic effect on *A. cepa* root cells. Aazza et al. (2014) attribute

the antiproliferative activity to the monoterpenes, thymol and carvacrol, which are predominantly present in thyme EO. Other medicinal species have also demonstrated antiproliferative activity of their essential oils, such as *Vitex negundo* L. (Issa et al., 2020), *Rosmarinus officinalis* L. (Cosme et al., 2022), *Origanum vulgare* L. (Husić et al., 2023) and *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. (Sousa et al., 2024).

Table 2 – Number of *Allium cepa* meristematic cells analyzed in the different treatments with *Thymus vulgaris* essential oil and their respective mitotic index (MI) and genotoxic index (GI), including cells in interphase, cells in division and cells with irregularities

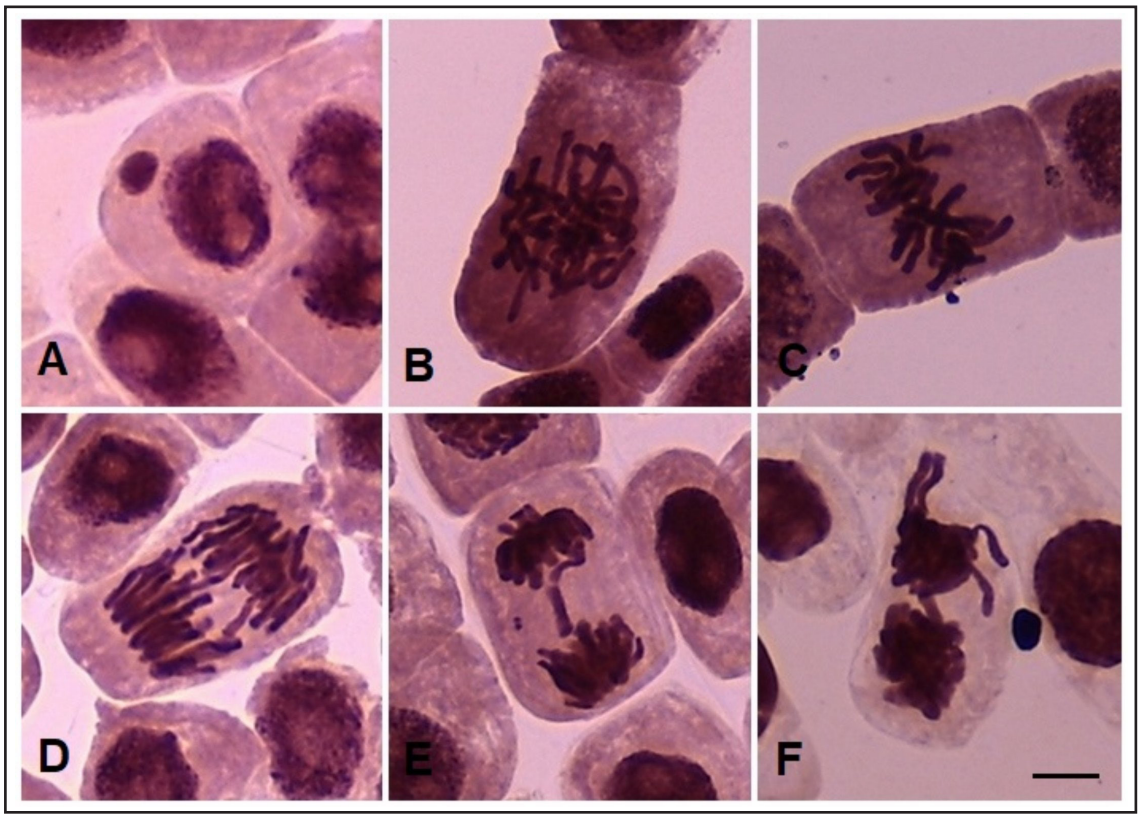
Treatments	Total of analyzed cells	Cells in interphase	Cells in division	Cells with chromosomal abnormalities	MI (%)	GI (%)
T1 - NC	4000	3802	198	0	4.95[a]	0[b]*
T2 - NCE	4000	3807	191	29	4.78[a]	0.73[a]
T3 - PC	4000	3876	122	47	3.05[b]	1.18[a]
T4 - EO010W	4000	3817	183	40	4.58[a]	1.00[a]
T5 - EO010S	4000	3819	180	43	4,50[a]	1.08[a]
T6 - EO025W	4000	3847	149	41	3.73[b]	1.03[a]
T7 - EO025S	4000	3804	195	46	4.88[a]	1.15[a]

NC = negative control; NCE = negative control in absolute ethanol; PC = positive control; EO010W = essential oil (0.10%) - extracted from fresh-frozen thyme plants cultivated in winter; EO010S = essential oil (0.10%) - extracted from fresh-frozen thyme plants cultivated in summer; EO025W = essential oil (0.25%) - extracted from fresh-frozen thyme plants cultivated in winter; EO025S = essential oil (0.25%) - extracted from fresh-frozen thyme plants cultivated in summer. *Values followed by different letters differ statistically by the Scott-Knott test ($p < 0.05$). Source: Organized by the authors

Regarding genotoxicity, all treatments with EO, as well as with NCE and PC controls, did not differ; however, all showed statistically significant differences when compared with the negative control in distilled water (NC). In other words, the EO treatments—regardless of concentration or climatic season tested—not only differed from the NC in water but also did not differ from the NC in ethanol (used as the oil diluent), nor from the positive control with glyphosate, a substance known to cause mutations. In this sense, although the chromosomal alterations found were low in number (an average of 1% of

the cells analyzed), it is possible to affirm that thyme EO is genotoxic. Figure 1 illustrates some chromosomal alterations found in the slide analysis.

Figure 1 – Chromosomal alterations visualized in meristematic cells of onion (*Allium cepa*) roots treated with thyme essential oil (*Thymus vulgaris* L.)



Source: Authors (2019)
Caption: A) Micronucleus; B) Disorganized metaphase; C) Metaphase with missing chromosome; D) Anaphase bridge; E) Anaphase with missing chromosome and micronucleus; F) Telophase bridge. Scale: 10 µm

Regarding the quality of the essential oil from thyme plants, a chemical analysis was performed through gas chromatography (GC). The chromatographic profile resulting from the GC performed on the winter-grown and summer-grown thyme EO can be seen in Table 3.

Table 3 – Chemical composition obtained by gas chromatography of the essential oil of *Thymus vulgaris* plants grown in greenhouse during the winter and the summer

Componente	KI_C	KI_L	% Winter	% Summer
α-Thujene	924	930	0.58[1]	1.50[1*]
α-Pinene	931	934	0.34[1]	0.72[1]
Benzaldehyde	959	958	0.16[1]	0.34[1]
Sabinene	975	974	0.65[1]	2.33[1]
β-Myrcene	989	988	0.28[1]	0.39[1]
α-Phellandrene	1004	1001	-	0.12[1]
α-Terpinene	1015	1015	1.77[1]	2.86[1]
β-Cymene	1023	1022	9.26[1]	8.35[1]
Limonene	1027	1027	0.99[1]	1.16[1]
Eucaliptol	1030	1031	0.14[1]	0.22[1]
γ-Terpinene	1057	1060	17.57[1]	23.04[1]
Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	1069	1069	0.21[1]	0.22[1]
β-Linalool	1099	1099	1.75[1]	1.43[1]
Camphor	1145	1145	0.71[1]	-
Borneol	1170	1172	0.55[1]	0.74[1]
1-Terpinen-4-ol	1179	1178	0.10[1]	0.11[1]
α-Terpineol	1193	1190	-	0.10[1]
Methyl thymyl ether	1237	1236	0.31[1]	0.46[1]
Thymol	1289	1290	52.41[2]	45.94[2]
Carvacrol	1296	1296	2.57[1]	2.65[1]
Thymol acetate	1344	1349	0.29[1]	0.32[1]
β-Caryophyllene	1418	1418	2.93[1]	2.32[1]
Geranyl propionate	1468	1473	-	0.14[1]
Germacrene D	1479	1480	0.29[1]	0.42[1]
δ-Cadinene	1516	1515	0.23[1]	-
γ-Cadinene	1511	1510	-	0.21[1]
τ-Cadinol	1640	1640	-	0.10[1]
TOTAL			**94,10%**	**95,99%**

KI_C: calculated kovats index; KI_L: literature kovats index (NIST, 2009). *Clustering by Tocher method. Source: Organized by the authors

The GC analysis was able to detect 94.10% on the winter-grown thyme EO, identifying 22 chemical substances present in the oil, with thymol being the most abundant, with 52.41%. The other major compounds were γ-terpinene (17.57%), β-cymene (9.26%), β-caryophyllene (2.93%), and carvacrol (2.57%). In the summer-

grown thyme EO, it was possible to identify 25 chemical compounds that represent 95.99% of the elements, with thymol again being the predominant substance, with 45.94%. The other major components differed only by the presence of α-terpinene instead of β-caryophyllene among the five most abundant elements, namely γ-terpinene (23.04%), β-cymene (8.35%), α-terpinene (2.86%), and carvacrol (2.65%). Thyme essential oil with a high thymol content is considered to be of excellent quality. According to the ISO 19817 (2017) standard, the thymol content in thyme essential oil should be between 35 and 55%. Therefore, thyme essential oil extracted under the conditions presented above provides excellent quality oil.

Corroborating the results found here, previous studies have already demonstrated the predominance of thymol in thyme essential oil (Dauqan & Abdullah, 2017; Sharangi & Guha, 2013), followed by carvacrol, p-cymene, γ-terpinene (Agili, 2014; Borugă et al., 2014; Gedikoğlu et al., 2019). This variation in the other majoritarian compounds may be linked to various environmental, nutritional, and climatic factors to which the thyme plants were subjected.

Although thymol was the majority component of thyme EO in both seasons tested, in winter it was present in even greater amounts, accounting for more than half of the chemical composition of the oil. This may be related to the cytotoxicity found only in EO from winter-grown plants. Commonly, the cytotoxic activity of some EOs is mainly due to the presence of substances such as alcohols, aldehydes and phenols (Bakkali et al., 2008), these which are part of the terpenes, being thymol a phenol of the monoterpene group.

Marco et al. (2012) tested the EO of *Lippia sidoides Cham*. (pepper rosemary), whose main component is thymol, on the germination of *Lactuca sativa* seeds and observed that there was an inhibitory cytotoxic effect. Experiments by Gedikoğlu et al. (2019) showed antimicrobial activity of thyme essential oil. Thymol can cause cell apoptosis and telomere shortening in *Candida albicans* (Kumar et al., 2025). Isolated thymol has also been shown to be active against *Salmonella* and *Staphylococcus*

bacteria (Dauqan & Abdullah, 2017). Essential oils of oregano and rosemary pepper, with carvacrol and thymol being the major constituents of these oils, respectively, showed greater antifungal activity against four fungi that cause post-harvest diseases in mangoes (Vilela et al., 2024).

The cytotoxicity of EO in mammalian cells is caused by the induction of apoptosis and necrosis (Bakkali et al., 2008). In this regard, studies by Sertel et at. (2011) have already shown that thyme EO inhibited the growth of human oral cavity squamous cell carcinoma and Kubatka et al. (2019), in an *in vitro* study, revealed the antiproliferative and pro-apoptotic effects of thyme EO on mammary carcinoma cells.

Characteristics such as cytotoxicity are of great interest as it sanctions the use of EOs and other phytoproducts in a variety of practices, such as in preserving food and other agricultural products, and especially in fighting pathogens. It has been shown that EOs and some of their isolated constituents are really effective in protecting against a wide range of organisms, and can be used as antiseptics and antimicrobials for personal use and for insecticidal use in the preservation of crops or food stocks (Bakkali et al., 2008).

In the experiments presented here, up to the 0.25% concentration, thyme EO induced chromosomal alterations, although in a small amount, and it is not possible to exempt thyme EO, and possibly thymol, from potential genotoxic activity, since thymol and carvacrol alone have already demonstrated genotoxic effect in previous studies (Alves et al., 2018; Pinheiro et al., 2015). Also, the low GI may also be due to the low concentration of the EO used in the treatments.

4 CONCLUSIONS

EO extracted from fresh winter-grown thyme shows antiproliferative cytotoxic potential only at the 0.25% concentration. In addition, EO shows, albeit low, genotoxicity at the concentrations tested. Thymol is the major substance in the EO of both winter and summer thyme plants, exceeding 50% of the oil composition in winter.

These results may serve as an indicator for future studies with thyme, in the search for practical benefits that take into account its wide range of biological activity, including the ability to cause cytotoxic effects, such as inhibition of cell division and consequent cell death, along with the reduced ability to induce chromosomal alterations.

ACKNOWLEDGEMENTS

I thank fellows Jéssica Mena Barreto de Freitas, Luísa Gonçalves Rodrigues and Julia de Senna Pereira for their help in transplanting the seedlings to the greenhouse.

REFERENCES

Aazza, S., Lyoussi, B., Megías, C., Cortés-Giraldo, I., Vioque, J., Figueiredo, A. C., & Miguel, M. G. (2014). Anti-oxidant, anti-inflammatory and anti-proliferative activities of Moroccan commercial essential oils. *Natural product communications, 9*(4), 587–594.

Adams, R. P. (2009). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Illinois: Allured Publishing Corporation

Agili, F. A. (2014). Chemical composition, antioxidant and antitumor activity of *Thymus vulgaris* L. essential oil. *Middle-East Journal of Scientific Research*, *21*(10), 1670–1676. https://doi.org/10.5829/idosi.mejsr.2014.21.10.85182

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. de M. & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6), 711–28.

Alves, T. de A., Pinheiro, P. F., Praça-Fontes, M. M., Andrade-Vieira, L. F., Corrêa, K. B., de Assis Alves, T., Cruz, F. A., Júnior, V. L., Ferreira, A. & Soares, T. C. B. (2018). Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in *Lactuca sativa* and *Sorghum bicolor*. *Industrial Crops and Products*, *114*(1), 59–67. https://doi.org/10.1016/j.indcrop.2018.01.071

Andriolo, J. L. (2017). *Olericultura Geral* (3 ed.). Santa Maria, RS: Editora da UFSM.

Asbaghian, S., Shafaghat, A., Zarea, K., Kasimov, F., & Salimi, F. (2011). Comparison of volatile constituents, and antioxidant and antibacterial activities of the essential oils of *Thymus caucasicus*, *T. kotschyanus* and *T. vulgaris*. *Natural Product Communications*, 6(1), 137–140. https://doi.org/10.1177/1934578X1100600133

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology*, 46(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Bonciu, E., Firbas, P., Fontanetti, C. S., Wusheng, J., Karaismailoğlu, M. C., Liu, D., Menicucci, F., Pesnya, D. S., Popescu, A., Romanovsky, A. V., Schiff, S., Ślusarczyk, J., Souza, C. P., Srivastava, A., Sutan, A., & Papini, A. (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia*, 71(3), 191–209. https://doi.org/10.1080/00087114.2018.1503496

Borugă, O., Jianu, C., Mişcă, C., Goleţ, I., Gruia, A. T., & Horhat, F. G. (2014). *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. *Journal of Medicine and Life*, 7(3), 56–60.

Carmo, L. R. do, Leal, L. S., & Ribeiro, L. R. (2020). *Allium cepa* e teste do micronúcleo como bioindicadores de citogenotoxicidade em extratos aquosos de plantas medicinais. *Brazilian Journal of Development*, 6(10), 82419–82430. https://doi.org/10.34117/bjdv6n10-610

Carvalho, A. C. B., Nunes, D. de S. G., Baratelli, T. de G., Shuqair, N., & Netto, E. M. (2007). Aspectos da legislação no controle dos medicamentos fitoterápicos. *T&C Amazonia*, 11(5), 26–32.

Combrinck, S., Regnier, T., & Kamatou, G. P. P. (2011). In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. *Industrial Crops and Products*, *33*(2), 344–349. https://doi.org/10.1016/j.indcrop.2010.11.011

Cosme, M. V., Nunes, N. M. F., Oliveira, A. P. de, & Oliveira, E. C. A. de. (2022). Evaluation of the cytotoxic and mutagenic potential of *Rosmarinus officinalis* L. essential oil through the *Allium cepa* Leach bioassay. *Acta Scientiarum. Biological Sciences*, 44, e62665. https://doi.org/10.4025/actascibiolsci.v44i1.62665

Cruz, C. D. (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy*, 35(3), 271–276.

Dauqan, E. M. A., & Abdullah, A. (2017). Medicinal and functional values of thyme (*Thymus vulgaris* L.) herb. *Journal of Applied Biology & Biotechnology*, 5(2), 17–22. https://doi.org/10.7324/jabb.2017.50203

Ferreira, D. F. (2014). Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia*, 38(2), 109–112. https://doi.org/10.1590/s1413-70542014000200001

Gedikoğlu, A., Sökmen, M., & Çivit, A. (2019). Evaluation of *Thymus vulgaris* and *Thymbra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science and Nutrition*, 7(5), 1704–1714. https://doi.org/10.1002/fsn3.1007

Guerra, M., & De Souza, M. J. (2002). *Como observar cromossomos: um guia de técnicas em citogenética vegetal, animal e humana*. Ribeirão Preto, SP: FUNPEC.

Hister, C. A. L., Andriolo, J. L., Tedesco, S. B. (20250. Effect of nitrogen and temperature on phytomass production and essential oil yield of thyme. *Disciplinarum Scientia. Série: Naturais e Tecnológicas, 26*(2), 89-101. https://doi.org/0.37779/nt.v26i2.5193

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: A review of *Thymus vulgaris*. *International Journal of Clinical Medicine*, 6(9), 635–642. https://doi.org/10.4236/ijcm.2015.69084

Husić, L., Parić, A., & Mesic, A. (2023). Allelopathic and toxicological effects of *Origanum vulgare* L. essential oil. *Caryologia*, 76(1), 97–102. https://doi.org/10.36253/caryologia-2132.

ISO (International Organization for Standardization). (2017). ISO 19817: Essential oil of thyme [*Thymus vulgaris* L. and *Thymus zygis* L.], thymol type. Geneva, Switzerland. https://www.iso.org/standard/66267.html

Issa, M., Chandel, S., Pal Singh, H., Rani Batish, D., Kumar Kohli, R., Singh Yadav, S., & Kumari, A. (2020). Appraisal of phytotoxic, cytotoxic and genotoxic potential of essential oil of a medicinal plant *Vitex negundo*. *Industrial Crops and Products*, 145, 112083. https://doi.org/10.1016/j.indcrop.2019.112083

Javed, H., Erum, S., Tabassum, S., & Ameen, F. (2013). An overview on medicinal importance of *Thymus vulgaris*. *Journal of Asian Scientific Research*, 3(10), 974–982.

Kubatka, P., Uramova, S., Kello, M., Kajo, K., Samec, M., Jasek, K., Vybohova, D., Liskova, A., Mojzis, J., Adamkov, M., Zubor, P., Smejkal, K., Svajdlenka, E., Solar, P., Samuel, S. M., Zulli, A., Kassayova, M., Lasabova, Z., Kwon, T. K., Pec, M., Danko, J., & Büsselberg, D. (2019). Anticancer activities of *Thymus vulgaris* L. in experimental breast carcinoma In Vivo and In Vitro. *International Journal of Molecular Sciences*, 20(7), 1749–1778. https://doi.org/10.3390/ijms20071749

Kumar, D., Ansari, A., Bajpai, A. B., Rai , N., & Kumar, N. (2025). *Thymus vulgaris* L. (Thyme): A herbal remedy against fungal infections. *Environment Conservation Journal, 26*(1), 219–225. https://doi.org/10.36953/ECJ.30233158

Lopes, V. C., Benato, E. A., Silva, B. M. da P., Veiga, J. C. da, Bron, I. U., & Cia, P. (2023). Antifungal activity of lemongrass and thyme essential oils and effect on gray mold control and postharvest quality of 'Italia' grape. *Bragantia*, 82, e20220202. https://doi.org/10.1590/1678-4499.20220202

Marco, C. A., Teixeira, E., Simplício, A., Oliveira, C., Costa, J., & Feitosa, J. (2012). Chemical composition and allelopathyc activity of essential oil of *Lippia sidoides* Cham. *Chilean Journal of Agricultural Research*, 72(1), 157–160. https://doi.org/10.4067/s0718-58392012000100025

Martelli, A., Esquisatto, M. A. M., Andrade, T. A. M., Aro, A. A., Mendonça, F. A. S., Santos, G. M. T. (2019). Phytotherapics in tissue healing and its interface with professionals of health in Brazil. *Brazilian Journal of Development*, 5(7), 10997-11016.

Morais, L. A. S. de. (2009). Óleos essenciais no controle fitossanitário. *In*: Bettiol, W. & Morandi, M. A. B. (Orgs.), *Biocontrole de doenças de plantas: uso e perspectivas* (p. 139–152). Jaguariúna: Embrapa Meio Ambiente.

NIST. (2009). *NIST/EPA/NIH Mass Spectral Library & Search Program*. Hoboken: John Wiley & Sons.

Pereira, J. de S., Hister, C. A. L., Ubessi, C., & Tedesco, S. B. (2022). Genotoxicity, cytotoxicity and phenolic compounds from aqueous extracts of *Phyllanthus tenellus* Roxb. cultivated under different light conditions. *Pakistan journal of biological sciences*, 25(7), 575–585. https://doi.org/10.3923/PJBS.2022.575.585

Pinheiro, P. F., Costa, A. V., De Assis Alves, T., Galter, I. N., Pinheiro, C. A., Pereira, A. F., Oliveira, C. M., & Fontes, M. M. P. (2015). Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays. *Journal of Agricultural and Food Chemistry*, 63(41), 8981–8990. https://doi.org/10.1021/acs.jafc.5b03049

Planta, M., Gundersen, B., & Petitt, J. C. (2000). Prevalence of the use of herbal products in a low-income population. Family medicine, 32(4), 252–257.

Raut, J. S., & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, 62, 250–264.

Sertel, S., Eichhorn, T., Plinkert, Peter, K., & Efferth, T. (2011). Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma. *Anticancer Research*, 31(1), 81–87.

Sharangi, A. B., & Guha, S. (2013). Wonders of leafy spices: Medicinal properties ensuring human health. *Science International*, 1(9), 312–317.

Sharifi-Rad, J., Sureda, A., Tenore, G. C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M. R., Ademiluyi, A. O., Sharifi-Rad, R., Ayatollahi, S. A., & Iriti, M. (2017). Biological activities of essential oils: From plant chemoecology to traditional healing systems. *Molecules*, 22(1), 70. https://doi.org/10.3390/molecules22010070

Sousa, M. A. A. de, Silva, F. S. L., & Orlanda, J. F. F. (2024). Genotoxic and antiproliferative effect of *Alpinia zerumbet* (Zingiberaceae) essential oil in *Allium cepa* biotest. *Ciência e Natura*, 46, e73445. https://doi.org/10.5902/2179460x73445

Tedesco, M., Kuhn, A. W., Frescura, V. D., Boligon, A. A., Athayde, M. L., Tedesco, S. B., & Silva, A. C. F. da. (2017). Assessment of the antiproliferative and antigenotoxic activity and phytochemical screening of aqueous extracts of *Sambucus australis* Cham. & Schltdl. (ADOXACEAE). *Anais da Academia Brasileira de Ciencias*, 89(3 Suppl.), 2141–2154. https://doi.org/10.1590/0001-3765201720150138

Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M. F., Saraiva, J. A., & Nunes, M. L. (2013). Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Industrial Crops and Products*, 43, 587–595. https://doi.org/10.1016/j.indcrop.2012.07.069

Vieira, L. F. A., & Silveira, G. L. (2018). Cyto(Geno)Toxic Endpoints Assessed via Cell Cycle Bioassays in Plant Models. *In:* Çelik, T. A. (Org.), *Cytotoxicity* (p. 117–129). London: IntechOpen.

Vilela, E. S. D., Terao, D., Queiroz, S. C. do N. de, Silva, A. M. da, Maia, A. de H. N., Fracarolli, J. A., Dorta, C., & Santos, L. da S. dos. (2024). Essential oils on the control of fungi causing postharvest diseases in mango. *Brazilian Journal of Microbiology*, 55, 689–698. https://doi.org/10.1007/s42770-023-01237-2

Who, World Health Organization (2019). WHO global report on traditional and complementary medicine 2019. https://www.who.int/publications-detail/who-global-report-on-traditional-and-complementary-medicine-2019

Authorship contributions

1 – Carmine Aparecida Lenz Hister

PhD in Agronomy from the Federal University of Santa Maria
https://orcid.org/0000-0003-1692-7698 • carmineh@gmail.com
Contribution: Conceptualization, Methodology, Software, Validation, Data curation, Formal analysis, Investigation, Visualization, Resources, Project administration, Writing - original draft, Writing – review & editing

2 – Nadia Helena Bianchini

PhD in Forestry Engineering from the Federal University of Santa Maria
https://orcid.org/0000-0002-7035-6075 • nhbianchini@gmail.com
Contribution: Methodology, Writing – review & editing

3 – Berta Maria Heinzmann

PhD in Natural Sciences from the University of Bonn
https://orcid.org/0000-0002-6509-949X • berta.heinzmann@gmail.com
Contribution: Supervision, Writing - review & editing

4 – André Schoffel

PhD in Agronomy from the Federal University of Santa Maria
https://orcid.org/0000-0002-2501-4834 • andre-schoffel@hotmail.com
Contribution: Software, Writing - review & editing, Data visualization

5 – Sidinei José Lopes

PhD in Agronomy from the University of São Paulo
https://orcid.org/0000-0002-7117-541X • sjlopes2008@gmail.com
Contribution: Supervision, Writing - review & editing

6 – Jerônimo Luiz Andriolo

PhD in Physiologie Bases de La Production Végétale from Université Montpellier 2 - Sciences et Techniques
https://orcid.org/0000-0002-7439-2604 • jeronimoandriolo@gmail.com
Contribution: Conceptualization, Methodology, Supervision, Writing - review & editing

7 – Solange Bosio Tedesco

PhD in Animal Science from the Federal University of Rio Grande do Sul
https://orcid.org/0000-0002-9673-1996 • solatedesco@gmail.com
Contribution: Conceptualization, Methodology, Supervision, Writing - review & editing

How to quote this article

Hister, C. A. L., Bianchini, N. H., Heinzmann, B. M., Schoffel, A., Lopes, S. J., Andriolo, J. L., & Tedesco, S. B. (2025). Chromatographic profile and cytogenotoxic activity of thyme (*Thymus vulgaris* L.) essential oil cultivated in two seasons. *Ciencia e Natura*, 47, e89744. DOI: 10.5902/2179460X89744. Available in: https://doi.org/10.5902/2179460X89744