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In vivo study of lavender and thymus essential oil vapour-phase on the antifungal potential and quality of strawberries stored for 7 days.

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Abstract
In the context of current agriculture, with the limitation of using synthetic compounds, finding for human and environment friendly alternatives is a priority. In our study, we utilized the property of essential oil evaporation by applying the method of enriching the storage atmosphere with essential oil-vapours for preserving strawberries in hermetically sealed containers at 10^0C. Lavender essential oil (EOL) and garden thyme essential oil (EOT), tested at two concentrations (C1=IC50; C2=2xIC50) served as working alternatives to appreciate the antifungal effect and the modification of fruits quality indicators: weight, firmness, acidity, and the content of total soluble sugars. After 7 days, the fungal spoilage disease index (FSD) was established. The highest value of the FSD index was recorded for the control, followed by the variants in the order TOL-C1 >EOL-C1 > EOT-C2 > EOL- C2. The total antifungal effect was determined in the variant EOL-C2. The double amount of essential oil stimulates the respiration of the strawberry fruits, which leads to water loss, modifying the texture and resulting in an increase in the firmness indicator.
Keywords: acidity, antifungal effect, firmness, fungal spoilage disease (FSD), TSS

Introduction

Essential oils enjoy great interest nowadays. Known since ancient times, they have been used for centuries in the curative treatment of various diseases, especially respiratory ones. In addition to their use in the medical field, especially in the last decade, a practical approach to them has also emerged and developed in agriculture, due to the protective effect they have shown against plant pests. [2,14,21]
So far, research in the field has demonstrated the insecticidal potential of essential oils, which are effective as repellents against store pests, constituting a natural, sustainable, environmentally friendly, and human healthy alternative for the storage of grains [3]. The antimicrobial effect of essential oils has been studied and demonstrated both in vitro and in vivo. The use of essential oils in preserving plant products represents a current approach, as post-harvest losses have reached alarming levels primarily due to the ubiquitous spread of spoilage fungi [12]. The preservation of fruits in the atmosphere enriched with essential oil-vapours is a very current topic, given the effectiveness of the antimicrobial action of essential oils [13]. Their application mainly aims to minimize losses due to the destructive action of spoilage microorganisms, which automatically has an impact on extending the shelf life [5,18].

Another important issue related to the use of essential oils concerns their effect on the nutritional and sensory qualities of the products. As such, consistent research is necessary regarding the exposure time and the amount of essential oil required so that the antifungal action can be achieved while also being feasible from a cost perspective, given that essential oils have a rather high production cost [7].

Strawberries are recognised as fruits that spoilage quickly. Their storage is recommended to be carried out at low temperatures, like refrigeration, which ensures a decrease in respiratory metabolism, contributing to the extension of the consumption period [16]. Changes in strawberries are caused by opportunistic fungi that colonise the fruits right from the plantation. The fungal load is greater when the technology used is based on low input, limiting chemical treatments for crop protection [17]. Consequently, the use of essential oils as an alternative for ensuring the integrity of fresh produce both in pre-harvest and post-harvest is considered friendly as essential oils are included in the category of generally regarded as safe (GRAS). Rhizopus, Fusarium, Mucor, and Botrytis are the main fungi responsible for the spoilage of strawberries [22]. They are saprophytic, mesophilic microorganisms which require temperatures above 7 degrees for spore germination. Numerous studies related to the preservation of strawberries have been conducted at low temperatures [10,15]. However, to demonstrate the antifungal effect of essential oil vapours on postharvest, studies at temperatures level that facilitate fungal spore germination and assess the negative or positive effects of essential oils acting through vapours are necessary.

In our study, we used the evaporative properties of lavender essential oil (EOL) and thyme essential oil (EOT), each applied in two concentrations (C1 similar IC50, C2 = 2*IC50) to determine the antifungal effect and the modification of the quality properties of strawberries preserved in an atmosphere enriched with essential oil vapours for a period of 7 days at 10^0C.

Material and Methods

In this study we used lavender essential oil, extracted by hydrodistillation from dried plants, cultivar 'Hidcote' from the 2022 harvest. The chemical composition was determined by GS-MS, and the proportion of the main compounds is presented in Table 1. The predominant compound is *gamma-Terpinene* (49,966%), a monoterpenoid that categorises this cultivar within the specific chemotype [5]. The compound *beta Caryophyllene* (26,852%) is specific to Lamiaceae family, a valuable source is also found in black pepper [21].

Table 1. The components of lavender essential oil (EOL)

No	Chemical compounds	*Lavender angustifolia* essential oil
1.	**alfa Thujene**	**5.807**
2.	beta Piniene	0.393
3.	beta.- Myrcene	4.603
4.	D-Limonene	6.062
5.	beta.-Phellandrene	1.411
6.	**gamma Terpinen**	**49.966**
7.	alfaThujona	1.459
8.	Camphor	2.208
9.	**beta Caryophyllene**	**26.852**
10.	Octanoic Acid	0.164
11.	Isothymol	0.602
12.	Thymol	0.472
	Total compounds identified (%)	**99,397**

The essential oil of *Thymus vulgaris* was purchased from the local producer SOLARIS and was analysed by GC-MS to determine the bioactive compounds. The major compound is *o-cymene* with a content of 32.920%, followed by *thymo*l at 19.988%, a compound specific to Thymus genera, and *γ-terpinene* at 14.311%, as shown in Table 2.

Table 2. The components of thymus essential oil (EOT)

No	Chemical compounds	*Thymus vulgaris* essential oil
1.	α-thujene	2,751
2.	α -pinene	2,193
3.	Camphene	1,688
4.	2-β-pinene	0,574
5.	1-octen-3-ol	0,811
6.	β-myrcene	2,740
7.	1-phellandrene	0,302
8.	α-terpinene	2,931
9.	**o-cymene**	**32,920**
10.	D-Limonene	1,344
11.	1,8-cineole	1,442
12.	**γ-terpinene**	**14,311**
13.	Sabinene hydrate	0,454
14.	Linalool	2,030

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15.	Camphor	0,375
16.	Borneol	1,144
17.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	0,571
18.	Endobornyl acetate	0,444
19.	Phenol, 5-methyl-2-(1-methylethyl)	3,332
20.	**Thymol**	**19,988**
21.	Trans-Caryophyllene	1,975
22.	δ-cadinene	0,261
23.	Caryophyllene oxide	0,488
	Total compounds identified (%)	**95,069**

To determine the antifungal effect, it is necessary to use the indirect method based on the action of essential oil vapours to inhibit spore germination and ensure the preservation of fruit [5]
The concentrations and the corresponding doses, Table 3, have been determined in line with the results of previous research related to the determination of the dose IC50 [19].

Eight healthy strawberries from organic farm, free from damage, were placed in a container with 800ml capacity (500ml net), made of glass with a hermetic seal. A filter paper with the essential oil generating vapours was attached to the lid. Each variant was done in 2 repetitions which also served to determine the quality indicators at the end of the storage period. In total, 10 containers were used and approximately 2 kg of healthy fruits, without any damages, treatments, discolorations, etc. The hermetically sealed containers of fruits were kept under controlled conditions, in darkness, at 10^0C. for. After 7 days, the number of affected fruits was recorded and the intensity of fungal degradation and the index of fungal spoilage disease of strawberries (FSD), was calculated according to the formula:

$$FSD = FSS \times (Ff \times 100)$$

where:
FSS- fungal spoilage severity (1- nonfungal spoilage; 2- till 20% fungal spoilage; 3- 21%-50% fungal spoilage; 4-50%-80% fungal spoilage; 5-over 81% fungal spoilage)
Ff- fungal frequency on strawberry fruits =number of spoilage strawberry / number of strawberry on repetition

Table 3. The amounts and concentrations of essential oil applied in the study

EO doses	C1(~IC 50)		C2 (2*IC50)	
	EO doses µL/jar	EO µL L^{-1} air space	EO doses µL/jar	EO µL L^{-1} air space
Control	0	0	0	0
EOL Lavender	434	868	868	1736
EOT Thymus	250	500	500	1000

Determining the qualitative parameters of strawberry fruits

The weight was determined gravimetrically, by weighing before and after storage in an essential oil-enriched atmosphere by formula G1-G7/G1x100, where G1- weight in day1, G7 – weight in day 7, [8].

The firmness was determined at the maximum diameter of the fruit, carrying out two perpendicular penetrations, at the initial moment and after preservation in a modified atmosphere. The penetrometer FORCE GAUGE PCE-FM200 was use, the results were expressed in maximum penetration force (N).

The acidity of the fruits, as assessed by pH value, was determined from the juice of strawberries using the portable pH meter from Hanna Instruments HI98190, employing reference electrodes that measure electric potential. This was done for each variant before incubation and after 7 days of storage. The analysis was conducted at a temperature of 25±2°C.

The total soluble sugar content (TSS) was determined from strawberry using the KRUSS - DR201-95 handheld refractometer, from juice of unspoiled fruits. The results were expressed in BRIX.

Statistical analysis.

The antifungal action of the essential oils was monitored for 16 fruits (n=16). The statistical analyses involved ANOVA variance for significance at $p<0.05$ (Microsoft Excel 2013) for weight indicator. Results of firmness, acidity and TSS content are expressed by average for eight fruits for each EOs treatment.

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Results and Discussion

Results regarding the in vivo antifungal potential of essential oil vapours of lavender and thyme

FSD is a synthetic index calculated based on the frequency of occurrence of fungi (Ff) and the fungal spoilage severity (FSS). It is evaluated by giving scores: 1- nonfungal spoilage; 2- till 20% fungal spoilage; 3- 21%-50% fungal spoilage; 4-50%-80% fungal spoilage; 5-over 81% fungal spoilage. Assessments are made for each repetition. The index FSD may have values ranging from 1 to 500, with values below 100 appearing when the degree of depreciation is low, and above 400 when there is a high degree of fungal depreciation. As can be seen in Figure 1, the highest value of the FSD index is 187.5 and was calculated for the control follow by variants in the order >EOT-C1 >EOL-C1 >EOT-C2 >EOL-C2.

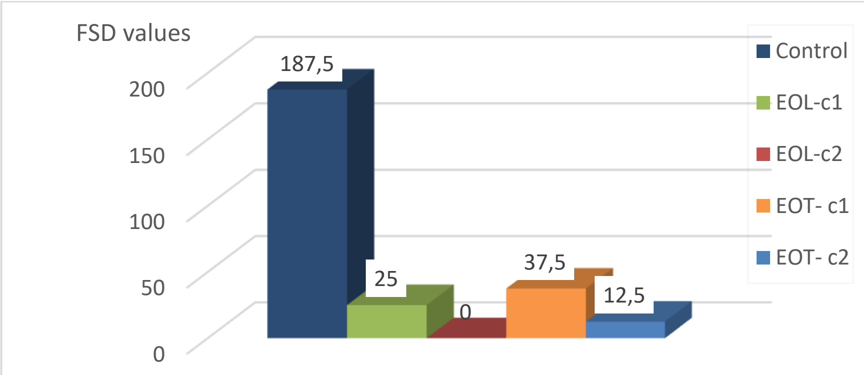

Figure 1. Values of the FSD index noticed after storing for 7 days of strawberries in atmosphere enriched with lavender essential oil (EOL-C1 and EOL-C2) and thyme essential oil vapours (EOT-C1 and EOT-C2)

Results regarding the evolution of quality indicators

Weight loss is an important indicator that correlates with storage temperature [10]. At low temperature of around 4^0C, the metabolic processes in fruits are minimized. Therefore, water loss through respiration is reduced and weight loss will be minimal.

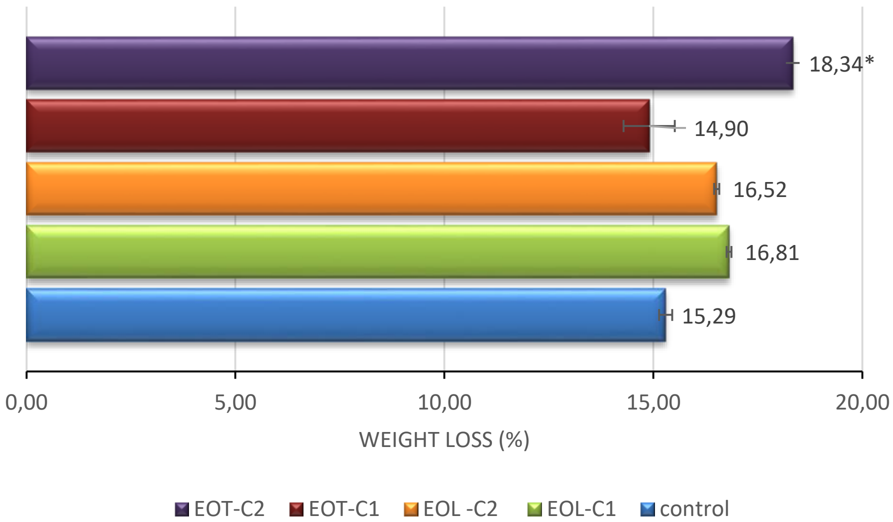

Figure 2. The weight losses recorded for strawberries after 7 days of storage in essential oil vapours of lavender essential oil (EOL-C1 and EOL-C2) and thyme essential oil vapours (EOT-C1 and EOT-C2); * significative positive difference compared to control at P<0.05

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Throughout the 7 days of storage, the fruits lost weight across all study variants. Statistically significant differences were noted for the variant EOT -C2, with a loss of 18.34% compared to the initial weight. For the rest of the variants, weight differences exist but are not statistically significant. The presence of oil vapours at a similar dose IC 50 provides partial antifungal protection and does not stimulate the fruits' respiration, with results comparable to the control.

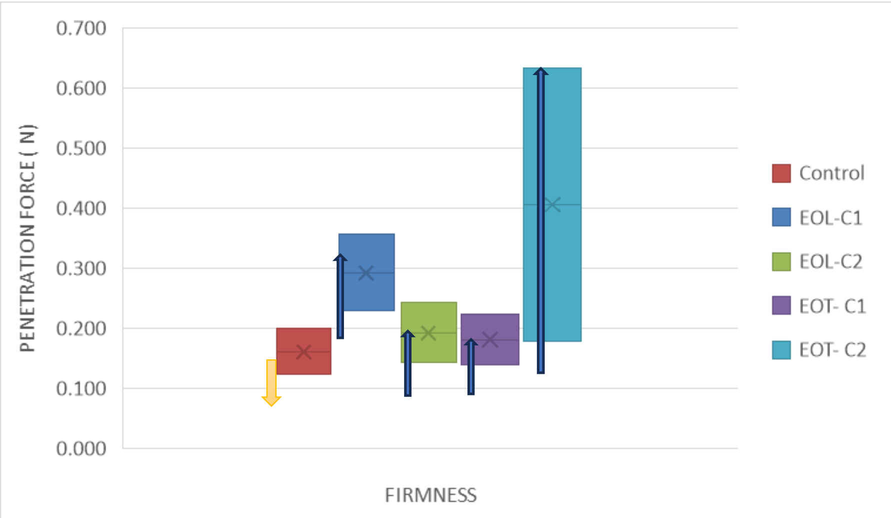

Figure 3. Firmness values for strawberry after 7 days of storage in essential oil vapours of lavender essential oil (EOL-C1 and EOL-C2) and thyme essential oil vapours (EOT-C1 and EOT-C2)

The results regarding the weight loss of strawberries are in line with other studies that have shown that along with the increase in temperature, respiration intensifies due to more accelerated metabolism. Consequently, changes occur in the carbohydrate content, which are the basic compounds that support respiration [15].

Upon exposure to essential oil vapours, the firmness of the strawberries increased while for control decreased, as shown in Figure 3. The greatest increase was observed for EOT-C2 variant, being three times higher, followed by EOL-C1.

Acidity contributes to the overall taste of fruits, adding a note of freshness and balancing sweetness. At full ripeness, fruits have low acidity, which is counterbalanced by a high quantity of sugars in a readily soluble form, Figure 4. Fruits with an optimal level of acidity are often perceived as being more flavourful and appealing. The explanation is that in the presence of acids, the aroma is intensified, becoming richer [4].

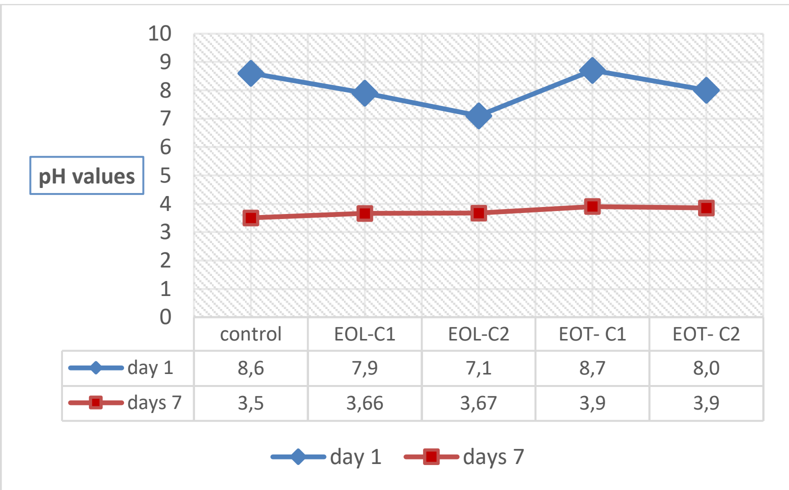

Figure 4. Values of strawberry acidity after 7 days of storage in essential oil vapours of lavender essential oil (EOL-C1 and EOL-C2) and thyme essential oil vapours (EOT-C1 and EOT-C2)

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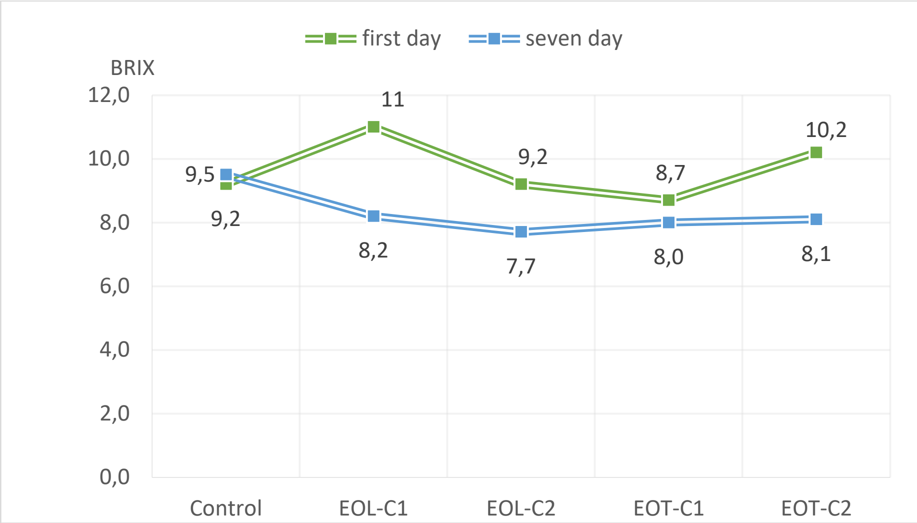

Figure 5. The content of total soluble sugar (TTS) in strawberries preserved in an atmosphere enriched with vapours of lavender essential oil (EOL-C1 and EOL-C2) and thyme essential oil vapours (EOT-C1 and EOT-C2).

A high level of acidity is beneficial for stored fruits, as acidity enhances the natural antimicrobial protection capacity, thus contributing to the prolongation of shelf life [1, 11]. This represents the main reason why fruits that require a long storage period should be harvested at technological maturity rather than full maturity [16].

The sugar content is the indicator that directly determines the sweetness of fruits, being the main attribute on which consumer acceptance and satisfaction depend. This was determined through TSS analysis and is expressed in BRIX. The moment of harvesting strawberries is a determining factor that influences their shelf life. It has been demonstrated that the time of harvesting tomatoes at the stage of physiological maturity is essential for quality and total soluble solids content, as the amount of sugar increases in the final stages of ripening [4]. The strawberries used in the study were fully ripe (red maturity) with an average TSS content of 9.7 BRIX. After 7 days, a decrease in soluble sugar content was recorded in all variants stored in an atmosphere enriched with oil vapours, except for control.

The decrease of TSS content is due to the accelerated respiration of the fruit, which causes the sugars to be involved in the respiratory process, resulting in the formation of carbon dioxide, water, and energy. Accelerated respiration of the fruit can be influenced by the storage temperature or elevated ethylene concentrations. As a result, the fruit loses water, dehydrates, and the metabolism alters, leading to a higher accumulation of organic acids such as malic and citric acids, which results in increased acidity [9].

Conclusions

The presence of essential oils in the form of vapours in the storing atmosphere of strawberries for 7 days at a temperature of 10 degrees produces an antifungal protective effect due to the inhibition of the germination of opportunistic fungal spores. The presence of oil vapours at a dose similar to IC 50 offers partial antifungal protection and does not stimulate the fruits' respiration, with results similar to the control. When the quantity of oil is doubled (2x IC 50), the total antifungal effect was noted for EOL-C2 from lavender. A double quantity of thyme essential oil, EOT-C2, stimulates the respiration of the fruits, resulting in water loss, which leads to an increase in firmness for the fruits stored in this variant. Therefore, by storing strawberries in an atmosphere enriched with essential oil vapours, we observe changes in quality indicators concerning sugar content, acidity, firmness, and weight loss because of increased respiration, and the total antifungal effect is achieved at double doses compared to IC50. Considering that some essential oils may affect the aroma or taste of the fruits, studies on sensory changes and consumer acceptance are necessary.

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