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Study on the Sustainability Potential of Thyme, Oregano, and Coriander Essential Oils Used as Vapours for Antifungal Protection of Wheat and Wheat Products

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Abstract: This study aims to highlight the antifungal, antimicotoxigenic potential and phytotoxic effect of three essential oils (EOs) of *Origanum vulgare* (OEO), *Thymus vulgaris* (TEO), and *Coriandrum sativum* (CEO) on wheat storage, but also the impact of EOs treatment on the sensory properties of bakery products obtained from the wheat seeds. The chemical composition of EOs was determined using GC-MS analysis; the fungal load was evaluated using the direct plating technique, while mycotoxin analyses were conducted using enzyme-linked immunosorbent assay (ELISA). A selective antifungal effect has been highlighted in terms of the action of EOs vapours. OEO and TEO are inhibited *Alternaria, Fusarium* and *Drechslera*, while *Saccharomyces* and *Cladosporium* have proven to be the most tolerant fungi. *Drechslera* is the most sensitive, the effect of all EOs being a fungicidal one. However, the fungicidal effect proved present in all EOs applied as vapours with values ranging between 0.2–0.4%. Regarding the phytotoxic effect of EOs vapours on the germination of the seeds, TEO and OEO had an inhibitory effect, especially at 0.4%. The effect is cumulative over time. The EOs inhibited deoxynivalenol (DON) occurrence; the maximum percentage of inhibition was obtained after 21 days of vapours exposure, being more effective in the case of 0.2%. EOs vapours treatment does not affect the quality of bread obtained from treated wheat seeds from a sensory point of view.

Keywords: bread; deoxynivalenol; fusarium; hormesis; phytotoxicity; sensory evaluation

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1. Introduction

The microflora of cereal seeds are diverse, determined by cultivation technology, variety, storage conditions, temperature and humidity levels during the vegetation period [1,2]. Cereal fungi are divided into two categories: (i) field fungi, which cause diseases during plant growth and (ii) storage fungi, involved in storage [3,4]. Often fungi involved in the mycotoxin infestation of cereal seeds are re-introduced by xerophilous species, metabolically adapted to humidity conditions around 14%. Field fungi have a chance to grow and multiply if the humidity is over 14%. At these humidity values, the activity of fungi affects the nutritional qualities of the seeds, being correlated with the increase of the temperature during the storage period [5]. Endophytic fungi, which survive during storage, can affect the quality of seeds and ensure their spreading in the following year.

Fusarium species fall into this category, fusarium blight being a disease that occurs for cereal plants during the growing season, affecting the quality of seeds during storage. Moreover, the genus is recognised, along with *Aspergillus* and *Penicillium*, as an essential producer of mycotoxins [4–8].

Great efforts have been made to control food deterioration by fungi, with synthetic fungicides being the most used storage strategy. However, despite the efficacy of these chemical substances, a significant number of them have led to the development of fungal resistance, proved to be toxic for the environment, and caused residual toxicity in grains [9].

Therefore, there is an increasing public demand for the development of new and safer antifungal agents for grain preservation. In recent years, many natural compounds have attracted the attention of scientists, such as plant extracts or essential oils (EOs) [10,11]. Given the positive impact of natural preparations on the environment and health, their use as antifungal agents in agriculture and food protection represents viable and sustainable alternatives to synthetic chemicals [11–15].

The establishment of natural alternatives with effective, efficient, and non-toxical preservative function derived from plants, whether extracts or oils, depends on several factors. Is discussed here about the chemical composition, concentration, possible synergies or antagonisms, forms of action (direct, vapours, nanoencapsulation), biological properties of the target microorganism and possible changes in the sensory properties of the preserved product [16–21].

EOs are hydrophobic substances of complex mixtures of volatile organic compounds obtained from different parts of aromatic plants. The antifungal activity of EOs has been extensively studied against *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., and *Verticillium* sp., among other economically important fungal genera [22–25]. However, the effectiveness of a particular EO as antifungal is determined not only by its fungitoxic effect but also by its ability to decrease or inhibit conidial production [26,27].

According to our previous studies, the treatment of wheat grains through direct contact with EOs from *Coriandrum sativum, Thymus vulgaris, Mentha piperita* determines lower mycotoxins occurrences belonging to Fusarium synthesis [28].

Considering the actual trends regarding the use of the three EOs: thyme (TEO), oregano (OEO) and coriander (CEO) in ecological agriculture, the present paper intends to clarify several aspects to assuring the sustainable use of EOs: (i) if the EOs can provide antifungal and antimicotoxigenic protection to wheat seeds during storage; (ii) if there is a phytotoxic effect of the essential oil vapours on the germination of the wheat seeds in the modified atmosphere; (iii) if the EOs provide antimicotoxigenic effect in wheat and if the mycotoxins are transferred in cereal products (flour, bran and bread); (iv) if the EOs treatment influences, from a sensory point of view, the bakery products obtained from EOs exposed wheat.

2. Materials and Methods

2.1. EOs Chemical Composition and the Working Variants

The essential oils used in the study come from the market (SOLARIS PLANT SRL, Bucharest, Romania).

The chemical characterisation of EOs was performed using GS/MS QP 2010 Plus (Shimadzu, Kyoto, Japan) equipped with AT WAX 30 m × 0.32 mm × 1 µm capillary column (Santa Clara, CA, United States). The program used to separate the compounds was: 40 °C for 1 min, a rate of 5 °C/min to 210 °C for 5 min. Helium was used as a carrier gas at a 1 mL/min flow rate. Injector and ion source temperatures were 250 °C and 220 °C, respectively. The injection volume was 1 µL at a split ratio of 1:50. The NIST 02, Wiley 275 spectra library has been used to identify the volatile compounds. The variants of the study are presented in Table 1.

Table 1. Quantities and concentrations used in the study of the sustainability of essential oils.

EOs		µL	% *
OEO1	*Oreganum sativum* EOs	800	0.2
OEO2	*Oreganum sativum* EOs	1600	0.4
TEO1	*Thymus vulgaris* EOs	800	0.2
TEO2	*Thymus vulgaris* EOs	1600	0.4
CEO1	*Coriandrum sativum* EOs	800	0.2
CEO2	*Coriandrum sativum* EOs	1600	0.4

* The EO concentrations are calculated for the volume of air enriched with essential oil vapours.

2.2. Estimation of Antifungal Potential of EOs

42 hermetically sealed containers (800 mL vol) were used to determine the antifungal potential of EOs used in vapours form for the storage of wheat seeds. To remove opportunistic microorganisms the wheat seeds, obtained by ecological technology (Antille variety), were washed with 1:9 (*v*/*v*) hypochlorite solution then rinsed three times with sterile distilled water and dried in a sterile hood at 23 + 2 °C to a relative humidity of 14%.

The wheat seeds thus prepared were distributed in containers 300g each and the adjacent air volume of 395 mL (78% nitrogen and 21% oxygen, 0.03%carbon dioxide, 0.97% argon, 32% humidity) was used to calculate the concentration of the vapours from the air enriched with EOs. The amount of EOs (see Table 1) was added to a sterile filter paper attached to the lid. The containers were kept in the dark and periodically have been shaken for mixing and uniformly exposing the seeds to EOs vapours. At 7, 14 and 28 days from the experiment begin 14 containers were opened (7 variants in 2 repetitions) for evaluation of fungal colonisation, seeds germination test, and mycotoxin content.

To detect the fungal load of wheat seeds exposed to EOs vapour the direct plating technique was used. In this regard, dichloran rose bengal chloramphenicol (DRBC) (Oxoid, CM0727, Thermo Fisher Scientific Inc., Altrincham, UK) with 2 repetitions and 56 Petri plates for each moment of determination were used in the 7th, 14th, and 28th days. The plates were incubated in the dark, with the lid upside down at 22 + 2 °C for 3–4 days, after which the number of colonised seeds was noted. The fungi were isolated in pure culture on the sixth day for identification at genus and species levels. For identification and confirmation of Fusarium species, transfer was made to a specific medium, Dichlorane Chloramphenicol Peptone Agar (DCPA), with a photoperiod of 12 h light/night [29].

We established the frequency of occurrence of the fungal genus from the total fungal genera isolated per variant using the formula:

$$\text{Fr (\%)} = (\text{NOG}/\text{TNS}) \times 100 \quad (1)$$

were,

NOG = number of occurrences of the genus
TNS = total number of fungi on sample

2.3. Estimation of the Phytotoxicity Effect of EOs on Wheat Seeds

As germination index used was the emergence of the radicles. The seedling (hypocotyl) and roots length (as a mean of 3 radicles) were recorded after 7 and 28 days of wheat exposure for each variant EOs vapours assay (Table 1). Briefly, 10 seeds were taken from each container and placed between 2 sheets of blotting paper in a Petri dish (Ø 90 mm) moistened with 10 mL of distilled water (2 Petri dishes were used per container). A total of 28 Petri dishes were used in each determination. After 4 days of incubating in the dark at temp 25 ± 2 °C, data were recorded.

2.4. Evaluation of Antimicotoxigenic Potential of EOs

Sample preparation and mycotoxin analyses were conducted using enzyme-linked immunosorbent assay (ELISA). The analysed mycotoxins were: deoxynivalenol (DON), fumonisin (FUMO), zearalenone (ZEA) and ochratoxin A (OTA).

For FUMO, OTA and ZEA the wheat samples (5 g) were extracted with 25 mL 70% methanol and for DON analysis 100 mL distilled water was used. The samples were extracted for 20 min using a high-speed shaker (IDL, Freising, Germany). The extracts were diluted at 1:13 for FUMO analysis and used directly for other mycotoxins analyses. R-Biopharm kits ELISA (Bio-Rad Laboratories, Redmond, WA, USA) were used to quantify mycotoxins. In this regard, 50 µL of filtrate and standard solutions were mixed with 50 µL of enzyme conjugate, then 50 µL antibody solution was added, mixed gently and incubated for 10 min. at room temperature. After washing three times with 250 µL distilled water, 100 µL substrate was added to each well and incubated for 5 min at room temperature. Finally, the resulting yellow colour's intensity was measured at 450 nm using an ELISA 96-well plate reader (PR-1100, Bio-Rad Laboratories, USA) after adding 100 µL stop solution to each well. The results are expressed in ppm or ppb depending on the type of mycotoxin. Table 2 presents the regression coefficient of calibration curves and the Minimum Detection Limit (MDL) for each mycotoxin.

Table 2. Parameters of mycotoxins determination.

Mycotoxins	Calibration Curve Parameters (r^2)	MDL *
DON	0.9959	0.08 ppm
ZEA	0.9884	5 ppm
FUMO	0.9946	0.015 ppm

* Minimum detection limit.

The mycotoxin content amplitude was evaluated using the formula:

$$\text{AMy (\%)} = (\text{IMC} - \text{TMC})/\text{IMC} \times 100 \quad (2)$$

were,

IMC—initial mycotoxin content (ppm)
TMC—mycotoxin content at time T (ppm)

2.5. The Obtaining of Flouring Derivative Products

The remaining wheat seeds were used to obtain flour, followed by the production of bread buns variants for mycotoxin analyses.

The wheat flour was obtained by grinding EOs fumigated wheat grains using the GM200 grinder (Retsch, Haan, Germany). The ground wheat was sieved to separate the flour from bran. The DON content was analysed for the flour and bran of each variant.

At 97 g flour obtained as above, 67 g of water and 2 g of yeast were added in granular form. The mixture was maintained for 20 min at room temperature and kneaded the dough again for another 8 min. The dough was left to ferment for 30 min at room temperature (23 °C), respectively, until the dough volume increased twice. This process was followed by modelling two buns weighing 83 g each and kept to the final fermentation (20 min). After baking, two bread buns weighing 62.95 g resulted for each EOs vapours treatment.

To highlight the transfer of mycotoxin occurring in endosperm or bran of seeds and the influence of heat treatment (baking) on the mycotoxin content in the bread, we experimented with wheat seeds contaminated with DON (6.45 ppm). From the 6 bread buns obtained with DON contaminated flour, 3 were baked at 180 °C and 3 at 250 °C, for 25 min.

2.6. Sensory Evaluation of Bread Derivates from EOs Fumigated Wheat

The sensory evaluation of bread buns was carried out only for wheat exposed 28 days to EOs vapours. The sensory evaluation of buns was evaluated by a panel of 38 assessors (males and females), non-smokers, without known cases of food allergies. A control sample (C) consisting of bread obtained from normal wheat flour was also evaluated with the other bread samples. Slices of bread with a thickness of 1 cm, with crust, were presented on sheet plates, coded with two digits, being served in random order, under normal lighting conditions and at room temperature. To evaluate the sensory attributes (appearance, texture, taste, flavour and overall acceptance), panellists used a five-point hedonic scale ISO 6658:2017 [30], with the following rates: 1 = strongly disliked; 2 = Slightly disliked; 3 = neither like nor dislike; 4 = Slightly liked; 5 = Strongly liked. The ranges of score and acceptability level were as follow: 1.00–1.49 = Not Acceptable (NA); 1.5–2.49 = Slightly Acceptable (SA); 2.50–3.49 = Moderately Acceptable (MA); 3.5–4.49 = Acceptable (A); 4.5–5.00 = highly acceptable (HA). All 38 panellists trained according to ISO 6658:2017 [30].

2.7. Statistical Analysis

All determinations were made in triplicate, and the results are reported as mean values ± standard deviation (SD). Differences between means were analysed with a one-way ANOVA, followed by multiple comparison analysis using the t-test (two-sample assuming equal variances). Differences were considered significant when p-values < 0.05. Data were processed with Statistica 10 (StatSoft, SAS Institute, Inc. Cary, NC, USA).

3. Results

3.1. GC-MS Composition of EO_S

The chemical composition of the analysed EOs is presented in Table 3. The results show that TEO contains Thymol (35.862%) and o-Cymene (31.957%) as major components, followed in percentage over 4% by Linalool (6.301%). The commercial CEO contains 72.231% Linalool and in smaller percentages Limonene (4.766%) and α-Pinene (6.071%). In commercial oils, minor compounds have also been identified. In OEO, 17 volatile compounds were identified, of which Carvacrol represents 37.349% and o-Cymene 28.750% of the total components. Four volatile compounds are found in percentages between 4–7% (Caryophyllene, β-Myrcene, γ-Terpinene and β-Linalool).

Table 3. Chemical composition (% of total) of EOs.

Compounds	Type	TR	EOs		
			OEO	TEO	CEO
α-Pinene	MH	11.314	2.403	1.918	6.071
Camphene	MH	12.641	-	1.675	0.308
β-Pinene	MH	13.468	0.594	3.477	0.143
B-Myrcene	MH	14.549	4.243	1.773	0.213
α-Phellandrene	MH	14.800	0.255	-	-
α-Terpinene	MH	15.159	2.143	0.725	-
D-Limonene	MH	15.245	3.713	2.388	4.766
γ-Terpinene	MH	16.504	4.121	-	1.335
cis- β-Ocimene	MH	16.850	-	-	0.237
p-Mentha-1,4(8)-diene	MH	17.224	-	-	0.281
Terpinolene	MH	17.225	1.048	-	-
Eucalyptol	MO	17.338	0.129	0.698	-
o-Cymene	MH	18.491	**28.750**	**31.957**	-
o-Cymol	MH	18.551	-	-	5.386
1-Octen-3-ol *		22.141	-	0.239	-
cis-Linalyl Oxide	MO	23.520	-	-	1.375
Trans-Linalool oxide	MO	24.441	-	-	1.162

Table 3. *Cont.*

Compounds	Type	TR	EOs OEO	EOs TEO	EOs CEO
β- Linalool	MO	24.583	4.786	6.301	**72.231**
Caryophyllene	SH	25.121	6.210	1.425	-
Thymol methyl ether	MO	25.701	-	1.041	-
Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)	MO	26.341	-	0.734	-
Bornyl acetate	MO	26.484	-	0.513	-
4-Terpinenol	MO	26.903	-	1.761	-
Camphor	MO	27,913	0.206	0.319	3.643
Geranyl acetate	MO	28.93	-	-	0.901
Borneol	MO	29.277	-	1.725	-
trans-Geraniol	MO	30.96	-	-	0.077
p-Propenylanisole	MO	32.462	-	-	0.948
p-Cymen-8-ol	MO	32.887	-	-	0.091
3,7-Octadiene-2,6-diol, 2,6-dimethyl-	MO	35.316	-	-	0.679
Caryophyllene oxide	SO	36.09	0.236	0.555	-
Caryophyllene	SH	36.419	3.573	3.380	-
Thymol	MO	37.378	-	**35.862**	-
Carvacrol	MO	38.198	**37.349**	1.524	-
1,7-Octadiene-3,6-diol, 2,6-dimethyl-	MO	38.275	-	-	0.1456
Viridiflorol	SO	40.635	0.234	-	-
Total major compounds **			98.343	96.213	95.969
Monoterpene hidrocarbonates (MH)			46.424	43.193	17.559
Monoterpenes oxygenate (MO)			42.136	48.215	78.410
Sesquiterpene hidrocarbonates (SH)			9.783	4.805	-
Sesquiterpene oxygenate (SO)			0.471	0.556	-

* Other compounds; ** The difference up to 100% represents unidentified compounds (values not presented in the table).

3.2. Estimation of Antifungal Potential of EOs

To express the frequency of occurrence of fungi for each moment of determination, we used the sum of the values of the occurrence frequencies noted for each EOs variant (Total Frequency-TFr%, Figure 1). The structure of the seed's microbiota has changed during 4-week. Thus, 5 dominant fungal genera have been identified, namely Saccharomyces (*S. cerevisiae*), Drechslera (*D. tritici*), Alternaria (*A. alternata*, *A. infectoria*), Fusarium (*F. graminearum*) and Cladosporium (*C. cladosporioides*).

After 7 days of exposure to EOs vapours, the frequency of occurrence of fungal genera has changed quantitatively but also qualitatively. Thus, all fungi were represented in the control version with values TFr between 5% and 30%. *Fusarium* and *Cladosporium* have appeared in all EOs treatments. The highest frequency for *Fusarium* (72%) was determined in the case of CEO2, followed by CEO1 with 46% and the lowest frequency was recorded for OEO1 and OEO2 with values of 20%. *Fusarium* is the most tolerant, with a total frequency of 237%.

After 14 days it is observed that the genus *Drechslera* is not found in any variant of EOs vapor treatment and *Alternaria* is totally inhibited at TEO1, registering the total frequency value of less than 34%. *Fusarium* appears with the highest frequency in the control version. The fungus with the highest TFr% is *Saccharomyces* with value of 379%. *Fusarium* was identified only in the control variant, the fungicidal effect of EOs concentrations in the vapor phase being responsible for this.

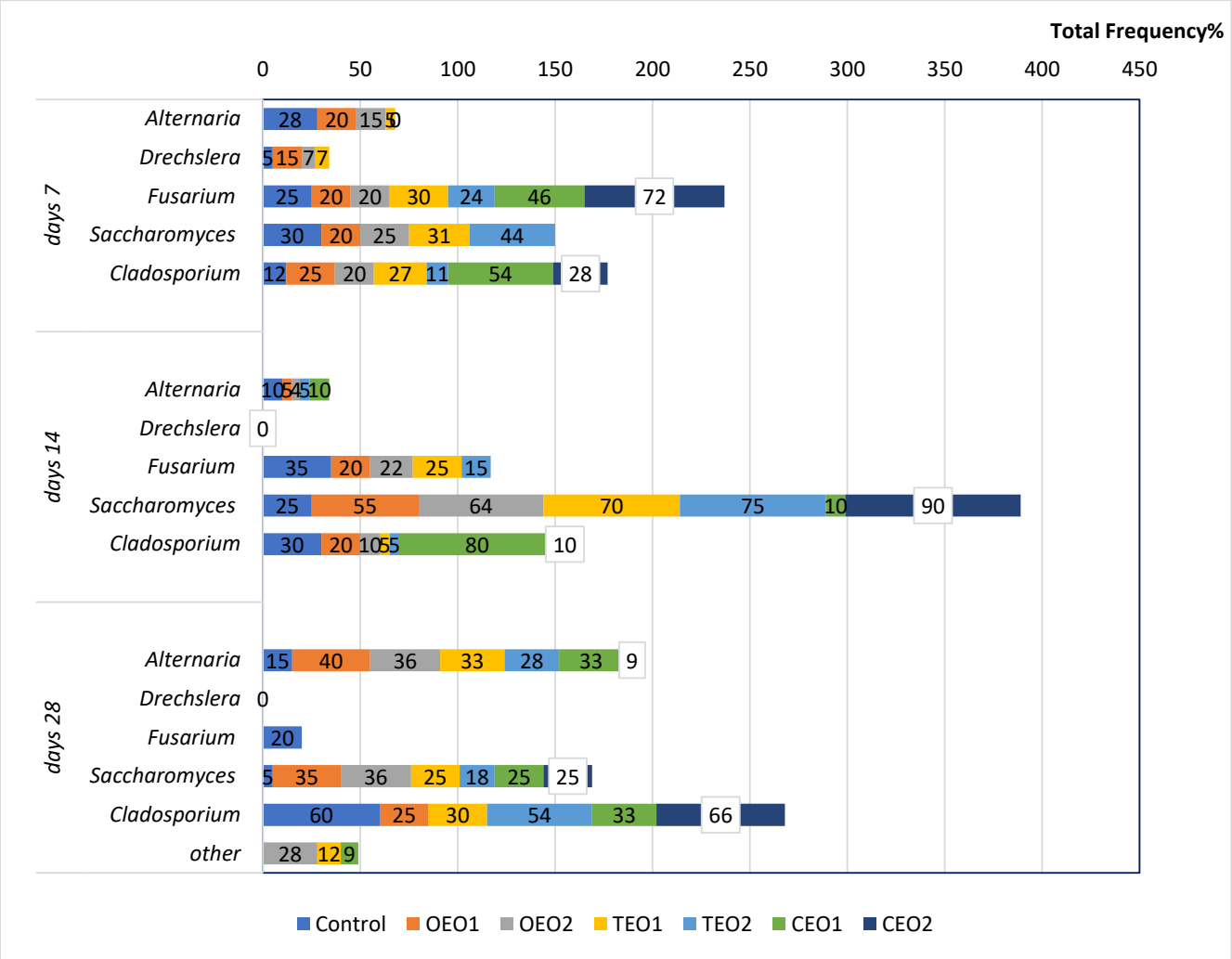

Figure 1. The Total Frequency (TFr%) of fungi isolated at 7, 14 and 28 days from seeds wheat stored in an atmosphere enriched with essential oil vapours. EOs concentrations are calculated for the volume of air enriched with essential oil vapours (OEO1, TEO1, CEO1 = 0.2%, OEO2, TEO2, CEO2 = 0.4%).

After 28 days the fungicidal effect of EOs is noticed for *Drechslera* and *Fusarium* which appear only in the control version. *Alternaria* reappears in all treatment variants alongside *Saccharomyces* and *Cladosporium*. *Cladosporium* is the genus with the highest frequency value of 268%.

3.3. Estimation of the Phytotoxicity Effect of EOs on Wheat Seeds

Due to the high volatilising properties of the oils and their persistence in closed environments, the evaluation of the impact on the germination capacity of wheat seeds is considered a fundamental analysis. Figure 2 shows the germination of wheat seeds stored in closed containers with a vapour-enriched atmosphere of EOs used in two concentrations. At first glance, the inhibitory effect of essential oil vapours can be observed. TEO1 and CEO1 applied in a concentration of 0.2% slightly affected the germination of seeds after 7 days of exposure, but after 28 days, the wheat germination is greatly affected. Seeds stored in atmospheres enriched with 0.2%/0.4% TEO and 0.4% OEO have lower germination values. In the case of CEO vapour, the highest values of all tested variants are noted for both concentrations at 70%.

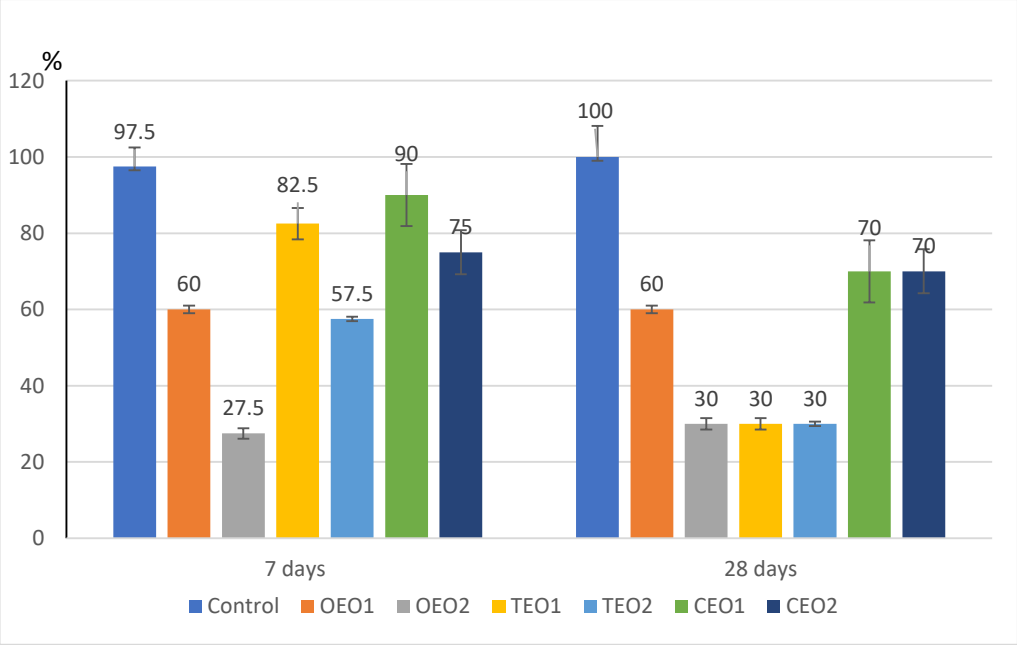

Figure 2. Wheat germination test determined at 7 and 28 days of seeds exposure to EOs in vapours-form. (n = 4).

Differences depending on concentrations of EOs-vapours are also noticeable at the length of the radicles and stem (Table 4). Overall, EOs vapours affect the growth of the radicles and stem in a differentiated way compared to the effect of the concentration. Thus, CEO1 stands out with appropriate control values for the length of the radicles, but significant differences occur for the size of the stems both at 7 days (3.00 ± 0.50 mm) and at 28 days (3.00 ± 0.58 mm) compared to the control sample (4.29 ± 0.41 mm). For CEO2, however, the negative effect on radicles growth occurs, both at 7 days and 28 days. The highest differences related to the radicle's length were found in the OEO2 (2.97 ± 0.40) and TEO2 (2.84 ± 0.22) variants compared to the control (2.84 ± 0.22 mm). The negative effect remained even after 28 days of storage in the atmosphere enriched with EOS vapours.

Table 4. Effects of EOs vapours-phase to radicles lengths and seedling lengths after 7 days and 28 days of seeds exposure.

Treatment	Radicles (mm)		Seedling (mm)	
	7 Days	28 Days	7 Days	28 Days
Control	11.50 ± 0.22 a	10.94 ± 0.55 a	4.29 ± 0.41 a	4.29 ± 0.41 a
OEO1	9.64 ± 1.52 b	5.52 ± 0.66 d	3.64 ± 0.66 b	3.65 ± 0.76 b
OEO2	2.97 ± 0.40 e	3.33 ± 0.00 e	2.17 ± 0.41 d	2.00 ± 0.00 e
TEO1	9.14 ± 0.62 c	9.07 ± 0.81 b	4.00 ± 0.86 a	3.84 ± 1.00 b
TEO2	2.84 ± 0.22 e	3.00 ± 0.00 e	2.75 ± 0.88 c	2.67 ± 1.15 c
CEO1	11.09 ± 0.44 a	11.03 ± 0.47 a	3.00 ± 0.50 c	3.00 ± 0.58 c
CEO2	6.19 ± 1.05 d	5.99 ± 1.07 d	2.38 ± 0.52 d	2.43 ± 0.53 d

Results as mean ± SE. Different letters (a,b,c,d,e) in columns reveal significant differences according to Tukey test at $p < 0.05$ for EOs vapours concentration.

3.4. The Effect of EOs Treatment on Mycotoxins Contamination in Wheat Samples and Derivative Products

The analysis of the mycotoxins content in the case of naturally contaminated samples showed that of the 4 mycotoxins analysed (DON, FUMO, ZEA and OTA), only DON was identified, both in the control sample and in the wheat samples treated with EOs (Table 5). The initial DON content in the contaminated natural wheat samples was 6.45 ppm. After 7 days of treatment, the highest content decrease is observed up to 4.863 ppm (inhibition

percentage of 24.6%, Figure 3) in the case of TEO1, respectively, at 4.23 ppm (decrease of 34.42%) when TEO2 was used. The fumigation with OEO1 leads to a slight increase in DON content (9.77%), respectively, a decrease in OEO2 of 20.78%. The CEO did not show inhibition of DON after 7 days at any of the concentrations tested. The DON content increased 29.61% for CEO1 and 39.53% for CEO2. There is a slight decrease in DON content of 5.74% in control compared with the initial value, Figure 3.

Table 5. The DON content at 7, 14 and 28 days from seeds wheat storage in an atmosphere enriched with essential oil vapours.

	DON Content (ppm)			
EOs Treatment	**Initial**	**7 Days**	**14 Days**	**28 Days**
CONTROL	6.45 ± 0.04 a	6.08 ± 0.06 f	7.07 ± 0.05 c	8.32 ± 0.10 g
OEO1	6.45 ± 0.07 a	7.08 ± 0.08 c	7.09 ± 0.08 c	2.88 ± 0.05 c
OEO2	6.45 ± 0.06 a	5.11 ± 0.09 a	4.22 ± 0.04 bd	3.62 ± 0.07 be
TEO1	6.45 ± 0.07 a	4.86 ± 0.06 a	3.76 ± 0.05 a	2.28 ± 0.04 a
TEO2	6.45 ± 0.08 a	4.23 ± 0.05 b	4.26 ± 0.06 b	3.88 ± 0.6 b
CEO1	6.45 ± 0.08 a	8.36 ± 0.07 d	4.01 ± 0.06 d	3.61 ± 0.06 de
CEO2	6.45 ± 0.07 a	9.00 ± 0.08 e	2.77 ± 0.03 e	4.79 ± 0.05 f

The values are expressed as mean ± SD (n = 2). A t-test was used to compare the mean differences among samples; data within the same column with different letters are significantly different ($p < 0.05$).

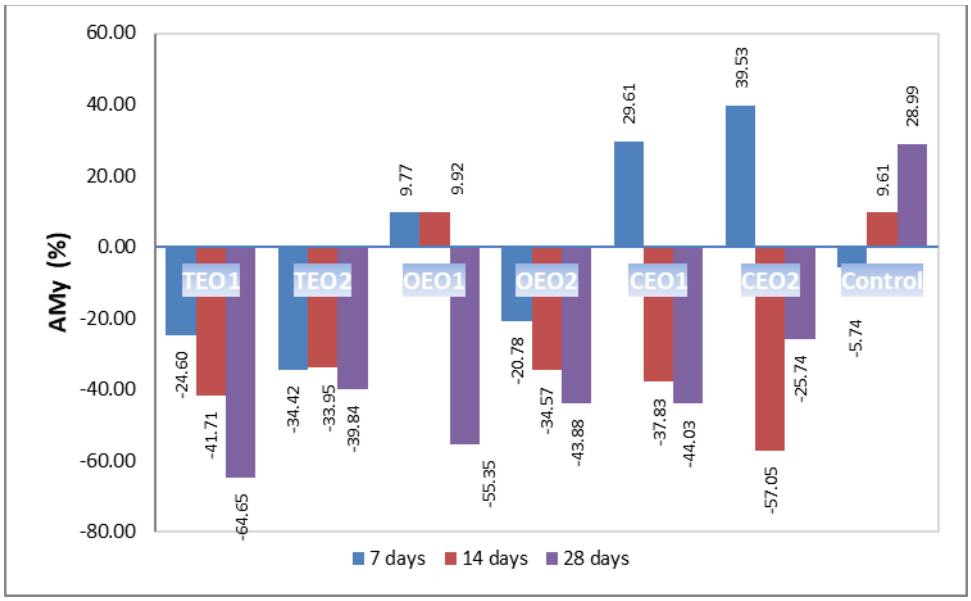

Figure 3. Amplitude of DON (AMy, %) at 7, 14 and 28 days from seeds wheat storage in an atmosphere enriched with essential oil vapours. EOs concentrations are OEO1, TEO1, CEO1 = 0.2%, OEO2, TEO2, CEO2 = 0.4% calculated for the volume of air enriched with essential oil vapours.

After 14 days of fumigation, an increase of DON concentration in control (9.61%) compared to the initial concentration, respectively, of 9.92% in the case of fumigation with OEO1, were observed. For the other EO_S analysed, regardless of the concentration tested, a decrease in DON content is observed, the highest inhibition being observed in the case of CEO2 (−57.05% compared to the initial concentration). A significant decrease is also noticed in the case of CEO1 (−37.83%), but also after fumigation with OEO2 (−34.57%) and TEO2 (−33.95%).

The effects of EOs fumigation after 28 days decrease compared to the effect after 14 days but show an essential percentage of inhibition related to the control for which the DON content increases by 28.99% compared to the initial one. For all EOs analysed, the

inhibitory effect was resisted after 28 days. The values vary between −25.74% in the case of fumigation with CEO2 and −64.65% for TEO1, according to Figure 3.

Figure 4 presents the DON content in the derivative products. The experiment was performed on the control sample naturally contaminated with DON (6.45 ppm) to highlight the transfer of DON occurring naturally in wheat in the endosperm and bran and the influence of heat treatment (baking) on the DON content in bread.

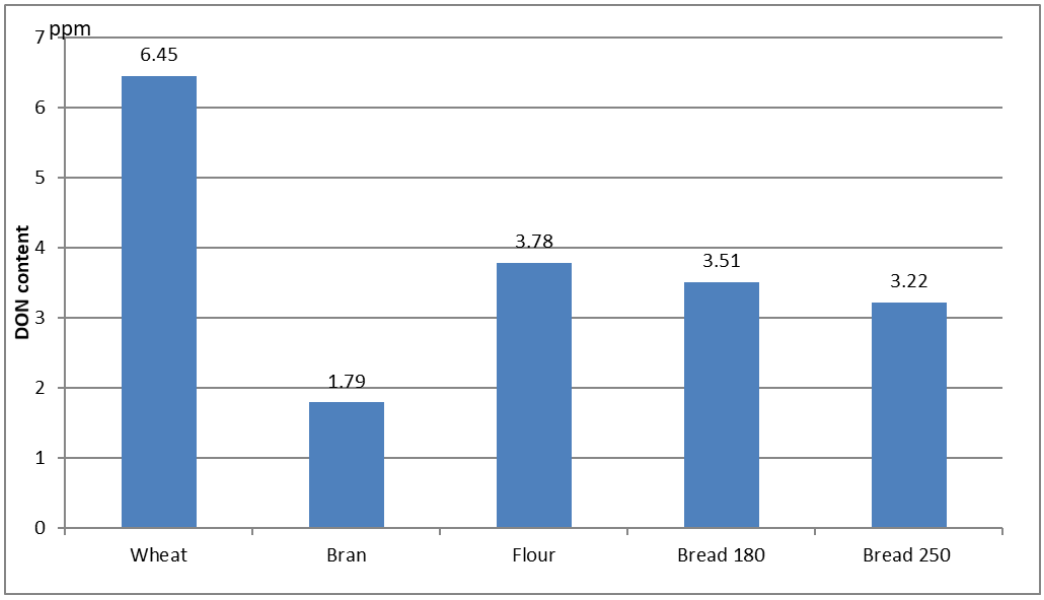

Figure 4. DON content in derivative products (wheat, bran, flour and bread buns baked at 180 °C and 250 °C). The values are expressed as mean ± standard deviations (n = 3). t-test was used to compare the mean differences registered among samples. The error bars indicate standard deviation. Different letters among samples indicate significant differences ($p < 0.05$) among values according to the t-test.

The obtaining data show the content of DON in flour was 3.78 ppm, while in bran was 1.89 ppm of DON. In bread, after baking at 180 °C, 3.51 ppm of DON was recovered, and 3.22 ppm when the baking temperature was 250 °C.

3.5. Results of the Sensory Evaluation of Bread Derivates from EOs Fumigated Wheat

The samples obtained (Figure 5) were analysed from a sensory point of view to estimate the printing capacity of the EO_S on taste and smell on the bread obtained from fumigated wheat.

Figure 6 shows the sensory evaluation results as mean and standard deviation differences. The most highly appreciated was TEO2, with 0.2% *Thymus vulgaris* oil, which obtained mean scores of 4.711 for overall acceptability, 4.842 for flavour and 4.816 for taste, falling within the 4.00–5.00 score range, which indicates high acceptability.

For the same attributes (overall acceptability, taste and flavour), the following high-scoring bread sample was TEO2 samples with 0.4% *Thymus vulgaris* oil, registering the following values: 4.632 (overall acceptability), 4.816 (flavour) and 4.789 (taste). However, in terms of appearance and texture, the scores were higher than in the TEO1 samples (Figure 6).

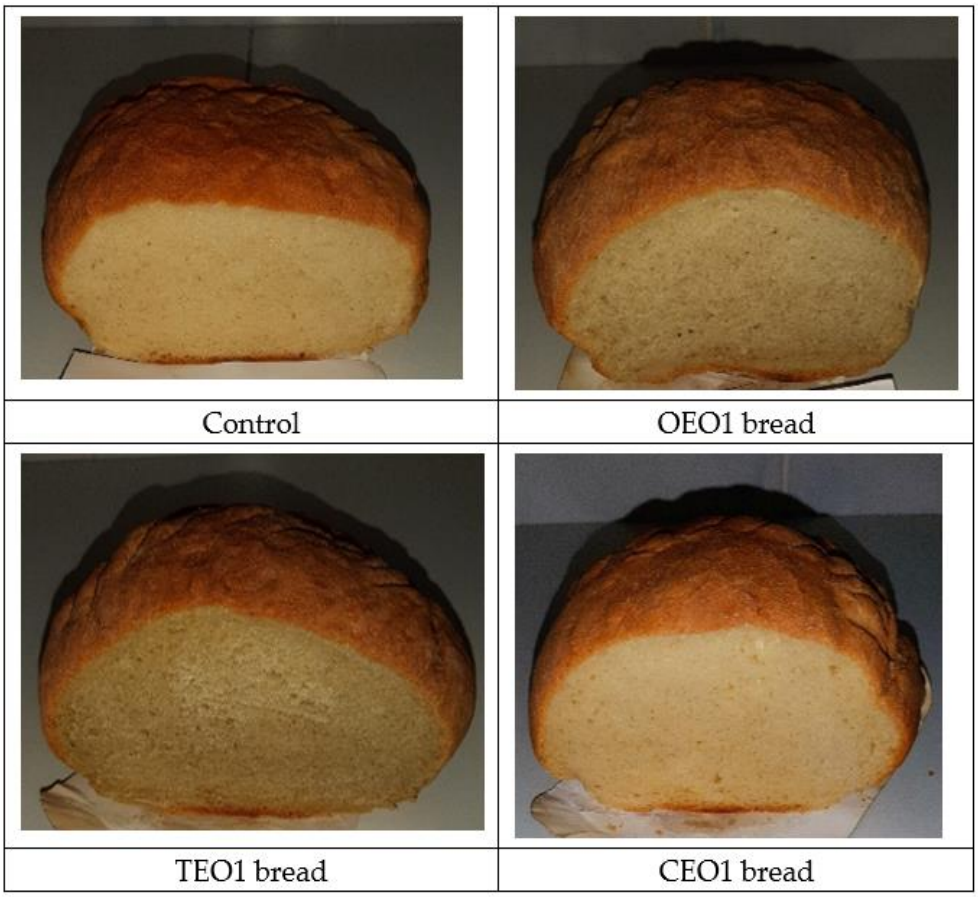

Figure 5. Bread samples obtained after fumigation with Eos.

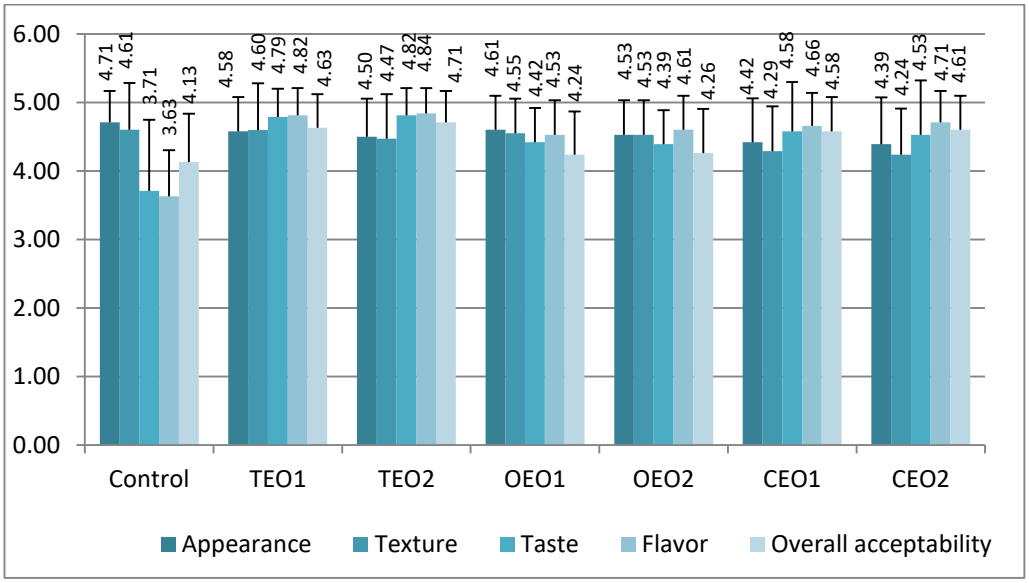

Figure 6. Mean values of the sensory evaluation (consumer acceptance) of bread with different oils (OEO1, TEO1, CEO1 = 0.2%, OEO2, TEO2, CEO2 = 0.4%) using 5-point hedonic scale (n = 38).

4. Discussion

4.1. Chemical Composition of Essential Oils

The content of volatile compounds and the dominant chemotype varies with the botanical species, geographical origin, and harvest time [28].

When TEO was used as an antifungal agent, thymol and o-cymene were the major components, with a sum of significant compounds over 60%. A smaller percentage of linalool was also detected (6.301%). These results agree with our previous study [11] regarding the chemical profile of TEO and which highlighted γ-terpinene and thymol as significant compounds. Other authors also reported thymol as a primary volatile compound [31–34], but o-cymene, γ-terpinene [35], camphor [35], carvacrol [32] and linalool [31] were found in significant quantities.

The chromatographic profile of CEO showed that linalool was the primary compound, followed by α-pinene and D-limonene. Our results are consistent with our previous investigations regarding the chemical composition of CEO obtained from Romania that reported the linalool as the principal constituent of CEO [36]. The content of linalool in CEO varied in the same range in other samples obtained from *C. sativum* seeds cultivated in Brazil (77.48% linalool) [37]. A high linalool content in CEO (60–80%) was also reported in other studies [38,39].

OEO analysed in our study was characterised by a high content of carvacrol and o-cymene (over 28%) and lower content (between 4–6.2%) of other compounds (caryophyllene, β-myrcene, γ-terpinene and β-linalool). Similar composition was reported by in the oregano leaf-flower oil, including carvacrol (30.73%), thymol (18.81%), p-cymene (10.88%), caryophyllene (7.73%), and 3-carene (4.06%) [40]. Carvacrol was the main compound (60%) in the fraction of OEO obtained after distillation at 140°C, while o-cymene represented the highest percentage (47.96%) of OEO obtained after distillation at 82 °C [41]. Higher content of carvacrol (70.2%) and similar minor compounds were reported by other authors [42].

4.2. Fungal Load of Wheat Seeds Kept in the Atmosphere Rich in Essential Oil Vapours

Research regarding the control of fungal contamination of cereals and cereal products is a significant focus; prevention of fungal infection during plant development, harvesting and storage of cereals are priorities in controlling the incidence of fungal infections and mycotoxins development [28]. Proper storage (humidity, temperature and insect control) and the addition of antifungal agents can reduce fungal growth and prevent the contamination of the sample. Boruga et al. [43] reported that the antimicrobial activity of EOs depends on their chemical components and is related to the presence of phenolic compounds and terpenic hydrocarbons, respectively [24,25,43].

The data resulting from the storage of wheat seeds in the atmosphere enriched with TEOs, CEOs and OEOs vapours show that the fungicidal or fungistatic effect is achieved depending on the type of oil and its concentration. The explanation lies in the antimicrobial compounds existing in each essential oil, whose diversity and weight vary depending on the plant, the plant part, the plant growth phase or the cultivation conditions [44].

Recent research has shown the different behaviour of essential oils applied in the form of vapours. The fact is that the antimicrobial activity of essential oils is more effective in the form of vapour-phase against bacteria [19,45–49].

This aspect was also highlighted in the case of our study. Therefore, when applying CEO1 and CEO2, *Fusarium* and *Cladosporium* recorded high occurrence frequency values after 7 days of seed fumigation. These high occurrence frequency values do not denote a weak antifungal effect of this EO for the concentrations taken in the study because in the weeks following, the frequency of occurrence for *Fusarium* was null. Based on these results, we can conclude that CEO acts over time, the fungicidal effect reaching the peak after 14 days of fumigation. The need for a more extended period to prove the irreversible antifungal effect in the case of EOs vapours has been reported by other authors [20].

The physical properties and chemical composition of significant compounds of EOs are responsible for the way of action and the achievement of the antimicrobial effect. Inouye et al. [50] highlighted that thymol and carvacrol are compounds with lower volatilisation and moderate solubility that deposit faster. With 35.862% thymol and OEO containing 37.349% carvacrol, our TEO study showed a more pronounced antifungal effect in the first week of fumigation. Linalool existing in the CEO in a high proportion (72.231%) considered

very volatile, along with 4.766% limonene, has a low solubility in aqueous environments, demonstrating a poorly applied antimicrobial effect in liquid form but has high persistence and a strong antifungal effect if used in the form of vapours [49,51].

Regarding the mechanism of action of EOs, it consists in the loss of semipermeable properties of the cytoplasmic membrane, which leads to imbalances and loss of the integrity of the microbial cell [47]. On the other hand, the penetrating capacity of microbial membranes can be reduced due to the hydrophobic properties of oils used in the form of microemulsions or applicated in liquid form, producing mycelium-mycelium aggregate and thus reducing the affinity for microorganisms [47]. There are possible modes of antimicrobial action of phenolic compounds that could be explained by the denaturation of enzymes responsible for germinating spores or by interfering with amino acids that play a role in germination [52].

EOs obtained from *Thymus vulgaris L.* were evaluated for biological activity and chemical composition. Various studies [53,54] reported significant bacteriostatic activity. Tian et al. in 2019 [24] as well demonstrated the antifungal potential of *Thymus essential* oil by a total inhibition of *Fusarium* mycelium growth at all investigated concentrations and a synergistic/antagonistic effect when sage essential oil was added [11].

Oregano extracts and OEO affect all stages of the peroxidative process by neutralising free radicals, blocking catalysis-peroxidase, and interrupting the lipid radical [55].

A selective antifungal effect has been highlighted in terms of the action of EOs vapours. For example, in the case of OEO and TEO, *Alternaria, Fusarium* and *Drechslera* sp. are inhibited. At the same time, *Saccharomyces* and *Cladosporium* have proven to be the most tolerant species and *Drechslera* the most sensitive, the effect of all EOs being a fungicidal one. However, the fungicidal effect was demonstrated in all EOs applied as vapours in 0.2–0.4%.

For *Alternaria* the effect of EOs vapours is fungistatic, this being certified by the appearance of fungus at 28 days. Short-term studies have shown that TEO applied as vapour-phase at 66.7 µL/L has proven an antifungal effect in protecting artificially inoculated cherry tomatoes with *Alternaria* sp. kept for 5 days [16]. In our study, within 28 days, the concentration of 0.4% TEO provides a fungistatic effect for Alternaria.

4.3. About Phytotoxicity Effect of EOs Vapours on Wheat

As it is known, EOs are an effective, eco-friendly alternative in the management of weeds in ecological technologies; numerous studies initiated in recent years confirm the potential of bioherbicide attributed to them [56–59]. This potential is attributed to the phenomenon of allelopathy, which is not selective, only if the seeds or plants have enzymatic mechanisms of annihilation of the toxicogenic potential [36,60]. Recent studies have shown that the phytotoxic effect of EOs by allelopathy is valid for weed seeds and crop plant seeds [57,61]. Generally, reaching the phytotoxicity limit depends on the type of EO, concentration, and the plant's sensitivity [62,63]. Using EOs vapours in wheat storage assay, it was demonstrated that exposure time is an essential factor in achieving the phytotoxic effect. As the seeds are exposed for a long time, in this case, 28 days, the germination capacity of the seeds is affected through direct action on the responsible enzymes, α-amylase being the main enzyme involved in initiating the germination process of monocots seeds [64]. However, even if germination has taken place, the negative effect of the essential oil vapours is evident in the seedling in the first step of growth.

Regarding the phytotoxic effect of EOs vapours on the germination of the seeds, TEO and OEO have an inhibitory effect, especially at 0.4%. The effect is cumulative over time.

According to the theory that the dose size makes the difference between good and bad, the effect on plants of EOs can be also stimulating or inhibiting. Phenomenon is characterised by a low-dose stimulation and a high-dose inhibition is known as hormesis [65]. Monoterpenes oxygenate from CEO determine the wheat germination release in a concentration-dependent and hormesis-type manner: high concentrations inhibit, and low concentrations enhance the induction of visible radicles. This could be due to the induction

of α-amylases in the endosperm of germinated cereal grains [66]. In our study, 0.2% CEO with 72.231% linalool does not affect the radicles release, but the inhibitory effect is noticeable in the emission of the seedling, which is an involvement in the synthesis of cytokinins. This statement is in agreement with previous studies that affirm the repressive effect of EOs may be manifested in the first step of maise growth if the treatment is applied to the seeds [67] radicles growth of lettuce exposed to thyme, marjoram, vervain, and caraway EOs. In contrast, thyme and oregano EOs inhibited germination and radicle elongation for cress seeds [68].

4.4. The Effect of EOs Treatment on Mycotoxins Contamination in Wheat Samples and Derivative Products

In stored cereals, the application of natural preservatives and EOs generates the inhibition of mycotoxins production [69]. On the other hand, the combination of chemicals and natural products can lead to a 90% reduction in deoxynivalenol (DON) [70,71].

In our experiment, the initial DON content of the naturally contaminated wheat in control was 6.45 ppm. The analysed sample does not correspond to the food consumption requirements for wheat, considering the current legislation in force which provides for DON content a maximum admitted limit of 1.250 ppm for cereals other than durum wheat, oats and maise. In the European Union, the presence of Fusarium toxins in foodstuffs is regulated by Regulation (EC) No 1126/2007 [72] amending Regulation (EC) No 1881/2006 [73], and by Recommendation 2013/165/EU [74].

The treatment of cereals with EOs leads to a significant decrease in DON levels in wheat samples, depending on the type of EOs, concentration and time of fumigation. Our results show that the maximum percentage of inhibition is obtained after 21 days from fumigation, being more effective in the case of the concentration of 0.2% EOs (Figure 3). Regardless of the type of oil used and the concentration, after 21 days of fumigation, the inhibition is over 50%. Even after 7 days of fumigation, there is an inhibition of the development of DON in the case of TEO and OEO2 between 20–35%. After 14 days of fumigation, the inhibition capacity increases, being noticed in all experimental variants except OEO1. After 28 days of fumigation, an inhibitory capacity is observed compared to the control in the case of using EOs, but lower than the values obtained after 21 days.

A previous study regarding the reduction of DON contamination using EOs in the storage phase highlighted that after 22 days of treatment, DON was undetectable in all wheat samples [28]. A similar effect of EOs on the growth rate of DON produced by *Fusarium* species was reported [69].

Since mycotoxins are generally located in the outer shell of cereal seeds, their removal can reduce the content of mycotoxins in the seeds, and the effectiveness of decontamination depends on the degree of penetration of the seeds. Grinding does not directly affect the mycotoxin content but changes the distribution between different cereal fractions. In the case of foods derived from dried and ground cereals, the distribution of mycotoxins in different fractions resulting from grinding depends on the fungal penetration of the endosperm. Thus, cereals with surface contamination will have a lower content of mycotoxins in the flour and a higher concentration in germs, bran and fiber. fibre.

Our results showed that the content of DON is higher in flour than in bran (Figure 4), which means that contamination has occurred because the field or that conditions of temperature, humidity, water activity in storage deposition favoured the penetration of mycotoxins into the endosperm of the grain.

Regarding the reduction of DON content by heat treatments, studies have shown that DON is very stable in the temperature range of 170–350 °C, without indicating a decrease in its concentration after 30 min at 170 °C. This feature is hazardous for human and animal health [75]. Our results show a slight decrease in DON content during the bread processing process, from a concentration of 3.78 ppm in wheat flour to 3.51 ppm when the baking process took place at 180 °C (7.14% reduction), respectively, 3.22 ppm at 250 °C (14.81% reduction). A reduction in DON concentration up to 52% was achieved in cereals using

hot steam at 185 °C for 6 min [76]. As the thermal decomposition products of the DON are not known, and the fact that no toxicity studies have been performed using these DON products, there is no clear evidence that these compounds resulting from the thermal instability of the DON lead to detoxification of food contaminated with DON.

Nowadays, different concentrations of OEO are used to control *Penicillium* sp. [77] and other fungal species such as *Aspergilus niger*, being stronger than rosemary or sage oil [78].

4.5. Sensorial Evaluation of Wheat Bread

Despite some positive studies on applying natural antimicrobials of plant origin, we have faced two significant problems in terms of food application: the change in smell occurring when using high concentrations and the additional costs that occur.

In this regard, we aimed to evaluate the effect of fumigation with EOs applied in wheat storage on the sensory properties of bread. Due to the low concentrations of EOs applied to fumigation, they do not have a negative effect on the smell or taste of the bread; on the contrary they were evaluated positively by the evaluators.

Regarding flavour attribute, the scores given by the evaluators increased in the order: TOE1 > TEO2 > CEO2 > CEO1 > OEO2 > OEO1 > Control suggesting that *Thymus vulgaris, Origanum vulgare* and *Coriandrum sativum* conferred to products an appreciated aroma by the evaluators. In terms of taste attribute, the scores increased in the following order TEO2 > TEO1 > CEO1 > CEO2 > OEO1 > OEO2 > Control and in terms of overall acceptability, the panellists ranked the bread samples as follows: TEO2 > TEO1 > CEO2 > CEO1 > OEO2 > OEO1 > Control.

If we refer to the difference in score between the two concentrations of essential oil used (0.4% and 0.2%), there are no notable differences between the evaluated attributes of the studied samples. Similar studies regarding consumer acceptability, in the case of the addition of essential oils in bakery products, have been carried out by Sikkhamondhol et al. [79] and Khalil et al. [80].

Previous studies highlighted that OEO could be used up to 2% in bread to improve nutritional and sensory qualities, specific volume and shelf life, having an inhibitory action on moulds [81]. In addition, natural antimicrobial compounds from OEO could influence the preservation of bread and other bakery products, being antimicrobial agents against fungal growth in many foods [55,82]. Oregano could be used successfully in producing graham bread, improving the taste and bread aroma [83].

5. Conclusions

In this study, we demonstrated the possibility of using essential oil of thyme, oregano, and coriander to control storage fungi and the prevention of DON mycotoxins occurring for two concentrations calculated at air volume, 0.2 and 0.4%, respectively. The fungicidal effect of EO vapours was achieved after 7 days for field fungi (e.g., Drechslera), while for Fusarium, the antifungal effect was proven after 14 days of EOs fumigation. The germination of the seeds was inhibited after 7 days of exposure; in the order, oregano > thymus > coriander, the effect of the EO vapours acting cumulatively over time and was affected both the emission of radicles and the seedling length.

There were no noticeable sensory differences between the two concentrations applied regarding the sensory evaluation of the bread obtained from seeds exposed for 28 days to EOs vapours. Concerning the overall acceptability, on the first place comes the variant of bread from seeds fumigated with TEO followed by the CEO and OEO.

TEO, OEO and CEO vapour-phase essential oils can be a sustainable alternative to preserving wheat seeds for baking, ensuring safety and protection against mycotoxin-producing fungi. Due to the negative effects on germination, it is not recommendable to apply them if the destination of the seeds is to obtain a field crop.

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Conflicts of Interest: The authors declare no conflict of interest.

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