applied sciences

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Essential Oils as Potential Alternatives to Synthetic Fungicides for the Control of Two *Fusarium* Head Blight Pathogens

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Abstract

Fusarium head blight of cereal, caused by fungi of the genus *Fusarium* spp., reduces yield and accumulates mycotoxins. The increasing resistance of pathogens to synthetic fungicides encourages the search for natural alternatives. This work aims to determine the chemical composition of essential oils (EOs) of caraway, sage, and pine and to evaluate their antifungal activity against *Fusarium graminearum* and *Fusarium culmorum*. The composition of EOs was determined by gas chromatography and gas chromatography–mass spectrometry, and activity was assessed by agar dilution to determine mycelial growth inhibition and EC_{50}, with the effect evaluated by two-way ANOVA. Caraway EO, belonging to the carvone chemotype (carvone 57.57%, limonene 35.86%), had the highest antifungal activity (EC_{50} = 727 ppm), sage EO (cis-thujone 29.59%, camphor 21.74%) had an average activity (EC_{50} = 1017 ppm), and pine EO, rich in α-pinene and δ-3-carene, had the lowest activity (EC_{50} > 3000 ppm). The antifungal activity was determined by the compounds' chemical nature, not by their amount. Low EO concentrations (125–250 ppm) elicited a biphasic response, promoting mycelial growth (zero effect point ≈ 269–295 ppm); therefore, insufficient dosage could increase the risk of mycotoxin contamination. Carvone-rich caraway EO exhibited the highest antifungal activity among the oils tested and is worthy of further investigation as a potential tool for sustainable control of *Fusarium* head blight in cereals.

Keywords: *Fusarium graminearum*; *Fusarium culmorum*; *Carum carvi*; *Salvia officinalis*; *Pinus sylvestris*; antifungal activity; EC_{50}

Academic Editor: Călin Jianu

Received: 14 July 2026
Revised: 3 August 2026
Accepted: 7 August 2026
Published: 10 August 2026

1. Introduction

Fusarium head blight (FHB) is one of the most common diseases of cereals (wheat and barley) worldwide, reducing yield and quality, increasing toxic loads, and causing economic losses [1,2]. Outbreaks of the disease are mainly recorded in temperate climate zones. The frequency of the disease depends largely on weather conditions, particularly during periods of heavy rainfall, when temperatures range from 16 to 30 °C and relative humidity exceeds 95% for 2–3 days, especially if the initial infection level is high [3]. Yield losses due to the disease can reach up to 80% of the crop [4,5]. Recent work [1,4,6] shows that climate change and fluctuations in meteorological conditions increase the risk of both

epidemics and mycotoxins. It is predicted that climate warming will not only increase the prevalence of the disease, but also the mycotoxin profile [7,8].

In temperate cereal production technologies, *F. graminearum* and *F. culmorum* are often considered among the most important pathogens of FHB, which are mainly associated with epidemics and mycotoxin contamination [9–11]. These species not only inevitably reduce yields and deteriorate the quality of grains and processed products (e.g., malts, etc.) but also synthesize B-type trichothecenes—deoxynivalenol (DON) and nivalenol (NIV)—and zearalenone (ZEA/ZEN) [9–12]. The resulting compounds pose a significant risk to animal and human health and may persist in the food and feed chain due to the slow degradation and thermal stability of trichothecenes during processing [12–15].

To date, triazole fungicides remain among the most effective preparations against *Fusarium* spp. pathogens, the long-term and intensive use of which poses more and more environmental and economic problems [11,12]. Also, even optimized triazole programs and DON often provide only partial control of the disease, and increasing their effectiveness with additional spraying is not always economically justified [9,16,17]. In addition, cases of reduced susceptibility are increasingly being recorded, concerns about pesticide residues in food products are growing [14], and the goals of the European Union's Green Deal encourage reducing the use of synthetic pesticides in agriculture.

In recent years, much attention has been paid to essential oils (EOs) of natural origin, which are complex mixtures of volatile secondary metabolites, exhibit antifungal properties, and are considered promising broad-spectrum antimicrobial mixtures [18–22]. Their antifungal activity is associated with the ability to damage fungal cell membranes, alter membrane permeability [20–22], disrupt ergosterol biosynthesis [20,22], promote oxidative stress [23,24] and inhibit fungal growth and sporulation [25].

Many plant EOs inhibit the growth of *Fusarium* spp. in vitro and in grain matrices and reduce mycotoxin formation. Nevertheless, the activity is highly dependent on the type of EO, concentration, and profile of dominant components [26–28]. For example, application of selected commercial EOs to maize seed reduced *F. graminearum*/*F. culmorum* growth (according to ergosterol as a biomass indicator) and, accordingly, significantly reduced deoxynivalenol and zearalenone [26]. Studies with various commercial EOs have shown that the fungistatic/antifungal effect on *Fusarium* species often coincides with the dominance of monoterpenoids; the antifungal role of thymol and citral is particularly emphasized [28]. EOs are rich in oxygenated monoterpenes (e.g., carvone from mint EO), which can achieve 80–100% inhibition at low doses (from 0.25 µL/mL) [27]. The phenolic monoterpenes thymol/carvacrol are associated with potent anti-*Fusarium* activity, and their mechanism of action is associated with membrane damage through disruption of ergosterol biosynthesis and lipid peroxidation [29–31]. Reviews related to monoterpenes also emphasize that camphor and other oxygenated monoterpenes increase membrane permeability, while hydrocarbon monoterpenes (e.g., limonene) may have a weaker effect individually but modulate the effectiveness of mixtures [32].

The sage EO (*Salvia officinalis*) exhibits a marked chemotype variability, but "typical" profiles are usually dominated by thujone isomers (including cis-/trans-), camphor, and 1,8-cineole, and their ratios depend on the origin and growing conditions [33–36]. The dominance of these main components has been confirmed in both empirical GC–MS profiling studies and chemotaxonomic analyses [33,34,36,37]. Researchers [36,38,39] also emphasize that the antifungal activity of sage EO depends on its composition and may be associated with the combined effects of several terpenoids (not a single compound), and that nanoformulation may enhance activity. In a broader context, monoterpenes and oxidized monoterpenes (e.g., carvone, limonene, α-pinene, δ-3-carene, thymol) are considered one

of the most promising groups of biopesticides; therefore, GC–MS chemotyping is necessary before interpreting the biological results of *Carum carvi* or *Pinus sylvestris* EO [32,39].

Although the effects of EOs on *Fusarium* spp. have been studied for several decades, comparisons of chemical profiles and biological activity are hampered by methodological heterogeneity. Studies have used different experimental models and evaluation criteria, for example, from inoculated maize seeds with ergosterol analysis to poisoned substrate tests with GC–MS analysis, disk diffusion method, and pre-sowing seed-soaking experiments, which also assess the effect on germination [18,19,21,40]. Recent studies have evaluated the effects of essential oils on *Fusarium* pathogens in various ways. Danielewicz et al. [41] studied the effects of essential oils of sage, cypress, cumin and thyme on *Fusarium* pathogens and germination rates of winter wheat and maize, using the oils as seed dressings and comparing them with prothioconazole. Grzanka et al. [42] found that different concentrations of essential oils have distinct effects on the mycelial growth of *Fusarium* species and can influence germination parameters in cultivated plants. Siber et al. [43] showed that the effects of volatile essential oil components on *F. culmorum* depend not only on concentration, but also on temperature. In comparative studies, thymol- and citral-rich EOs are usually dominant, and consistently exhibit the highest activity against *F. graminearum* and *F. culmorum* [19,40]. Meanwhile, *Salvia officinalis* is rarely included in such studies, and *Carum carvi* and *Pinus sylvestris* EOs have not been directly compared with *Salvia officinalis* under the same experimental conditions [18,21]. Analysis of individual compounds shows that their antifungal activity differs (e.g., citral is more active than limonene), and sub-lethal concentrations, in some cases, can even promote mycotoxin biosynthesis [19,20,22]. Therefore, it is necessary to apply standardized GC–MS, MIC, and MFC protocols and use uniform sets of *Fusarium* isolates to reliably link the chemical composition of EOs with antifungal activity against both species.

For this study, essential oils of three plants—sage, caraway, and pine—were selected, which contain different classes of biologically active compounds: oxidized monoterpenes dominate in caraway and sage oils, and monoterpene hydrocarbons in pine oil. Such a choice allows us to compare the effects of compounds of different chemical nature on *Fusarium* spp. under the same experimental conditions. In addition, all three plants are readily available in Lithuania and other European regions; therefore, if they are sufficiently effective, they could be evaluated as raw materials of local origin for the development of plant-based plant protection products.

Therefore, this study aimed to determine the chemical composition of sage, caraway, and pine EOs and to evaluate their antifungal activity against *isolates of F. graminearum* and *F. culmorum*. It also aimed to determine the possible relationships between the main components of EOs and their antifungal efficacy, and to assess the potential of these natural products for the sustainable control of *Fusarium* head blight in cereals.

2. Materials and Methods

2.1. Plant Materials and Chemicals

The plant-origin essential oils (EOs) selected for this research:

- Common sage (*Salvia officinalis* L.) plant material, originating from the Mediterranean region, was grown in the experimental garden of the Institute of Horticulture of the Lithuanian Agricultural and Forestry Sciences Center (Babtai, Kaunas district, Lithuania). The essential oil from dried leaves was isolated by hydrodistillation at the Food Aroma Research Laboratory of the Department of Food Science and Technology, Kaunas University of Technology.
- Caraway (*Carum carvi* L.) commercial EO (Frey+Lau GmbH, Henstedt-Ulzburg, Germany), obtained from caraway fruits (seeds) by steam distillation.

- Pine (*Pinus sylvestris* L.) commercial EO (UAB "MĖTA", Vilnius region, Lithuania), according to the manufacturer's data, obtained by steam distillation from pine needles, twigs and young saplings growing in Lithuanian forests.

The common sage plant material used in this research was dried under natural conditions, protected from direct sunlight and with adequate air circulation. The dried leaves were separated from the stems and ground in a laboratory ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany), using a 0.5 mm mesh sieve.

Pentane (for residue analysis, ≥99.9% GC) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and ethanol (96%) was from JSC Stumbras (Kaunas, Lithuania). HIQ helium (grade 6.0, 99.9999% purity) and detector helium (grade 5.0, 99.999%) were obtained from Linde Gas UAB (Vilnius, Lietuva). The following reference compounds (95–99% purity) for the identification of volatiles were purchased from Fluka Chemicals (Steinheim, Germany), Sigma-Aldrich (St. Louis, MO, USA) or Supelco Analytical (Bellefonte, PA, USA): limonene, *p*-cymene, α-terpineol, carvone, thymol, linalool, eucalyptol, caryophyllene oxide. A standard mixture of C7–C30 n-alkanes (Supelco Analytical, Bellefonte, PA, USA) was used to determine the Kováts retention index (KI).

2.2. Isolation of Essential Oil by Hydrodistillation

Dried and ground sage leaves (100 g) were poured with 2 L of distilled water and subjected to hydrodistillation in a Clevenger-type apparatus (TECHNOSKLO s.r.o., Držkov, Czech Republic) for 3 h under atmospheric pressure [44]. The obtained EO was separated from the aqueous phase, collected in sealed dark glass bottles, and stored at 4 °C until further analysis. Distillation was performed in triplicate. The EO yield was calculated on a dry weight basis (v/w).

2.3. Gas Chromatography with Flame Ionization (GC–FID) and Time-of-Flight Mass Spectrometry (GC–TOF/MS) Detectors

The quantitative and qualitative composition of EOs was determined using gas chromatography with a flame ionization detector (GC-FID) and gas chromatography–time-of-flight mass spectrometry (GC-TOF/MS), respectively. The GC-FID and GC-TOF/MS workflow charts are presented in Figure 1a,b.

The conditions for each step are described in detail in Sections 2.3.1 and 2.3.2.

2.3.1. GC-FID

Quantitative analysis of EOs was performed on a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer, Shelton, WA, USA) equipped with a flame ionization detector (FID) and an Elite-5 (5% diphenyl, 95% dimethylpolysiloxane) fused silica capillary column (30 m length × 0.25 mm id × 0.25 µm film thickness) according to Šulniūtė et al. [45]. The EOs were diluted with pentane (5 µL mL^{-1}) before analysis. The carrier gas was helium at a flow rate of 1.3 mL min^{-1} (15 psi at 50 °C). The injector and detector temperatures were 260 °C and 300 °C, respectively. The oven temperature was programmed from 50 °C to 280 °C (10 min hold) at a rate of 5 °C min^{-1}. One µL of sample was injected into a split/splitless injector of the chromatograph using a split mode 1:10. Analyses were performed in four replicates and expressed as a mean of peak area percentage.

The experimental Kováts retention index was calculated using the standard formula:

$$KI = 100n + 100 \cdot \frac{\log RT(x) - \log RT(Cn)}{\log RT(Cn+1) - \log RT(Cn)} \quad (1)$$

where KI—Kováts retention index; n—number of carbon atoms in the n-alkane; $RT(x)$—retention time of unknown compound on the Elite-5 column; and $RT(Cn)$ and $RT(Cn + 1)$—retention times of the n-alkanes eluting before and after the unknown compound.

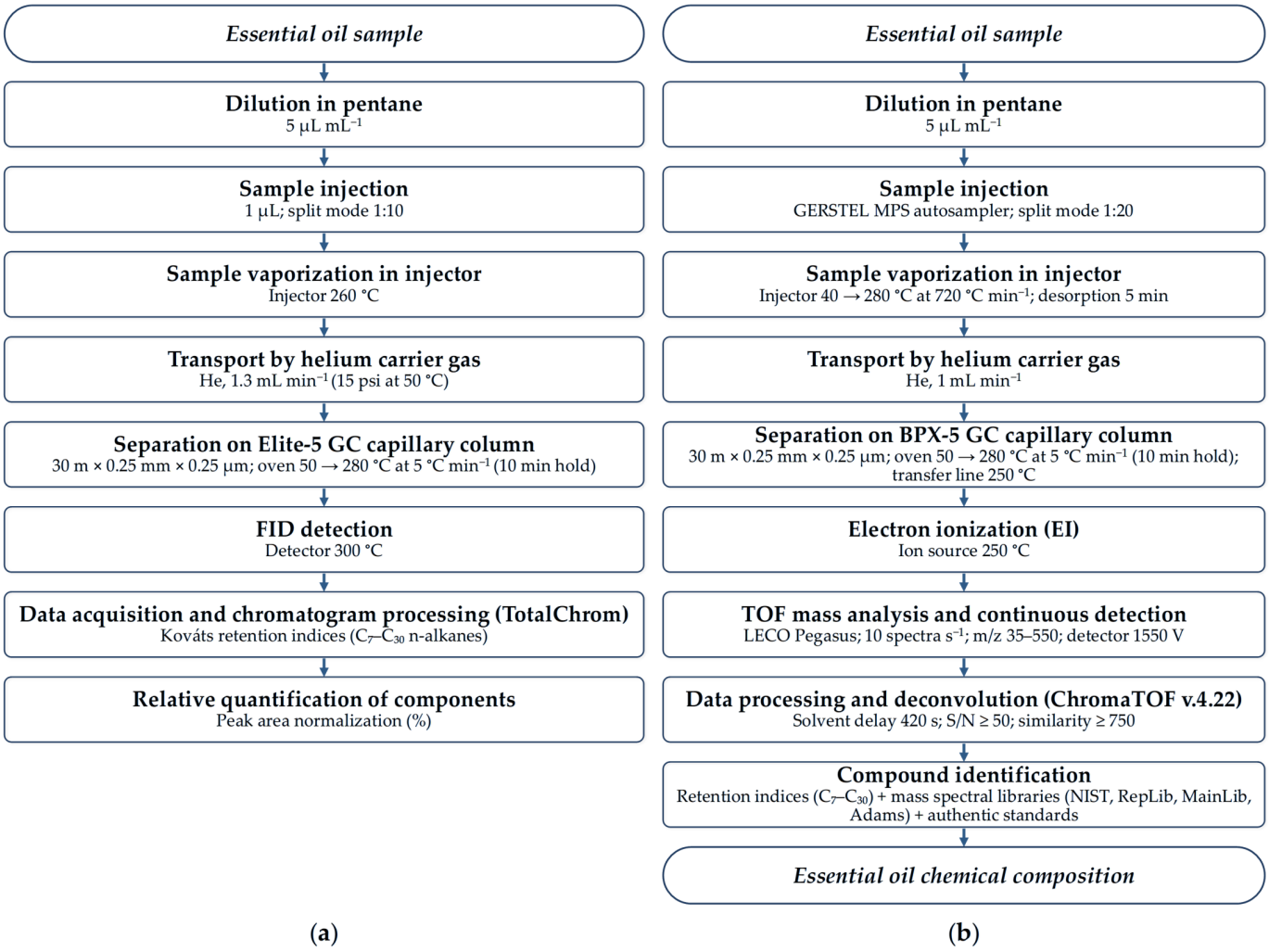

Figure 1. Workflow of GC–FID (**a**) and GC–TOF/MS (**b**) analyses used for characterization of essential oils.

2.3.2. GC-TOF/MS

The qualitative composition of EOs (diluted in pentane at concentration 5 µL/mL) was analyzed on a LECO Pegasus 4D GC×GC-TOF/MS system (LECO Corporation, St. Joseph, MI, USA), consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA), a GERSTEL Multipurpose sampler MPS (Gerstel GmbH, Mulheim an der Ruhr, Germany), a high-speed TOF/MS detector (LECO Corporation, St. Joseph, MI, USA) and four-jet cryogenic modulator (Zoex Corporation, Houston, TX, USA) according to Šulniūtė et al. [45] with slight modification. The column set consisted of a primary column BPX-5 (30 m, 0.25 mm id, 0.25 µm film thickness) (SGE Analytical Science Pty Ltd., Ringwood, Australia) connected to a secondary BPX-50 column (2.0 m, 0.10 mm id, 0.1 µm). The primary oven programming was 50 °C, which was then ramped to 280 °C at 5 °C/min (10 min hold); the secondary oven programming was 65 °C, which was then ramped to 295 °C at 5 °C/min (10 min hold). The transfer line temperature was 250 °C. The GC injector port was set to 40 °C, and then ramped to 280 °C at 720 °C/min, with a desorption time of 5 min. The TOF/MS acquisition rate was 10 spectra/s, and the mass range used for identification was from 35 to 550 m/z units. The detector voltage was set

at 1550 V, and the ion source temperature was 250 °C. Data from the GC×GC-TOF/MS system were collected by ChromaTOF software v.4.22 (LECO Corporation, St. Joseph, MI, USA) after a solvent peak delay of 420 s; the carrier gas helium was set at 1 mL/min in split mode 1:20; the signal-to-noise threshold was set to 50, and the minimum accepted similarity was 750.

The identification of volatile components was assigned by comparing their Kováts retention indices (KI) relative to C_7–C_{30} *n*-alkanes, obtained on nonpolar Elite-5 and BPX-5 columns, with those provided in the literature [46] and by comparing their mass spectra with data from the NIST, RepLib, MainLib, and Adams mass spectral libraries. Additionally, the identities of some compounds were confirmed by co-injection with reference compounds. Positive identification was assumed when a good match of mass spectrum and KI was achieved.

2.4. Fusarium spp. Isolates

Five isolates of the genus *Fusarium* spp. (Table 1), previously isolated from different plant hosts and stored in the collection of the Microbiology Laboratory of the Institute of Agriculture of the Lithuanian Agricultural and Forestry Sciences Center (LAMMC), were selected for this study. Fungi were isolated from plant tissues on potato dextrose agar (PDA), and pure cultures were obtained by single-spore isolation. Species were identified morphologically and confirmed by species-specific PCR. A detailed description of the origin, isolation, and identification of isolates is given in previous work [47].

Table 1. *Fusarium* spp. isolates selected for this study.

Fungal Code	Host	Latin Name	Fungal Species
18P	Shepherd's Purse	*Capsella bursa-pastoris*	*F. culmorum*
21P	Black Bindweed	*Fallopia convolvulus*	*F. culmorum*
23P	Spring wheat	*Triticum aestivum*	*F. culmorum*
24P	Spring wheat	*Triticum aestivum*	*F. culmorum*
29P	Shepherd's Purse	*Capsella bursa-pastoris*	*F. graminearum*

Before experiments, all isolates were grown on potato dextrose agar medium (PDA, Liofilchem, Italy) for 7 days at 25 ± 2 °C in the dark.

2.5. Evaluation of Antifungal Activity of Essential Oils

Antifungal activity was assessed using a modified poisoned food technique, which is widely used in primary studies of the effects of essential oils and herbal preparations on *Fusarium* spp. [28,48]. Sterile PDA medium was cooled to 47–50 °C and then supplemented with Tween 20 (0.1 mL per 100 mL^{-1} of medium) and the appropriate amount of essential oil to obtain final concentrations of 125, 250, 500, 1000, and 3000 ppm (Table 2). The medium was continuously stirred to ensure uniform distribution of the essential oil. Concentrations were selected according to the doubling principle (125, 250, 500, and 1000 ppm) used in the primary screening studies, and the highest concentration (3000 ppm) was included to verify that the maximum inhibitory effect was achieved.

Table 2. Essential oil concentrations in the medium.

Essential Oil	Amount of Essential Oil in 100 mL of Medium, µL
Sage	12.5/25/50/100/300
Caraway	12.5/25/50/100/300
Pine	12.5/25/50/100/300
Control (no EO)	0

Note: All media, including the control, contained the same amount of Tween 20.

The prepared medium was dispensed into 90-mm-diameter Petri dishes at a volume of 12.0 ± 2.0 mL. Mycelial discs of 10 mm diameter were cut from the edge of the colony of 7-day-old actively growing *Fusarium* spp. cultures and transferred to the center of the PDA medium with the mycelium facing down. Control plates used the same PDA medium with the same amount of Tween 20 emulsifier (0.1 mL per 100 mL^{-1}) but without essential oil. This ensured that the observed effects were attributable to the essential oil rather than the emulsifier.

All experiments were performed in triplicate. The plates were incubated at 25 ± 2 °C in the dark for 5 days or until the mycelium of the fungal colony reached the edge of the Petri dish in the control plates. The diameter of the fungal colony was measured every 24 h in two perpendicular directions, and the average of these measurements was used for further analysis.

The mycelial growth inhibition (MGI) was calculated based on the results of the last measurement, obtained after the control fungal colony reached the edge of the Petri dish or at the end of the 5-day incubation period:

$$\mathrm{MGI} = \frac{a - b}{a} \cdot 100 \quad (2)$$

where MGI—mycelial growth inhibition, %; a—diameter of the fungal colony in the control plate, mm; and b—diameter of the fungal colony in the medium with EO, mm.

MGI values were calculated using Abbott's formula for evaluating the antifungal activity of herbal preparations against *Fusarium* spp. in poisoned media [48]. For descriptive purposes, the response of the isolates was categorized as low (MGI < 20%), moderate (MGI 20–50%), or high (MGI > 50%) inhibition. Since there is no universally accepted classification of mycelial growth inhibition levels for essential oils, these categories are operational definitions used in this study and were intended only to facilitate the presentation of results. The final MGI assessment was performed on the last day of measurement, when the growth of the control isolates reached the edge of the plate. Negative MGI values were interpreted as stimulation of fungal growth compared to the control.

2.6. Data Analysis and Statistical Evaluation

For each condition (EO × isolate × concentration), three Petri dishes (biological replicates) were used, each measuring fungal mycelial growth in two perpendicular directions (technical replicates, the means of which were used for further analysis). Data are summarized as means ± standard deviations (SD), $n = 3$.

The dependent variable in all analyses was mycelial growth inhibition (MGI, %). One-way analysis of variance (ANOVA) with Tukey's HSD and Dunnett's post hoc tests was used to assess the effect of EO concentration on MGI. Two-way ANOVA was used to assess the effect of two factors—EO concentration and *Fusarium* isolate—and their interaction on MGI. Differences between treatment means (EOs, concentrations, and isolates) were assessed using one-way and two-way analyses of variance (ANOVAs) with Tukey's HSD post hoc test; significant differences are distinguished by different lowercase letters. To assess whether the treatment groups differed statistically significantly from the control group (without EOs), a one-way ANOVA with Dunnett's post hoc test was used; significant differences from the control are marked with "*". The strength of the effect of factors was assessed by the effect size (η^2). Differences were considered significant when $p < 0.05$ (95% probability level).

To compare the effectiveness of essential oils, EC_{50} values (concentration that inhibits mycelial growth by 50%) were calculated according to a four-parameter log-logistic (Hill) model [49], where the variables were EO concentration and mycelial growth inhibition

(MGI). For pine EO, because 50% inhibition was not achieved within the concentration range studied, linear regression was applied.

To assess the hormetic effect of low concentrations, a zero effect point (ZEP) was determined—the concentration at which MGI transitions from negative values (growth promotion) to positive (inhibition). ZEP was calculated by interpolating the concentration dependence of MGI in the low-concentration range.

Calculations were performed using IBM SPSS Statistics 20 and Microsoft Excel. Graphical diagrams were prepared using Microsoft Excel software.

3. Results

3.1. Chemical Composition of Essential Oils

The yield of EO isolated from the common sage leaves was 1.75 ± 0.08% (*v*/*w*). In total, 51 components were identified in sage EO, accounting for 97.71% of the total integrated GC peak area; the main components were cis-thujone (29.59%), camphor (21.74%), trans-thujone (14.39%), and eucalyptol (8.62%). A total of 26 components were identified in caraway EO, accounting for 98.35% of the total oil. The most prevalent components were carvone (57.57%) and limonene (35.86%), together constituting 93.43% of the EO. Thirty-seven components were identified in pine EO, the content of which was 97.65%; the main ones were α-pinene (42.17%) and δ-3-carene (38.87%). The compounds were identified by Kováts retention indices and mass spectra, and co-injection was used to compare the main compounds with reference materials. The class, formula, exact mass, and experimental and literature Kováts indices of each compound are presented in Table 3, and the distribution of component classes is presented in Figure 2. The GC-TOF/MS chromatograms of all three essential oils are presented in Figure S1 (Supplementary Materials).

Table 3. Main chemical volatile compounds of sage, caraway and pine essential oils (>1%, content in % of the total GC peak area, mean ± SD, *n* = 4). KI-E—Kováts index experimental; KI-L—Kováts index literature; tr—trace amount (<0.1%); dash (–) indicates that the component was not detected in the specific oil.

Compound	Identification	KI-E	KI-L	Class	Exact Mass	Formula	% of the Total GC Peak Area		
							Sage	Caraway	Pine
α-Pinene	KI, MS	939	939	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	2.79 ± 0.02	tr	42.17 ± 0.05
Camphene	KI, MS	955	954	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	2.70 ± 0.02	–	0.75 ± 0.01
β-Pinene	KI, MS	980	979	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	–	tr	2.06 ± 0.01
2-Pentyl furan	KI, MS	990	988	Other (Furan derivative)	138.1045	$C_9H_{14}O$	–	–	1.89 ± 0.01
δ-3-Carene	KI, MS	1012	1011	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	–	tr	38.87 ± 0.03
Limonene	KI, MS, Co-I	1031	1029	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	1.25 ± 0.01	35.86 ± 0.17	4.51 ± 0.01
Eucalyptol	KI, MS, Co-I	1033	1031	Oxygenated monoterpene	154.1358	$C_{10}H_{18}O$	8.62 ± 0.03	–	–
Terpinolene	KI, MS	1089	1088	Monoterpene hydrocarbon	136.1252	$C_{10}H_{16}$	–	–	2.70 ± 0.01
cis-Thujone	KI, MS	1106	1102	Oxygenated monoterpene	152.1201	$C_{10}H_{16}O$	29.59 ± 0.10	–	–
trans-Thujone	KI, MS	1117	1114	Oxygenated monoterpene	152.1201	$C_{10}H_{16}O$	14.39 ± 0.07	–	–
Camphor	KI, MS	1146	1146	Oxygenated monoterpene	152.1201	$C_{10}H_{16}O$	21.74 ± 0.02	–	tr
Borneol	KI, MS	1168	1169	Oxygenated monoterpene	154.1358	$C_{10}H_{18}O$	3.77 ± 0.01	–	–
Carvone	KI, MS, Co-I	1244	1243	Oxygenated monoterpene	150.1045	$C_{10}H_{14}O$	–	57.57 ± 0.14	–
α-Humulene	KI, MS	1457	1454	Sesquiterpene hydrocarbon	204.1878	$C_{15}H_{24}$	1.95 ± 0.02	–	–
Viridiflorol	KI, MS	1593	1592	Oxygenated sesquiterpene	222.1984	$C_{15}H_{26}O$	2.76 ± 0.03	–	–
Manool	KI, MS	2057	2057	Diterpene	290.2610	$C_{20}H_{34}O$	1.06 ± 0.01	–	–
Total identified, %							97.71 (51 compounds)	98.35 (26 compounds)	97.65 (37 compounds)

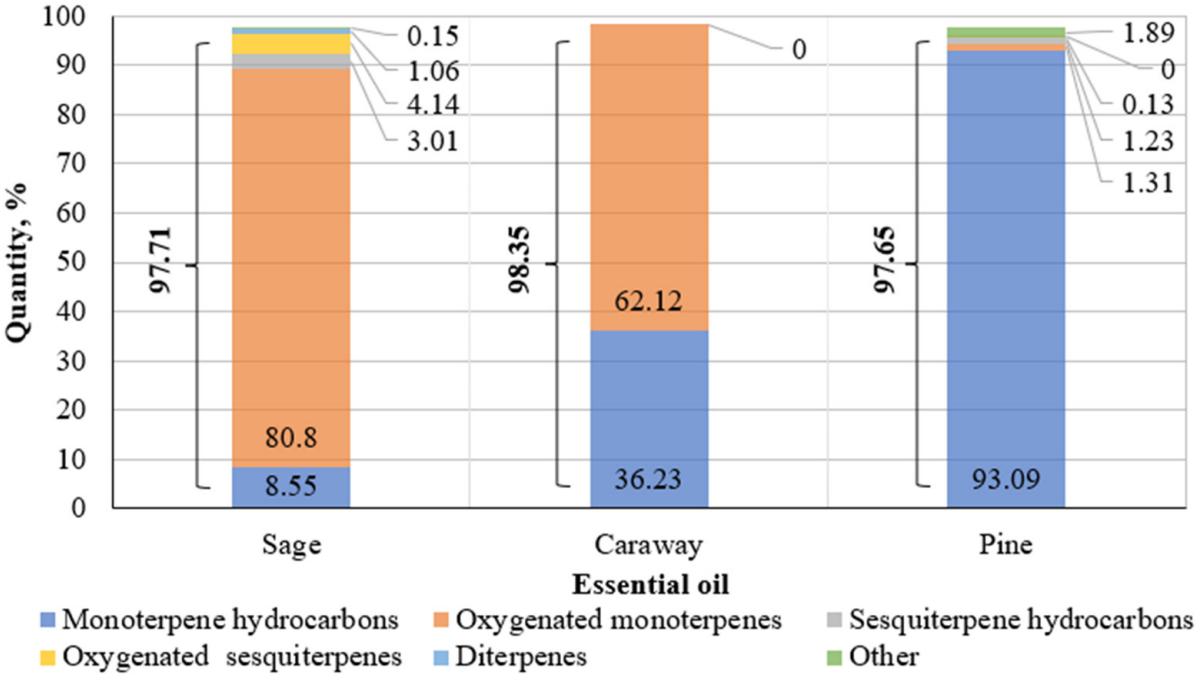

Figure 2. Distribution of component classes of the studied essential oils (%).

Sage EO belongs to the thujone–camphor chemotype, caraway EO to the carvone chemotype (carvone/limonene ratio 1.60), and pine EO to the α-pinene and δ-3-carene chemotype.

The data presented in Figure 2 show that oxygenated monoterpenes predominate in sage and caraway EOs (80.8% and 62.1%, respectively), whereas pine EO is rich in monoterpene hydrocarbon components (93.1%).

3.2. Antifungal Activity of Essential Oils

The antifungal activity of all three essential oils (EOs) increased with increasing concentration. The highest activity was observed for caraway EO, which, at a concentration of 3000 ppm, almost completely inhibited the mycelial growth of all tested *Fusarium* isolates (MGI—85.83–88.05%). Sage EO showed moderate antifungal activity (MGI—49.80–61.75%), while pine EO was the least active (MGI—13.33–33.60%). Mycelial growth inhibitions, statistical significance groups (Tukey HSD), and the overall heat map are presented in Figure 3. In the case of all three EOs, increasing concentration led to a statistically significant increase in antifungal activity (Tukey HSD, $p < 0.05$), although in the case of pine EO, the effect of some concentrations was not statistically significant.

Sage EO began to inhibit the growth of *Fusarium* isolates from a concentration of 500 ppm (MGI: 10.02–17.02%). When the concentration was increased to 1000 ppm, the inhibition increased (MGI: 30.36–44.79%), and the maximum effect was observed at 3000 ppm (MGI: 49.80–61.75%). Meanwhile, concentrations of 125 and 250 ppm promoted the growth of some isolates, with negative MGI values observed. According to this classification, sage EO exhibited low inhibition at 500 ppm, moderate inhibition at 1000 ppm, and moderate-to-strong inhibition at 3000 ppm.

Low concentrations of sage EOs were most effective in promoting the growth of isolate 21P, while isolates 23P (500 ppm), 21P (1000 ppm), and 24P (3000 ppm) were most resistant to increasing concentrations. However, the response of all isolates to increasing EO concentrations was similar (Figure 4a,b). At 250 ppm, a more intense pigmentation of the fungal mycelium of some isolates was visually observed, which may indicate a stress response of the fungus. In the literature, sublethal stress in *F. graminearum* is associated with

increased deoxynivalenol (DON) synthesis [50], and caraway EO causes oxidative stress in this species and affects DON biosynthesis [51]. Direct confirmation of this association requires measurements of mycotoxin content, which were not performed in this work.

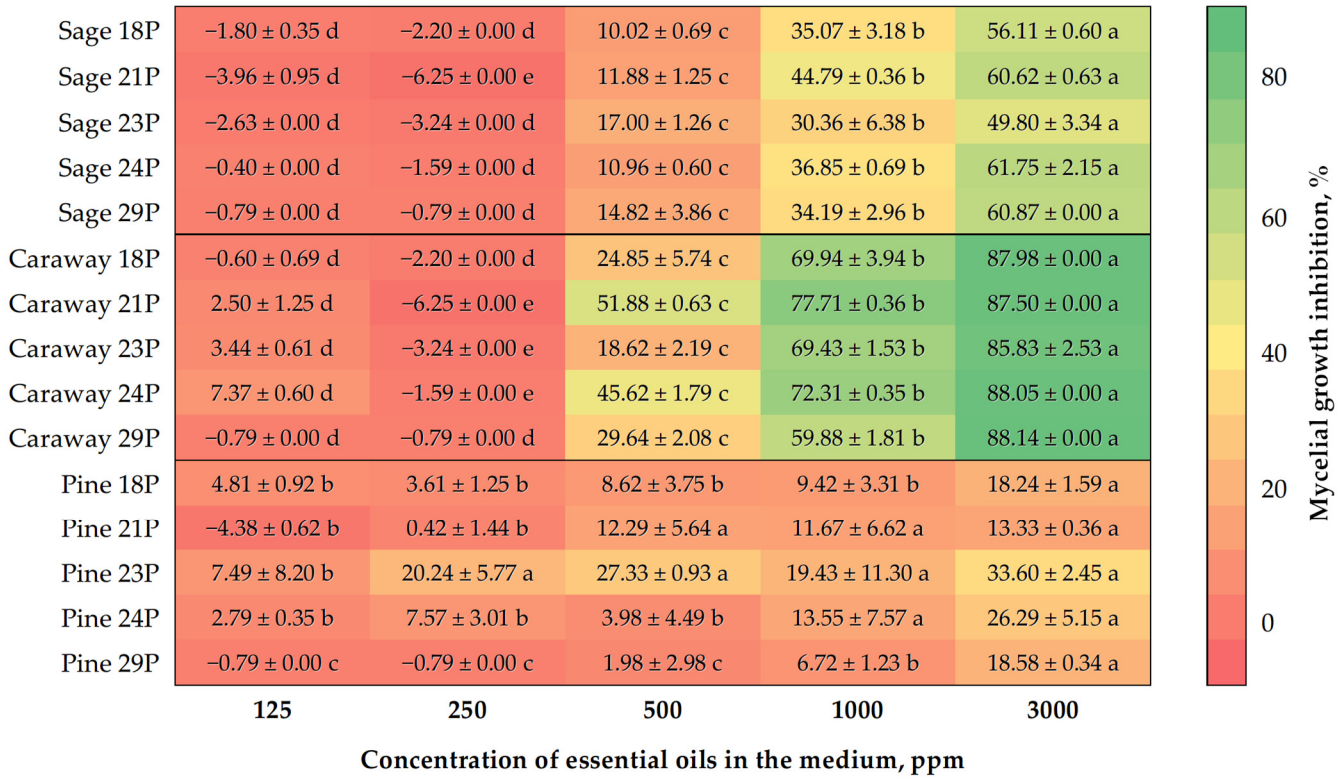

Figure 3. Heat map of the antifungal activity of essential oils against *Fusarium* isolates (mycelial growth inhibition, %, mean ± SD, $n = 3$). Green indicates strong inhibition of mycelial growth, red indicates weak inhibition or growth promotion. Different letters in the same row indicate statistically significant differences between concentrations (Tukey HSD, $p < 0.05$). The value ± 0.00 means that the results of all replicates coincided. In the case of pine EO, the standard deviations were larger than those of the other EOs, indicating a lower and more uneven antifungal effect.

Caraway EO had the highest antifungal activity. Although concentrations of 125 and 250 ppm were not yet sufficiently effective, a significant growth inhibition was observed at 500 ppm (MGI—18.62–51.88%), and at 3000 ppm, the growth of all isolates was almost completely stopped (MGI—85.83–88.14%). Thus, the active concentration of caraway EO was 500 ppm, which was lower than that of sage EO (1000 ppm). Visually (Figure 4c,d), at a concentration of 3000 ppm, fungal mycelium did not grow at all, while at 250 ppm, pigmentation changes and slower colony growth were observed.

Pine EO had the weakest antifungal activity and the greatest variability in effect among isolates. At low concentrations, the growth of some isolates was slightly inhibited, while that of others was stimulated. Inhibition of most isolates increased with increasing concentration, but the response of 21P and 23P was not linearly dependent on concentration. No changes in the pigmentation of the fungal mycelium were visually observed (Figure 4e,f). However, since mycotoxins were not measured, the effect of pine EO on their synthesis cannot be confirmed or refuted in this work. In addition, the growth of *F. graminearum* isolates was more uneven than that of *F. culmorum*; therefore, it can be assumed that this species is more sensitive to the effects of pine EO.

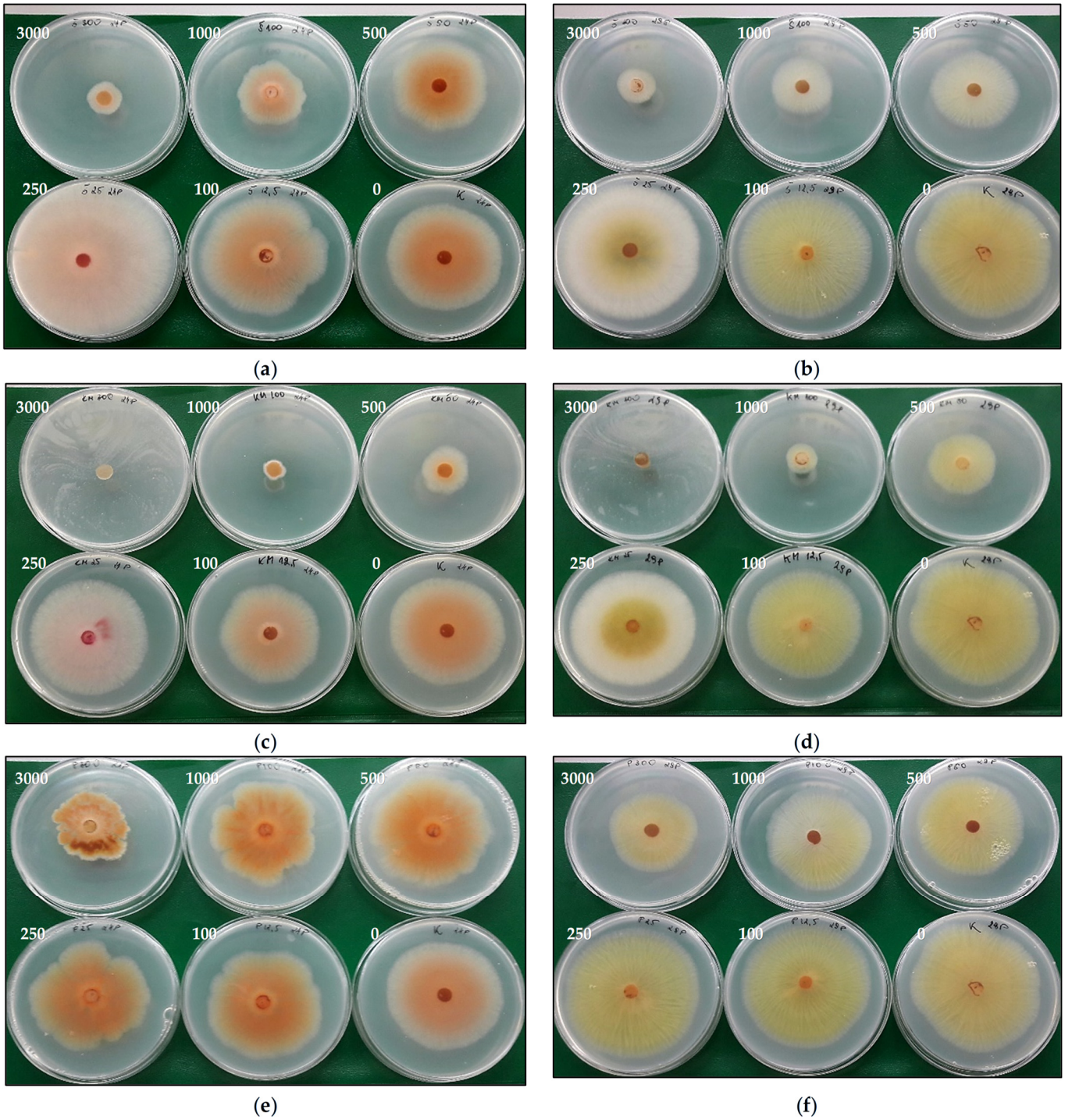

Figure 4. Mycelial growth of *F. culmorum* (29P) (**a**,**c**,**e**) and *F. graminearum* (24P) (**b**,**d**,**f**) isolates exposed to different concentrations of essential oils of sage (**a**,**b**), caraway (**c**,**d**) and pine (**e**,**f**) (3000, 1000, 500, 250, 125 ppm and 0 ppm—control) on the third day of measurement. All Petri dishes are 90 mm in diameter, which serves as the scale reference.

To determine the minimum effective concentration of essential oils (EO), the mean effect of each concentration (averaged across all isolates) was compared with the control group (without EO) using Dunnett's test (Figure 5). The lowest tested concentrations of all three EOs (125 and 250 ppm) did not differ significantly from the control group ($p > 0.05$), while from 500 ppm and higher, the mean MGI values were significantly different from the control group ($p < 0.05$). However, it is important to emphasize that this applies to the mean

response across isolates. At the individual isolate level (Figure 3), the response to pine EO was significantly more variable: at 500 ppm, the MGI values for isolates 24P and 29P were low, with standard deviations above the mean, and did not differ significantly from the control group. Therefore, a concentration of 500 ppm can be considered the minimum effective concentration for sage and caraway EO, but not for pine EO, as consistent effects for all isolates were observed only at the highest concentration tested.

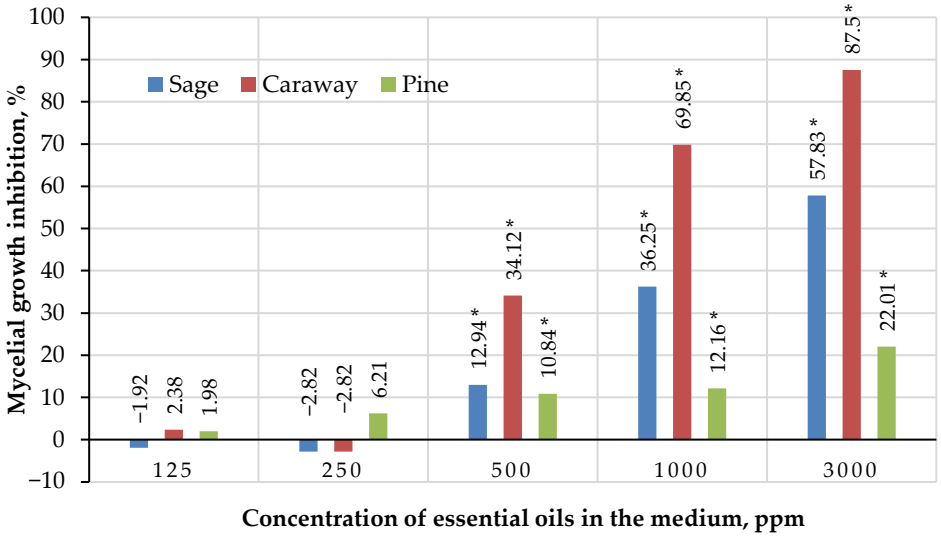

Figure 5. Mean mycelial growth inhibition (MGI, %) induced by different essential oils, calculated from the average of all five *Fusarium* spp. isolates (n = 3 for each isolate). * Indicates a statistically significant difference from the control group (Dunnett's test, $p < 0.05$). Significance refers to the mean response across isolates; individual isolate responses are shown in Figure 3.

Comparing the effects of different essential oils on the average inhibitory coefficient (MGI) values of all tested *Fusarium* isolates (Figure 5), it was found that the antifungal activity of all EOs increased with increasing concentration. The highest activity was observed for caraway EO, which achieved the highest inhibitory effect at a concentration of 3000 ppm (MGI—87.57%). Sage EO showed moderate antifungal activity (MGI—57.83%), while pine EO was the least effective (MGI—22.01%).

To assess the influence of essential oil concentration and *Fusarium* isolate on antifungal activity, a two-way analysis of variance was performed (Table 4).

Table 4. Results of two-factor analysis of variance (ANOVA): Effects of EO concentration, *Fusarium* isolate and their interaction on mycelial growth inhibition (MGI).

Essential Oil	Source of Variation	Degrees of Freedom (*df*)	Fisher's Criterion (*F*)	Significance (*p*)	Effect Size (η^2)
Sage	Isolate	4	9.07	<0.001	0.003
	Concentration	4	2661.41	<0.001	0.976
	Interaction	16	10.98	<0.001	0.016
	Error	50			
Caraway	Isolate	4	76.76	<0.001	0.009
	Concentration	4	7891.72	<0.001	0.967
	Interaction	16	44.02	<0.001	0.022
	Error	50			
Pine	Isolate	4	34.03	<0.001	0.330
	Concentration	4	45.39	<0.001	0.440
	Interaction	16	2.79	<0.001	0.108
	Error	50			

Note: *df*—degrees of freedom; *F*—Fisher's F-ratio (variance ratio from ANOVA); η^2—effect size (part of the variance explained by the factor). The effects of all factors (isolate, concentration, and their interaction) were statistically significant; $p < 0.001$ for most factors, and for the pine EO interaction—$p = 0.003$ (two-way ANOVA).

It was found that both the concentration of the essential oil and the isolate had a statistically significant effect on mycelial growth inhibition across all essential oils tested ($p < 0.001$). A significant interaction between concentration and isolate was also observed ($p < 0.001$), but its magnitude varied across the essential oils. Effect size analysis (η^2) showed that the antifungal activity of sage and caraway essential oils was primarily determined by concentration, explaining 97.6% and 96.7% of the variance in mycelial growth inhibition, respectively. At the same time, the isolate's influence was minimal ($\eta^2 \leq 0.009$). Meanwhile, for pine essential oil, the concentration explained 44.0% of the variance, and the influence of the isolate was significantly stronger ($\eta^2 = 0.330$), indicating greater sensitivity of different *Fusarium* isolates to this essential oil.

3.3. Dose–Response Analysis and EC_{50} Estimation

The dose–response relationship was assessed using a four-parameter log-logistic model (Figure 6). The calculated EC_{50} values allow for an objective comparison of EO activity: caraway EO was the most active (EC_{50} = 727 ppm), sage was average (1017 ppm), and pine EO EC_{50} exceeded the tested range (>3000 ppm). The 95% confidence intervals for caraway and sage EO do not overlap; so, their difference is significant (Table 5).

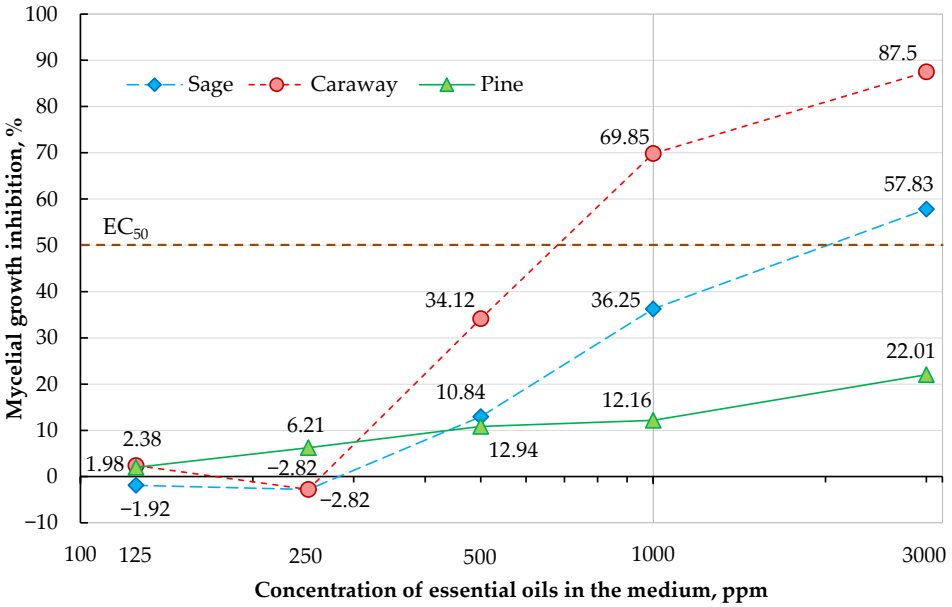

Figure 6. Dose–response curves of different essential oils against *Fusarium* spp. isolates. The dotted line represents the 50% mycelial growth inhibition level (EC_{50}).

Table 5. Calculated EC50 values of essential oils with 95% confidence intervals (CI).

Essential Oil	EC_{50} (ppm)	95% CI (ppm)	R^2	Relative Activity
Sage	1017	936–3927	0.851	Medium
Caraway	727	672–794	0.980	The largest
Pine	>3000	—	0.818 *	Smallest

R^2—coefficient of determination describing the goodness of fit of the dose–response model. * For pine essential oil, linear regression was used because 50% inhibition of mycelial growth was not reached within the tested concentration range.

The lowest EC_{50} value was observed for caraway EO (727 ppm), indicating the highest antifungal activity. The EC_{50} of sage EO reached 1017 ppm, showing lower activity than that of caraway EO. Pine EO did not reach 50% inhibition of mycelial growth in the

tested concentration range; therefore, its EC_{50} exceeded the highest tested concentration (>3000 ppm), confirming the weakest antifungal effect (Table 4).

The dose–response curves (Figure 6) showed that the antifungal activity of caraway EO increased most rapidly—the 50% inhibition limit was reached between 500 and 1000 ppm concentrations. This limit was reached for sage EO only at higher concentrations, while pine EO remained below the 50% inhibition limit throughout the tested concentration range.

The 95% confidence intervals showed that the EC_{50} values of caraway and sage EOs were statistically significantly different, as their confidence intervals did not overlap. The dose–response model described the experimental data well (R^2 = 0.851–0.980). In the case of pine EOs, a linear regression model was applied because even the highest concentration tested did not achieve 50% inhibition of mycelial growth.

3.4. Mycelial Growth Kinetics and the Promoting Effect of Low Concentrations

Caraway EO was chosen for kinetic analysis because it exhibited the highest antifungal activity (lowest EC_{50}) and most clearly reflected both a strong inhibitory effect at high concentrations and a stimulatory (hormetic) effect at low concentrations. The kinetics of the other two oils were less informative: pine EO showed a weak effect, and sage EO an intermediate effect. Mycelial growth kinetics analysis showed that the effect of caraway EO was concentration-dependent (Figure 7). At a concentration of 3000 ppm, fungal mycelial growth was almost completely stopped from the first day. In contrast, concentrations of 500 and 1000 ppm significantly slowed down colony growth. Meanwhile, at concentrations of 125 and 250 ppm, mycelial growth was similar to, or in some cases even more intense than, that of the control.

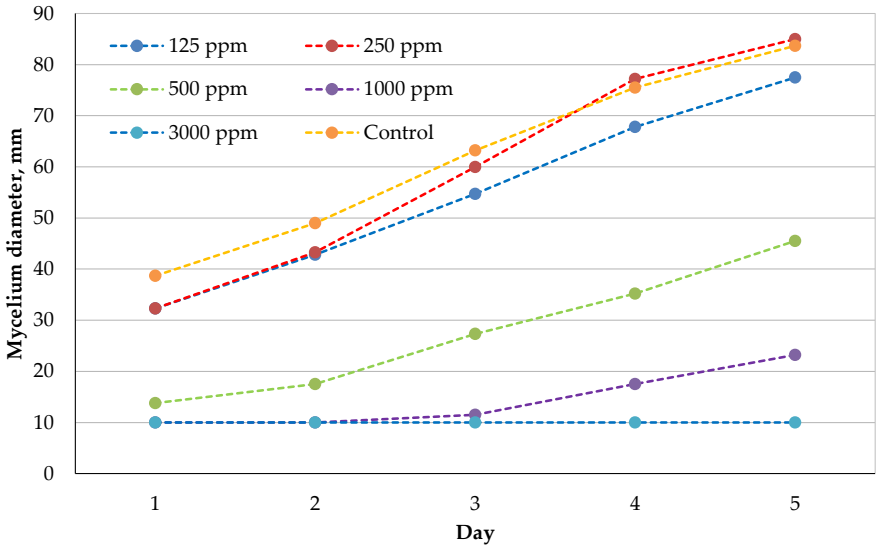

Figure 7. Mycelial growth kinetics of a typical *Fusarium* isolate (24P) at different concentrations of caraway essential oil, selected as the most active oil to illustrate the concentration-dependent and biphasic (hormetic) response.

The stimulatory effect at low concentrations (125–250 ppm) is consistent with the negative mycelial growth inhibition (MGI) values observed for some *Fusarium* isolates (Figure 3), suggesting a hormetic response to the essential oils. The zero effect point (ZEP), which marks the transition from growth promotion to inhibition, was estimated at approximately 295 ppm for sage EO and 269 ppm for caraway EO. In addition, low concentrations of sage and caraway EOs induced changes in fungal mycelial pigmentation, possibly reflecting a stress response of the fungus [50], while pine EO did not. Since

mycotoxins were not detected in this work, direct conclusions about their synthesis cannot be drawn.

3.5. Relationship Between Chemical Composition and Antifungal Activity

Comparing the chemical composition of essential oils with their antifungal activity at all tested concentrations (Figure 8), it was found that antifungal activity depends not only on the total amount of dominant compounds, but also on their chemical nature.

Although the dominant compounds of pine EO constituted 81.04% of the total composition, this EO was characterized by the lowest antifungal activity in the entire concentration range tested. Meanwhile, the highest antifungal activity was observed in caraway EO, whose dominant compounds were carvone (57.57 ± 0.14%) and limonene (35.86 ± 0.17%), which together constituted 93.43% of the oil composition. Sage EO, dominated by cis-thujone (29.59 ± 0.10%), camphor (21.74 ± 0.02%), trans-thujone (14.39 ± 0.07%) and eucalyptol (8.62 ± 0.03%), had moderate antifungal activity.

Figure 8 shows that a higher content of dominant compounds does not, in itself, ensure higher antifungal activity. Pine EO, which was dominated by α-pinene (42.17 ± 0.05%) and δ-3-carene (38.87 ± 0.03%), inhibited the growth of *Fusarium* spp. mycelium less than caraway and sage EO at all tested concentrations. Meanwhile, caraway EO, dominated by carvone and limonene, exhibited the highest antifungal activity. These results indicate that antifungal activity is determined not only by the content of dominant compounds, but also by their chemical nature and their interaction with each other (synergism or antagonism).

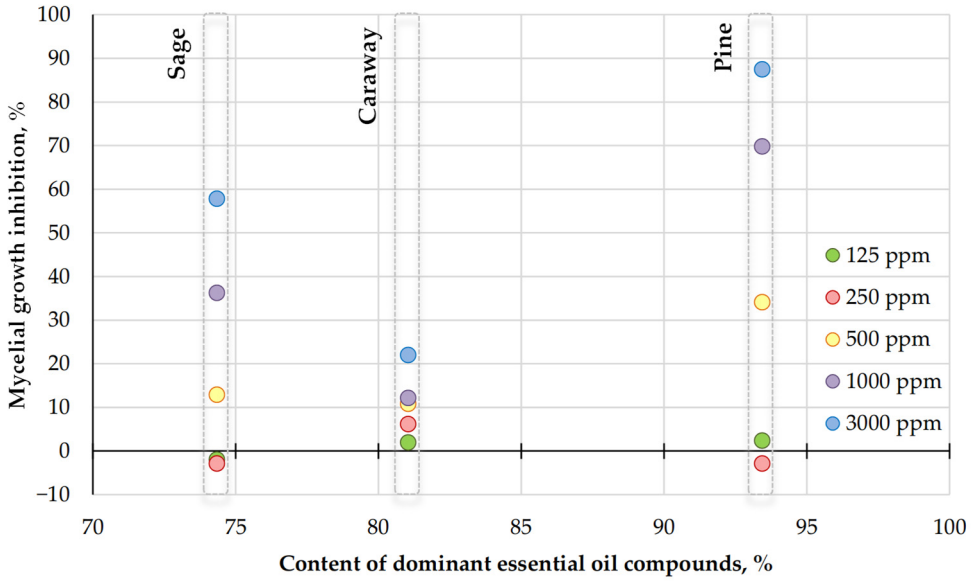

Figure 8. Relationship between the total amount of dominant essential oil compounds (%) and antifungal activity (mycelial growth inhibition, MGI, %) at different essential oil concentrations (125–3000 ppm).

4. Discussion

GC-FID and GC-TOF/MS analyses enabled reliable identification and quantification of essential oil components, providing the basis for chemotype identification and its association with antifungal activity. The antifungal activity of the tested essential oils clearly differed: the highest was that of caraway EO (average MGI at 3000 ppm—87.57%; EC_{50} = 727 ppm), the average was that of sage EO (MGI 57.83%; EC_{50} = 1017 ppm), and the lowest was that of pine EO (MGI 22.01%; EC_{50} > 3000 ppm). This difference was not determined by the total amount of dominant compounds, but by their chemical nature.

In this work, caraway EO belonged to the carvone chemotype: carvone accounted for 57.57%, and limonene for 35.86% (carvone/limonene ratio 1.60). This differs from the EO of annual caraway grown in Serbia, which was dominated by limonene, with a carvone/limonene ratio of only 0.58; therefore, this oil was assigned to the limonene chemotype [52]. Such an inverse ratio indicates that the dominance of carvone and limonene is strongly determined by the genotype (annual or biennial form), origin, and growing conditions, and that a carvone-rich chemotype is important for achieving higher antifungal activity [53].

The high activity of caraway EO (active at concentrations as low as 500 ppm, with near-complete inhibition at 3000 ppm) can be attributed to the action of carvone. Saremi et al. [54] showed that caraway EO and pure carvone inhibit ergosterol biosynthesis in a dose-dependent manner, disrupt the membrane structures of fungal hyphae and organelles such as mitochondria, when acting on *Aspergillus fumigatus*. Our results complement this mechanism, showing that carvone-rich caraway EO is also effective against the cereal pathogens *F. graminearum* and *F. culmorum*, and the lowest EC_{50} (727 ppm) among the three tested oils is consistent with the strong antifungal effect of carvone described in the literature.

In total, 51 components were identified in the EO of *Salvia officinalis*, among which cis-thujone (29.59%), camphor (21.74%), trans-thujone (14.39%), and eucalyptol (1,8-cineole) (8.62%) were predominant. A similar but not identical composition was found in the EO of Tunisian *S. officinalis*: 49 components, with camphor (25.14%), α-thujone (18.83%), and 1,8-cineole (14.14%) being predominant [55]. Thus, both oils belong to the thujone–camphor type, but in this work cis-thujone dominated rather than camphor, and trans-thujone was present in greater proportion. This dominance of cis-thujone is closer to the cis-thujone chemotype of sage grown in Europe (including Serbia) [33,34,36], while camphor and 1,8-cineole are more common in samples from the Mediterranean region [55,56]. These differences confirm the pronounced variability in *S. officinalis* chemotypes depending on the origin [57].

The antifungal activity of sage EO was moderate (active from 1000 ppm; EC_{50} = 1017 ppm). Khedher et al. [55] also found that among the fungi tested, sage EO had the strongest effect on *F. oxysporum* (MIC = 0.156 mg/mL), while Zaccardelli et al. [58] confirmed the activity of sage EO against plant pathogenic fungi and bacteria. Although direct numerical comparison is limited by differences in indicators (MIC and EC_{50}), methods (broth and agar dilution), and *Fusarium* species, the general trend is consistent—sage EO has a noticeable but not very strong effect on the genus *Fusarium*. The relatively lower activity than caraway EO is because thujone, camphor, and eucalyptol are weaker antifungal compounds than carvone. However, eucalyptol (1,8-cineole) acts on fungi by promoting the production of reactive oxygen species (ROS) and inhibiting ergosterol biosynthesis genes [59], thus contributing to the overall effect of sage EO.

The pine EO was dominated by α-pinene (42.17%) and δ-3-carene (38.87%). This profile corresponds to the α-pinene–δ-3-carene chemotype of pine needle oil growing in Lithuania, although the proportion of the main compounds in our commercial oil was higher than in needle oils, in which α-pinene and δ-3-carene constituted 7.0–16.1% and 5.2–14.3%, respectively [60]. Despite the large proportion of dominant compounds, pine EO was the least active—even at 3000 ppm, it inhibited growth by only about 22%, and the EC_{50} exceeded the tested range. This is consistent with Semerdjieva et al. [61] reported that α-pinene-rich *Pinus* (including *P. sylvestris*) EOs have relatively weak inhibitory effects on *Fusarium* mycelium growth, confirming that monoterpene hydrocarbons are less effective than oxygenated monoterpenes.

Summarizing the relationship between composition and activity, our results show that antifungal activity is not determined by the amount of dominant compounds, but by their chemical nature: oxidized monoterpenes (carvone, eucalyptol) are more effective than hydrocarbon monoterpenes (α-pinene, δ-3-carene, limonene individually). The antifungal effect of EO is usually manifested by damage to the cytoplasmic membrane and organelles, changes in membrane permeability, inhibition of ergosterol biosynthesis, and oxidative stress [20–23,30,62]. This may explain why pine EO, although its dominant compounds constituted a large part of the total composition, was the least effective, while carvone-rich caraway EO was the most effective.

Two-way ANOVA revealed that the activity of caraway and sage EOs was almost entirely determined by concentration (η^2 = 0.967 and 0.976), with minimal influence of isolate ($\eta^2 \leq 0.009$). In contrast, in the case of pine EOs, the influence of isolate was much greater (η^2 = 0.330 compared to concentration η^2 = 0.440), indicating that the efficacy of the weakly acting oil was more strain-dependent. Other authors have observed similar variability in the sensitivity of *Fusarium* isolates or strains to EOs [55,61]. This is important when choosing EOs for broad- or narrow-spectrum control.

When evaluating the results in a broader context, it is important to note the difference between the active concentrations of essential oils and those of synthetic fungicides. In our previous study [47] the same *Fusarium* isolates were tested with triazole fungicides, the active concentrations of which are several orders of magnitude lower than the EC_{50} values of essential oils determined in this work (727–1017 ppm). This indicates that essential oils cannot directly replace synthetic fungicides but can be evaluated as an additional tool in integrated plant protection, especially considering the increasing resistance of *Fusarium* to triazoles and the goals of the European Union's Green Deal. Similarly, Danielewicz et al. [41], who studied the effects of essential oils of sage, cypress, cumin and thyme on *Fusarium* pathogens and compared them with prothioconazole, found that essential oils can be an alternative to synthetic fungicides, but must be properly selected; thyme oil was the most effective in inhibiting *Fusarium* growth.

One of the most important observations of this work is the stimulatory (biphasic, hormetic) effect of low EO concentrations (125–250 ppm): for some isolates, they did not inhibit but promoted mycelial growth (negative mycelial growth inhibition values) and changed the pigmentation of the fungal mycelium. The calculated zero effect point (ZEP $\approx$ 269 ppm for caraway and $\approx$295 ppm for sage EO) quantitatively defines the transition from promotion to inhibition. This result has direct practical significance: it has been found that, specifically in the case of *F. graminearum*, sublethal doses of fungicides cause the formation of hydrogen peroxide (H_2O_2), which promotes the production of deoxynivalenol (DON) [50], and sublethal concentrations of EO compounds can similarly increase the synthesis of mycotoxins [24]. Mycotoxins were not quantified here; so, this effect was not directly assessed. Oxidative stress and fungal secondary metabolism are closely linked, and H_2O_2 plays an important role in modulating mycotoxin production [63], with the direction of effect (promotion or inhibition) depending on the concentration and fungal species. Therefore, insufficient EO concentrations in the field could inadvertently increase the risk of mycotoxins, and *F. graminearum* and *F. culmorum*, as producers of trichothecenes and zearalenone, pose a particular threat to food and feed safety [11,12]. Unlike sage and caraway EO, pine EO did not induce a pronounced stimulatory response and color changes in the fungal mycelium, though this was not verified, as mycotoxins were not measured.

Carvone-rich caraway EO is the most promising natural alternative studied, which can complement or partially replace synthetic fungicides in sustainable crop protection strategies and thus reduce the problems of resistance, residues, and environmental pollution, especially considering the growing resistance of *Fusarium* to groups of active ingredients

and the goals of the European Union's Green Deal [17,64]. At the same time, the identified biphasic response indicates that the application of EO is ambiguous. While providing ecological benefits, it requires precise dosing, as an insufficient concentration may not only fail to protect the crop but also increase the risk of mycotoxin contamination. Therefore, the practical use of EO is inseparable from ensuring appropriate formulation, stability, and sufficient active concentration under field conditions, which is significant for food and feed safety.

However, the results of this study are limited by several circumstances: the evaluation was performed only in vitro, three biological replicates were used (n = 3), and the activity was assessed by growth inhibition, EC_{50} and stimulatory effect indices, without minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations and without direct quantification of mycotoxins. In the case of pine essential oil, the EC_{50} exceeded the highest concentration tested; so, the dose–response curve for this oil was estimated less accurately than for the other two. Also, this study did not include a positive control (synthetic fungicide); so, this work does not provide a direct comparison of the effectiveness of essential oils and chemical preparations. In addition, fungal viability was not assessed, and no regrowth study was performed; therefore, the observed effect should be considered growth-inhibitory rather than fungicidal. In future studies, it is appropriate to determine MIC and MFC, measure the concentrations of ergosterol and mycotoxins (DON, NIV, ZEA), investigate the synergy of active compounds (e.g., carvone), evaluate the effect of essential oils on spore germination and germ tube growth, evaluate the effect of volatile components of essential oils using the fumigation method, verify the effect of EO in the grain matrix and under in vivo conditions, include a positive control (synthetic fungicide) in the study design, as well as include more isolates and replicates to confirm the potential of essential oils for practical control of *Fusarium* head blight in cereals.

5. Conclusions

The antifungal activity of the tested essential oils against *Fusarium graminearum* and *Fusarium culmorum* was determined not by the amount of dominant compounds, but by their chemical nature: the highest effect was observed for carvone chemotype caraway EO (EC_{50} = 727 ppm), medium for cis-thujone and camphor chemotype sage EO (EC_{50} = 1017 ppm), and the lowest for α-pinene and δ-3-carene rich pine EO (EC_{50} > 3000 ppm). A biphasic response induced by low concentrations (125–250 ppm), when caraway and sage EO promoted mycelial growth (ZEP ≈ 269–295 ppm), was found, suggesting that insufficient dosage may fail to suppress fungal growth in vitro; its effect on mycotoxin accumulation was not assessed. Among the tested oils, carvone-rich caraway EO was the most active and therefore the most promising candidate for further evaluation in sustainable *Fusarium* head blight management. In future studies, it is appropriate to determine MIC and MFC, quantify the levels of ergosterol and mycotoxins (DON, NIV, ZEA), investigate the effect of essential oils on *Fusarium* spore germination, and verify the effect of EOs under in vivo conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app16167959/s1, Figure S1: GC-FID chromatograms of sage (a), caraway (b) and pine (c) essential oils. The main compounds (>1%) are labelled above the peaks; their full identification and relative contents are given in Table 3. Chromatographic conditions are described in Section 2.3.

Author Contributions: Conceptualization, R.Ž.; Methodology, R.Ž. and R.B.; Formal Analysis, R.Ž., R.B., D.Č., D.Ž. and S.P.; Investigation, R.Ž. and R.B.; Data Curation, R.Ž., R.B. and S.P.; Writing—Original Draft Preparation, R.Ž., R.B., D.Č., D.Ž. and S.P.; Writing—Review & Editing, R.Ž., R.B.,

D.Č., D.Ž. and S.P.; Visualization, S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in this article.

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

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