

Article

Chemical Composition, Antioxidant and Antimicrobial Effects of Essential Oils Extracted from Two New *Ocimum basilicum* L. Varieties

Cătalina Stan (Tudora)^{1,2,*}, Florin Nenciu^{2,*}, Adriana Muscalu², Valentin Nicolae Vlăduț², Floarea Burnichi³, Carmen Popescu⁴, Florentina Gatea⁵, Oana Alina Boiu-Sicuia^{1,6} and Florentina Israel-Roming¹

¹ Department of Plant Sciences, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464 Bucharest, Romania

² National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry-INMA, 6 Ion Ionescu de la Brad Avenue, 013813 Bucharest, Romania

³ Vegetable Research and Development Station Buzau, 23 Mesteacanului Street, 120024 Buzau, Romania

⁴ S C Hofigal Export Import SA, 2 Intrarea Serelor, 042124 Bucharest, Romania

⁵ National Institute for Biological Sciences, 296 Splaiul Independenței, 060031 Bucharest, Romania

⁶ Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Avenue, 013813 Bucharest, Romania

* Correspondence: cmc_tudora@yahoo.com (C.S.); florin.nenciu@inma.ro (F.N.)



Citation: Stan (Tudora), C.; Nenciu, F.; Muscalu, A.; Vlăduț, V.N.; Burnichi, F.; Popescu, C.; Gatea, F.; Boiu-Sicuia, O.A.; Israel-Roming, F. Chemical Composition, Antioxidant and Antimicrobial Effects of Essential Oils Extracted from Two New *Ocimum basilicum* L. Varieties. *Diversity* **2022**, *14*, 1048. <https://doi.org/10.3390/d14121048>

Academic Editors: Ekaterina Kozuharova, Ina Yosifova Aneva and Luca Rastrelli

Received: 4 November 2022

Accepted: 25 November 2022

Published: 29 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Recent agricultural research aims to identify novel applications for plant extracts as efficient defenses against weeds, insects, or phytopathogenic agents. The main goal of the present study was to evaluate two new varieties of basil (*Ocimum basilicum* L., fam. *Lamiaceae*), Yellow basil, “Aromat de Buzau” variety and Red-violet basil, “Serafim” variety. The basil varieties were studied under different aspects regarding biochemical characterization using GC-MS, antioxidant activity evaluated under three methods (DPPH, ABTS, FRAP), and antimicrobial properties (for three G-bacteria: *Pertobacterium carotovorum*, *Pseudomonas marginalis*, *Pseudomonas syringae* and three phytopathogenic fungi: *Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea*). The results showed that linalool (39.28%; 55.51%), estragole (31.48%; 9.78%), and eugenol (5.42%; 7.63%) are the main compounds identified in the extracts, validating the high antioxidant activity of the studied essential oils and floral waters. When applied in high concentrations, essential oils have shown a potential bactericidal effect on *P. carotovorum* as well as a potential fungicidal effect on *R. solani* and *B. cinerea*.

Keywords: new varieties of basil; essential oils; floral water; chemical composition; antimicrobial activity

1. Introduction

Medicinal and aromatic plants (MAPs) play an important role in the strategic priorities for rural development, according to the basic principles of multifunctionality, sustainability, and diversification. In this context, the research and development of new varieties of MAPs can be approached from several perspectives, including new uses, botanical and phytochemical characterization, crop management, with new economic and industrial implications. In the context of climate change, the MAPs exposure to (a)biotic stress, including extreme temperatures, drought, flooding, diseases caused by pests, etc., affects the functionality of cellular and metabolic processes, limiting their growth and development, and finally reducing the yield and quality of the productions obtained [1–4]. Agrochemicals have been used to avoid losses associated with biotic stress; however, the irrational use has led to their accumulation in the food chain, with implications for human health and causing several environmental problems [5]. Studies have documented the use of EOs as pesticides, since they are presenting high volatile potential, low persistence, and low toxicity to vertebrates, which is safer in terms of health issues and environmental impact than chemicals [6–8].

MAPs belonging to the *Ocimum* genus generically called basil, include more than 30 species of plants and shrubs, originating in the tropical and subtropical regions of Asia, Africa, Central and South America [9]. Among the species of this genus, *Occimum basilicum* L.—common sweet basil is cultivated in many countries and represents the most important crop for obtaining EOs worldwide. Basil EO is considered an important source of aromatic compounds since it possesses a series of biological activities (insecticides, repellents, nematocides), antibacterial, antifungal and antioxidant activities [10,11]. A market growth of EOs obtained from *Ocimum* species is estimated, from USD 186.5 million (2019) with an annual growth rate of 8% until 2023, Europe holds the largest share of this market [12]. Sweet basil is botanically described as an annual plant, growing as a branched bush between 0.3 and 1.3 m in height, with opposite, light green silky leaves containing many oil glands in which EOs are stored. Basil flowers are colored from white to purple and arranged in a terminal spiciform inflorescence [13,14]. Basil cultivars have the genetic ability to generate and maintain different sets of chemical compounds, leading to a wide variety of chemotypes within the same species. This phenomenon occurs since the crop is highly pollinated by bees [15,16].

Depending on the chemical composition, basil EOs have been classified into several chemotypes [11]:

- the European chemotype with the main compounds linalool of very good quality in a proportion of at least 50%, eugenol 4–5%, and methyl chavicol (estragole) 2–3%;
- tropical chemotype rich in methyl cinnamate 50–70%, linalool 8–30%, and high content of camphor;
- reunion chemotype, characterized by high concentrations of methyl chavicol (estragole);
- eugenol-rich chemotype, characterized by high concentrations of eugenol (>5%).

The flavor of different basil families can vary significantly since they contain a varied mixture of chemical elements in varying ratios. The strong clove flavor of sweet basil is given by eugenol (a chemical compound also present in cloves), while the lemon flavor is given by the higher content of citral (compound present in mint, lemon) and limonene. African blue basil has a strong camphor flavor due to the high proportion of camphor and camphene. Other chemicals involved in producing the distinct flavors of basil varieties, depending on the proportion are cinnamate, citronellol (compound found in geranium, rose), geraniol (geranium), methyl-chavicol (tarragon), myrcene (bay, myrtle), pinene (pine oil), ocimene, terpineol, etc.

As an alternative to potentially hazardous chemical products used in the food industry, a range of different chemical and synthetic substances have been proposed as antimicrobial agents to suppress pathogens. Food scientists are examining the efficacy of a range of inhibitory compounds, especially essential oils, which are a complex combination of molecules, mainly monoterpenes, sesquiterpenes, and their corresponding oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides). Some studies [17] have demonstrated that basil EO possesses antibacterial and antifungal properties, while other researches [18] have examined in more detail the compounds found in oils that have the most intense antimicrobial action. The antibacterial action of basil EOs may be primarily attributed to the presence of the major component linalool [19,20], considering that Gram-positive bacteria strains are more sensitive to basil EOs.

It is remarkable that different European regions have comparable basil customs and medicinal use. Romania has a rich ethnobotanical and ethnomedical heritage related to basil, a plant that has been used for various practices [21]. Table 1 lists the ethnomedical applications of basil, taking into account the primary pharmacological treatments and a wide range of health conditions it addresses, the data being gathered from the literature [22–26].

Table 1. Main methods of using basil in traditional medicine.

The Method of Use	Treatment Method	The Conditions Addressed
Powders—grinding of dried inflorescences followed by sieving.	1.5 g of basil plant remains under the tongue for 2 min, then swallowed with water (3–4 times/day on an empty stomach).	
Hot infusion—10 g of stems with crushed flowers are scalded in a cup of boiling water, and after 15 min of infusion are filtered.	It is usually consumed hot for maximum effect (antispasmodic, hyperthermic, and relaxing purposes).	
Combined infusion—combines cold extraction with hot infusion. 2–3 teaspoons of chopped basil flowers and leaves are left to soak in 1/2 cup of water (from evening to morning), then filtered. Another portion of chopped plants is scalded with another 1/2 cup of hot water for 20 min, after which it is left to cool and then filtered. The two extracts are combined.	It is consumed before each meal (15 min before).	Nutritional disorders Atherosclerosis Myocardial infarction Bronchial asthma Infection with carcinogenic papilloma viruses Mental stress Neurovegetative disorders Chronic fatigue syndrome Nightmares, sleep disorders Adjuvant in depression, Anxious depression, Neurosis Headaches Respiratory viruses, Flu Acute bronchitis Corneal rhinitis Sinusitis Rheumatoid arthritis Infectious eczema Menstrual cramps etc.
Tincture—after filling 1/2 of the jar with basil powder, the rest of volume is filled with 50 °C alcohol. It is left to macerate for 8 days, after which it is filtered and stored in dark bottles to avoid the light.	It is used 2–3 times a week.	
Volatile essential oil—obtained by industrial processes, steam entrainment, etc.	It is used for internal applications, administered 3 drops dissolved in honey or in water, 2–4 times/day (cure of 5–14 days). For children between 8 and 12 years old, the dose is halved, and for those between 5 and 8 years old, 1 drop/2 times a day is usually administered.	
Therapeutic baths—the basil is left to soak in 3–5 L of warm water for 12 h, after which it is filtered. The obtained maceration is set aside, and the remaining plant is scalded with 1 L of water, then left to infuse for 15 min.	Basil baths last 20–30 min, with physical and mental tonic effects, helping the body fight against infections and diseases associated with cold.	

The main objective of the present research was to identify the chemical composition, antioxidant and antimicrobial effects of EOs and FWs extracted from basil. Therefore, we assessed the chemical composition for the essential oils and floral waters obtained from two new varieties of basil (*Occimum basilicum* L., fam. *Lamiaceae*); namely, Yellow basil, the “Aromat de Buzau” variety and Red-violet basil, the “Serafim” variety. The primary three compounds identified in the chemical characterization stage (linalool, estragole, and eugenol) were dosed quantitatively under controlled conditions, in order to test their antimicrobial activity. The quantitative dosage of the three compounds of interest was established by GC-MS analysis, while the evaluation of the antioxidant capacity was carried out by three methods: DPPH, ABTS, and FRAP. The antimicrobial capacity of the two EOs was studied against three phytopathogenic bacteria G- (*Perctobacterium carotovorum*, *Pseudomonas marginalis*, *Pseudomonas syringae*) and three negative bacteria (*Rhizoctonia solani*, *Fusarium oxysporum*, and *Botrytis cinerea*).

2. Materials and Methods

2.1. Plant Materials

The two varieties used in the experiments are part of the Collection of Germplasm Resources of the Buzau Vegetable Research-Development Station. EOs and FWs were produced by processing the plant material collected from the two novel varieties, that were grown under the 2018 growing season agro-meteorological conditions.

Yellow basil (*Ocimum basilicum* L., fam. *Lamiaceae*), “Aromat de Buzau” variety (Certificate no. 1367/2006), semi-late variety, with a well-defined genetic constitution, very well adapted to the climatic conditions existing in Romania (Figure 1a).



Figure 1. Yellow basil (*Ocimum basilicum* L., fam. Lamiaceae), “Aromat de Buzău” variety (a) and Red-violet basil (*Ocimum basilicum* var. *purpurascens* L., fam. Lamiaceae), “Serafim” variety (b).

Red-violet basil (*Ocimum basilicum* var. *purpurascens* L., fam. Lamiaceae), “Serafim” variety (Certificate no. 4432/2017), semi-early variety of violet-purple color, intense, due to the high content of anthocyanins, a variety resistant to specific diseases and pests (Figure 1b).

The plant materials represented by the two new varieties of basil were created by SCDL Buzău, the voucher specimens being submitted to USAMV Bucharest, Faculty of Biotechnology, with registration numbers ‘RO-042’. *O. basilicum*, “Aromat de Buzău” and ‘RO-043’. *O. basilicum*, “Serafim”.

The vegetable raw materials needed for processing have been obtained within INMA Bucharest Institute, Baneasa area (44°30′01″ N; 26°04′19″ E, altitude 90 m), climate: transitional continental temperate, soil: reddish-brown, the average temperature during the growing season varied between 19.4 °C (June) and 21.7 °C (August), and the average value of precipitation: 83.63 mm (with a “peak” of precipitation of 155.9 mm in June, but also a deficit in August (34.2 mm) and September (26.3 mm)). The cultivation respected the specific recommended technological operations (land preparation, planting seedlings, culture maintenance works, harvest). Two harvests/year were obtained, during June to September 2018, and the production varied depending on the climatic conditions, 1100 kg ha^{−1} and respectively 900 kg ha^{−1} of green vegetable raw material.

2.2. EO_S and FW_S Extraction

In order to obtain the oils and floral waters, basil plants were harvested at the time of flowering, cutting inflorescences, young shoot tips and leaves. Harvesting was carried out mechanically, using a specialized equipment for cutting medicinal plants. The cutting height of the plants was made at 25 cm from the top, and the hydrodistillation was performed within 24 h from harvesting, without prior shredding or washing. Hydrodistillation was used as an extraction method, in series of 10 kg of green plants, for a distillation time of 2.5 h/series. The installation used to obtain EOs and FWs was the Aura Distillateur Equipment, 130 L tank capacity, equipped with an electric steam generator MA 18—15 Kw, 0.1 bar steam, constructed of stainless steel. The total volume of water used in the hydrodistillation process was 12.5 L/batch. The obtained EOs and FWs were stored in dark bottles and remained at 4 °C until their use (for GC-MS analysis, antioxidant and antimicrobial activity assays).

The amount of oil obtained from vegetable material was calculated using Equation (1):

$$\text{Oil (\%v/w wet base)} = \frac{\text{Observed volume of oil (mL)}}{\text{Weight of sample (g)}} \times 100 \quad (1)$$

2.3. EO_S and FW_S Analysis

The chemical composition and concentration of the compounds was determined by gas chromatography, coupled to mass spectrometry (GC-MS). The equipment used was a 7890 A-Agilent Technologies gas chromatograph coupled to 5975 C Mass Selective detector MS produced by Agilent Technologies, California, USA and Macrogol Column 20,000 R (30 m × 0.25 mm ID, Bonded 0.50 µm). The carrier gas was helium with a flow rate of 1.5 mL/min; temperature regime of −250 °C (10 degrees/min) up to 280 °C (const. 5.5 min); injector temperature was set to 220 °C; detector temperature 235 °C; mobile phase 1 mL/min; split injector (split ratio—1:100); automatic injection system for the sample; volume used for the analysis: 1 mL of essential oil. Prior to injection, EO was dissolved 100 times in n-Hexane, and 15 mL of undiluted FW was extracted into 10 mL of n-Hexane, then dried over anhydrous sodium sulfate R. The standards used were linalool (97% purity), estragole (98% purity), and eugenol (99% purity), purchased from Sigma-Aldrich. Reference solutions were prepared as follows: linalool: 0.1; 0.2; 0.5; 1.0; 2.0 mg/mL; estragole: 0.1; 0.5; 1.0; 2.0; 4.0 mg/mL; eugenol: 0.1; 0.5; 1.0; 2.0 mg/mL. Scan range used during the GC-MS analysis was established in the interval of 0–70 min. One sample was analyzed for each EO and FW. Using the retention times and spectra of the reference solutions, the compounds were located in the chromatograms for each testing probe. The individual constituents were identified by their similar retention indices, referring to compounds found in literature [27]. Identification was based on standard library Wiley Registry 10th Edition/NIST 2014.

2.4. Analysis of EO_S and FW_S Antioxidant Activity

The use of three assessment methods provides a better overview of the antioxidant activity of EOs and FW_S of basil. The fundamental principles of each method and their complementarity enable a more accurate assessment of the chemicals.

- A. The scavenger activity of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is based on the ability of antioxidants to reduce the DPPH radical. The percentage of DPPH remaining in the solution is calculated according to Equation (2):

$$\%DPPH = \frac{A_{\text{control sample}} - A_{\text{sample}}}{A_{\text{control sample}}} \times 100 \quad (2)$$

where $A_{\text{control sample}}$ is the absorbance of the control sample and A_{sample} is the absorbance of the sample.

The number of samples required to reduce DPPH absorbance by 50% is called IC₅₀. All samples were used in triplicate to determine IC₅₀, 5 concentrations being tested for each sample.

- B. The scavenger activity of the ABTS radical (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid))—the method is known as Trolox equivalent antioxidant capacity (TEAC) since the expression of the antioxidant capacity of the extracts can be associated with Trolox equivalents. The antioxidant capacity was expressed in mM Trolox using 3 calibration curves of the Trolox standard.
- C. Ferric ion Reducing Antioxidant Power (FRAP)—is based on the ability of antioxidants to reduce the yellow-colored tripyridyltriazine—Fe³⁺ (Fe(III)-TPTZ) complex to the blue-colored tripyridyltriazine—Fe²⁺ (Fe(II)-TPTZ) complex, by the action of electron release by antioxidants. The samples were worked in triplicate, and the FRAP values of each sample were expressed in mM Trolox g^{−1} for EOs and L^{−1} h for FWs, respectively.

2.5. Microbial Strains and Growth Conditions

The three strains of Gram-negative bacteria were *Perctobacterium carotovorum* (sin. *Erwinia carotovora*), *Pseudomonas marginalis*, and *Pseudomonas syringae*. All strains are natural isolates with high plant pathogenic activity. The bacterial strains were provided by the Research and Development Institute for Plant Protection, Bucharest, Romania.

Pseudomonas marginalis is a bacterium with phytopathogenic potential for vegetable plants [28,29] and some ornamental plants. The strain can produce effects during vegetation, after harvest, or during storage [30,31].

Pseudomonas syringae (LMG5090) is a phytopathogenic bacterium with an extremely varied host range. Due to this aspect, the species are used as a model organism in numerous studies [32], in order to understand the pathogenicity mechanisms encountered by the bacteria. Over 50 pathological varieties have been identified for these bacterial species, these pathovars can infect almost all plants of economic interest. Bacterial inoculum was obtained from fresh cultures, prepared in Luria Bertani (LB) broth at 28 °C, under orbital shaking at 150 rpm. For this bacterium, three compounds (linalool, estragole, eugenol) were tested using the same protocol, using high purity substances.

Pectobacterium carotovorum (sin. *Erwinia carotovora*) infects many vegetable plants, such as carrots, onions, potatoes, tomatoes, lettuce, etc., but also decorative plants, such as tulips, irises, calla lilies, etc. [33,34]. Bacterial inoculum was obtained from fresh cultures, prepared in LB broth at 28 °C, under orbital shaking at 150 rpm.

Other three strains of fungal phytopathogens, *Rhizoctonia solani* (DSM63002), *Fusarium oxysporum* (ZUM 2407), and *Botrytis cinerea* (natural isolate) are able to produce high economic losses in agriculture. *Rhizoctonia solani* is a cosmopolitan soil born fungi with a large spectrum of host plants. *Fusarium oxysporum* comprises more than 120 special forms and resistance breeds, and most of them are pathogenic to plants of agricultural and horticultural interest, with a very wide range of host plants. Sometimes it can also develop saprophytically, on plant debris and in the soil, or as an asymptomatic endophyte, harmless to the host [35]. The fungal inoculum was prepared as mycelia plugs, 6 mm in diameter, collected from 14-day-old cultures obtained on Potato-Dextrose-Agar. *Botrytis cinerea* is responsible for the gray mold, which can infect over 200 plant species. The fungal inoculum was prepared as mycelia plugs, 8 mm in diameter, collected from 14-day-old cultures obtained on Potato-Dextrose-Agar.

2.6. Evaluation of Essential Oils Emulsions

For antimicrobial assays, the EOs were tested for the following 4 concentrations: $C_1 = 100\%$, $C_2 = 75\%$, $C_3 = 50\%$, and $C_4 = 25\%$. Emulsions were prepared in 10% DMSO supplemented with 0.5% Tween 80 [36]. This solvent reveals no influence on the microbial growth. The tests performed for the EOs were undiluted as in $C_1 = 100\%$; in $C_2 = 75\%$, which represents 3/4 ratio (or three quarters of EO and the rest is solvent); in $C_3 = 50\%$, which represents 1/2 ratio (or half of EO and half of solvent); and in $C_4 = 25\%$, which represents 1/4 ratio (one quarter of EO and the rest three quarters of solvent). The solvent was a mixture of 10% DMSO with 0.5% Tween 80 in water.

2.7. Antibacterial Assay

The antibacterial potential of EOs was tested and analyzed in vitro conditions. Non-ventilated, sterile, polypropylene Petri dishes were used in this study. Each plate was filled with 20 mL of LB agar and plated with fresh bacterial suspension (10^8 CFU/mL). EOs were placed equidistantly and spotted (10 µL/spot) four times on each plate. Four replicate plates were prepared for each concentration of the tested oils. Positive controls, without EOs, were also prepared for phytopathogenic bacterium. All plates were sealed with parafilm, incubated at 28 °C. For each pathogen, the following control plates were prepared: a control only with the test microorganism (without the solvent); a control in which the test microorganism was grown toward the solvent (mixture of 10% DMSO with 0.5% Tween 80 in water). The volume of bacterial inoculum consisted in 100 µL of fresh culture per plate of 9 cm in diameter. Biometric measurements were taken after 1 to 24 h and 3 days after inoculation. Similarly, the three commercial compounds (linalool, estragole, and eugenol), used undiluted ($C_1 = 100\%$) or in the proportion of 1/2 ($C_3 = 50\%$) and 1/4 ($C_4 = 25\%$), were tested. Antibacterial activity was estimated based on the clear areas where the pathogen could not colonize the growth substrate.

2.8. Antifungal Assay

The antifungal assay was performed in similar conditions as the previous test. However, to sustain the fungal growth, PDA medium was used. The plates were inoculated in the center with mycelia plugs, 8 mm in diameter. Four sterile paper disks, 5 mm in diameter, were placed equidistantly at 2 cm distance from the fungal inoculum. Each disk was filled with 10 µL of EOs emulsion. Four concentrations were tested, one concentration per plate, each plate in four replicates. Positive controls, without EOs, were also prepared for each plant pathogenic fungi. For each pathogen, the following control plates were prepared: a control only with the test microorganism (without the solvent); a control in which the tested microorganism was grown toward the solvent (mixture of 10% DMSO with 0.5% Tween 80 in water). Tests carried out with the solvent for fungi, showed that the solvent reveals no influence on the microbial growth. An example of evaluating the effect of the solvent on fungi is shown in Figure 2a,b.

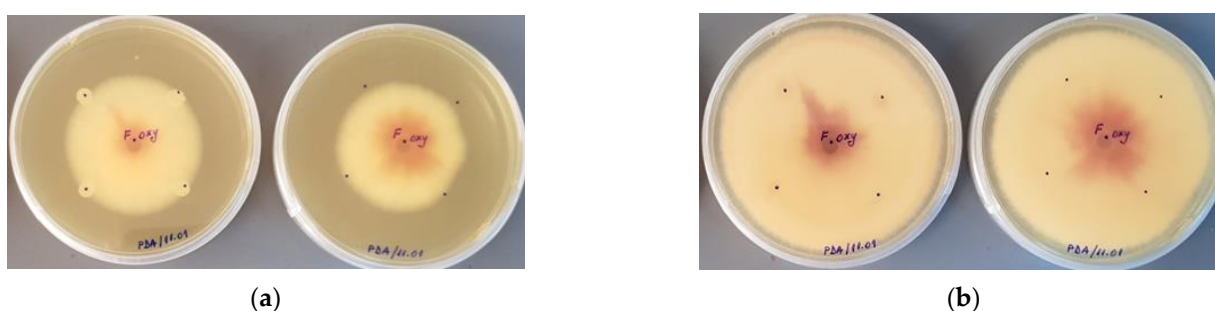


Figure 2. *F. oxysporum* grown after 5 days on PDA (a) and grown after 10 days on PDA (b). In the presence of the solvent 10% DMSO with 0.5% Tween 80 (at left), without the solvent (at right).

Plates were sealed with parafilm, incubated at 26 to 28 °C and analyzed on a daily basis for the first 10 days after inoculation. For bacteria, the incubation was carried out at 28 °C as it is the optimum growth temperature for this species. Fungi were incubated at 26 °C as their growth is more proper at this temperature. Samples were maintained in an incubator, at 26 °C in the case of fungi, only in the first 10 days after inoculation, during this time in the control plates the fungi were able to completely colonize the surface of the growth medium on the entire plate. This is the maximum active growth that can be quantified by biometric measurements. After the 10 days, plates were taken out from the incubator (at constant 26 °C). Subsequently, after 10 days of maintenance at room temperature, the plates were visually analyzed to confirm the antifungal activity. Biometric analyses were carried out to the fungal growth, in order to evaluate the antifungal potential of the EOs.

Fungal inhibition efficacy (E, %), was calculated according to the formula proposed by [37]:

$$E = \frac{R_c - R_T}{R_c} \times 100 \quad (3)$$

where R_c is the radius of the fungal colony in control plates and R_T is the fungal radius in the test plates.

The two EOs were tested in four concentrations (25%, 50%, 75%, and 100%), while the pure substances (linalool, estragole, and eugenol) were tested in three concentrations (25%, 50%, and 100%). Antifungal activity of linalool, estragole, and eugenol was similarly evaluated against *B. cinerea* pathogen. Microbial growth in the control and test plates was also subjected to light microscopy studies, in order to detect potential cells and mycelia abnormalities.

2.9. Statistical Analysis

The purpose of the analysis was to determine the statistical average temporal efficacy for the two EOs, depending on the tested concentrations, against *F. oxysporum* and *B. cinerea*.

The statistical analysis was performed with the Matchcad 2000 program and Excel from MS Office 2007 package. For each reading across time, four repetitions of the essential oils were conducted, and the results were analyzed using simple statistical estimators including arithmetic means, medians, and quartiles.

3. Results

3.1. EOs and FWs Chemical Composition Evaluation with GC-MS

The results showed that EOs are characterized by the presence of linalool in high concentrations (32.66% and respectively 52.18%), when compared to the other compounds. According to some studies [38–41], secondary metabolites of medicinal plants are prone to qualitative and quantitative variations, depending on a number of factors: agrometeorological conditions, season and harvest period, phenological stage of development of the plant at the time of harvesting, etc.

The chemical composition of the EOs of Yellow basil, variety “Aromat de Buzau” and Red-violet basil, variety “Serafim” is shown in Table 2. For both EOs, 16 main compounds were identified of which 13 are common compounds (found in both EOs, but in different percentages), representing 99.65% and respectively 99.99% of the total separated compounds.

Table 2. Chemical composition of the EOs isolated from aerial plant part of the basil (*Ocimum basilicum* L., fam. *Lamiaceae*).

Compound	Molecular Formula	Molecular Weight (g/mol)	RT	EO ₁ *(Area %)	EO ₂ *(Area %)
Pinene	C ₁₀ H ₁₆	136.24	9.68	nd	0.92
Myrcene	C ₁₀ H ₁₆	136.23	13.62	nd	0.95
Cineol	C ₁₀ H ₁₈ O	154.24	15.09	2.63	7.29
Camphor	C ₁₀ H ₁₆ O	152.23	25.28	0.60	nd
Linalool	C ₁₀ H ₁₈ O	154.25	26.32	32.66	52.18
Linalyl acetate	C ₁₂ H ₂₀ O ₂	196.29	26.35	0.45	2.74
Bornyl acetate	C ₁₂ H ₂₀ O ₂	196.28	27.04	0.38	nd
Bergamontene	C ₁₅ H ₂₄	204.33	27.17	nd	1.13
α-Guaiene	C ₁₅ H ₂₄	204.35	27.28	2.64	3.54
Caryophyllene	C ₁₅ H ₂₄	204.36	27.39	1.19	2.87
Estragole	C ₁₀ H ₁₂ O	148.20	29.30	42.71	3.93
Terpineol α	C ₁₀ H ₁₈ O	154.25	29.66	0.40	0.84
Germacrene D	C ₁₅ H ₂₄	204.35	30.05	4.47	4.76
Guaiadiene	C ₁₅ H ₂₄	204.35	30.25	2.18	2.87
Elemene α	C ₁₅ H ₂₄	204.36	30.55	1.15	2.40
Elemene β	C ₁₅ H ₂₄	204.35	31.19	2.00	5.10
Cubenol	C ₁₅ H ₂₆ O	222.37	31.23	2.31	nd
Eugenol	C ₁₀ H ₁₂ O ₂	164.20	39.34	0.81	5.31
Cadinol	C ₁₅ H ₂₆ O	222.37	39.65	3.07	3.16
Total of major compounds	16 compounds identified (13 compounds common for both varieties representing over 99.65% and 99.99%)				
	Classes			EO₁	EO₂
	Monoterpenes hydrocarbons			3.46	11.90
	Monoterpenes oxygenated			76.37	56.95
	Sesquiterpene			19.01	25.83
	Others			0.81	5.31

* EO₁—Essential oil of Yellow basil ‘Aromat de Buzau’ variety; EO₂—Essential oil of Purple basil ‘Serafim’ variety; RT—Retention time; Area—the values were expressed as (area percentage); nd—not detected.

The main compounds identified for EOs (Table 2) from “*Aromat de Buzău*” variety were linalool (32.66%) and estragole (42.71%), while for the “*Serafim*” variety was linalool (52.18%). The variability of the species, the weather parameters specific to year 2018, and the pedological conditions were the major factors influencing the compound variations.

The chemical composition of the FWs of Yellow basil, variety ‘*Aromat de Buzău*’ and Red-violet basil, variety ‘*Serafim*’ is shown in Table 3. A total of 14 compounds were determined for FW₁ and 12 for FW₂, 12 of them being common compounds (found in both FWs although in different percentages), accounting for 98.92% and respectively 99.88%, of the total isolated compounds.

Table 3. Chemical composition of the FWs isolated from aerial part of the basil (*Ocimum basilicum* L., fam. *Lamiaceae*).

Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT	FW ₁ *(Area %)	FW ₂ *(Area %)
Cineol	C ₁₀ H ₁₈ O	154.24	14.27	5.98	10.19
Fenchone	C ₁₀ H ₁₆ O	152.23	21.23	0.13	0.59
Linalool oxide	C ₁₀ H ₁₈ O ₂	170.25	23.84	0.08	0.06
Camphor	C ₁₀ H ₁₆ O	152.23	24.58	1.91	1.24
Linalool	C ₁₀ H ₁₈ O	154.25	25.65	68.08	59.58
α-Guaiene	C ₁₅ H ₂₄	204.35	27.23	0.21	0.45
Phenylacetaldehyde	C ₈ H ₈ O	120.15	28.28	0.08	nd
Estragole	C ₁₀ H ₁₂ O	148.20	28.59	9.54	0.58
Terpineol α	C ₁₀ H ₁₈ O	154.25	29.45	1.53	1.51
Nerol	C ₁₀ H ₁₈ O	154.25	32.80	0.72	0.57
Eugenol	C ₁₀ H ₁₂ O ₂	164.20	39.21	7.73	24.77
Chavicol	C ₉ H ₁₀ O	134.18	41.94	0.75	0.08
Diethyl phthalate	C ₂₄ H ₃₈ O ₄	390.55	43.66	2.02	nd
Cumarina	C ₉ H ₆ O ₂	146.14	44.25	0.16	0.08
Total of major compounds		14 compounds identified (12 compounds common for both varieties representing over 98.92% and 99.70%)			
Classes				FW₁	FW₂
Monoterpenes hydrocarbons				6.19	10.84
Oxygenated monoterpenes				81.78	63.42
Sesquiterpene				0.93	1.22
Others				10.02	24.22

* FW₁—Floral water of Yellow basil ‘*Aromat de Buzău*’ variety; FW₂—Floral water of Purple basil ‘*Serafim*’; RT—Retention time; Area—the values were expressed as (area percentage); nd—not detected.

The antimicrobial effect produced by the main compounds found in the two types of basil was tested afterwards separately. The subsequent dosage of the three compounds of interest (linalool, estragole, and eugenol, Table 4), showed that linalool is the major compound (39.29%; 55.51% for EOs and 13.70%; 0.09% for FWs), followed by estragole (31.48%; 9.78% for EOs and 1.04%; 0.30% for FWs) and eugenol (5.42%; 7.63% for EOs and 0.43%; 0.32% for FWs).

3.2. Antioxidant Activity of the EOs and FWs

The methods used to evaluate the antioxidant activity (DPPH, ABTS, and FRAP) were selected to best cover the different mechanisms of antioxidant activity. These methods can act primarily through hydrogen atom transfer, electron donation, or transition metal chelating ability.

Table 4. Quantitative dosage of the main three compounds (linalool, estragole, eugenol) found in basil extracts.

Compound Name	EO ₁ * (g, %)	EO ₂ * (g, %)	FW ₁ * (g, %)	FW ₂ * (g, %)
Linalool	39.29	55.51	13.70	0.09
Estragole	31.48	9.78	1.04	0.30
Eugenol	5.42	7.63	0.43	0.32

* EO₁—Essential oil of Yellow basil ‘Aromat de Buzău’ variety; EO₂—Essential oil of Purple basil ‘Serafim’ variety; FW₁—Floral water of Yellow basil ‘Aromat de Buzău’ variety; FW₂—Floral water of Purple basil ‘Serafim’.

As illustrated in Table 5, the highest antioxidant capacity obtained using the DPPH method was in the case of EO obtained from Red-violet basil, variety ‘Serafim’ ($0.85 \pm 0.01 \text{ g L}^{-1}$), compared to EO obtained from Yellow basil ‘Aromat de Buzău’ ($15.47 \pm 0.50 \text{ g L}^{-1}$). Therefore, the lower the IC₅₀ value, the higher the antioxidant capacity of the analyzed sample.

Table 5. Antioxidant capacity of EO_S and FW_S obtained from new variety of basil (*Occimum basilicum* L., fam. *Lamiaceae*).

Methods	EO ₁ *	EO ₂ *	FW ₁ *	FW ₂ *
DPPH ^a (IC ₅₀ g L ^{−1})	15.47 ± 0.5	0.85 ± 0.01	893.63 ± 1.51	124.50 ± 0.33
ABTS ^a (mM Trolox g ^{−1})	0.08 ± 0.00	0.20 ± 0.00	0.45 ± 0.01	1.08 ± 0.02
FRAP (mM Trolox g ^{−1})	22.46 ± 0.72	28.45 ± 0.46	0.11 ± 0.00	0.24 ± 0.00

* EO₁—Essential oil of Yellow basil ‘Aromat de Buzău’ variety; EO₂—Essential oil of Purple basil ‘Serafim’ variety; FW₁—Floral water of Yellow basil ‘Aromat de Buzău’ variety; FW₂—Floral water of Purple basil ‘Serafim’; ^a Values are expressed as average $\pm$ SD ($n = 3$).

3.3. Antibacterial Activity of the EOs

The two EOs obtained by hydrodistillation, were tested against the pathogenic G-, *Perctobacterium carotovorum*, *P. marginalis*, and *P. syringae*.

The EOs extracted from both basil varieties expressed wider inhibition areas when applied in C₃ = 50% against the tested bacteria. It is assumed that, at this concentration, the emulsion contained sufficient solvent to ensure a good dispersion of the active ingredient, and sufficient essential oil for bacterial inhibition.

In addition, three pure compounds of linalool, estragole, and eugenol were tested for *P. syringae*.

Tests showed that linalool has bacteriostatic activity only at high concentrations (C₃ = 50% and C₁ = 100%) and the inhibitory activity is maintained only during the first 24 h of incubation (Figure 3). However, in the case of using estragole (Figure 4) and eugenol (Figure 5), the antibacterial activity is observed for all concentrations (25–100%), both during the first 24 h of incubation, and is maintained 3 days after applying the oil.

When tested against *P. carotovorum*, the EOs extracted from ‘Aromat de Buzău’ and ‘Serafim’ varieties, at C₃ = 50%, revealed inhibition areas of 2.3 and 2.13 cm in diameter, after 24 h of incubation. After 7 days of incubation, the inhibition areas decreased to 2.2 and 1.53 cm, respectively. Longer incubation times provided the opportunity for viable bacteria cells to multiply and colonize the area spotted with oil sample, starting from the edge toward the center. However, no colonies developed starting from inside the treated areas. These indicate a bactericidal activity only when the volatile oils are in direct contact with the bacterial cells; otherwise, the effect is bacteriostatic.

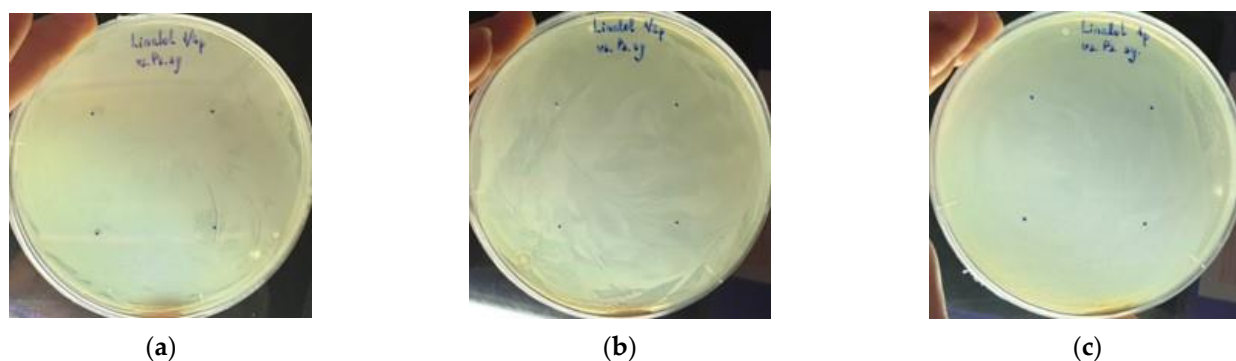


Figure 3. *Pseudomonas syringae* cultures grown in the presence of linalool (after 3 days), in different concentrations. (a) $C_1 = 25\%$; (b) $C_2 = 50\%$; (c) $C_3 = 100\%$.

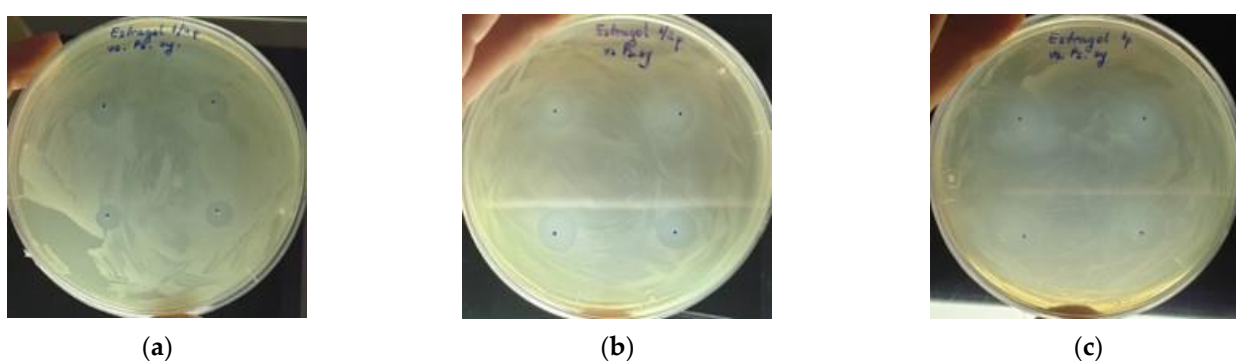


Figure 4. *Pseudomonas syringae* cultures grown in the presence of estragole (after 3 days), in different concentrations. (a) $C_1 = 25\%$; (b) $C_2 = 50\%$; (c) $C_3 = 100\%$.

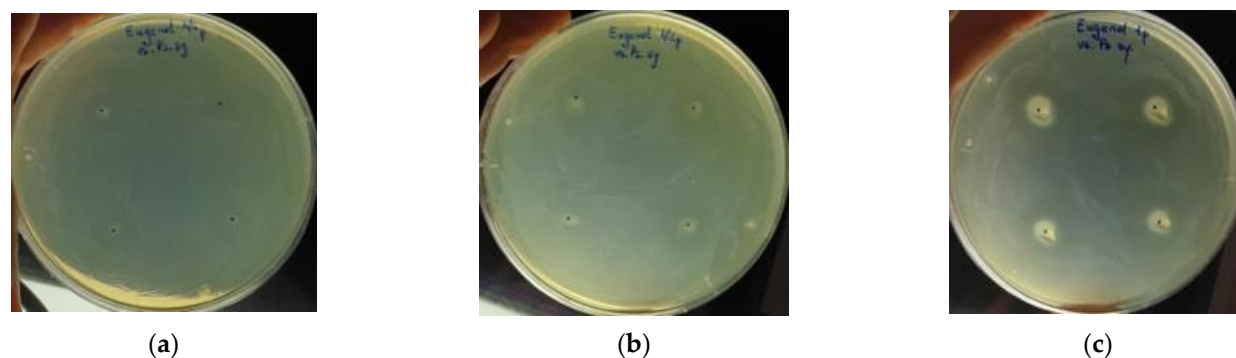


Figure 5. *Pseudomonas syringae* cultures grown in the presence of eugenol (after 3 days), in different concentrations. (a) $C_1 = 25\%$; (b) $C_2 = 50\%$; (c) $C_3 = 100\%$.

Similar analysis carried out against *P. marginalis* showed a bacteriostatic effect of the EOs against this pathogen. This was considered based on the fact that on the initially clear area, with no bacterial growth in the first 24 h, some small bacterial colonies appeared after 7 days of incubation. In the case of undiluted basil oils, the bacterial pigment production was visibly diminished. After 7 days of incubation, the inhibition areas decreased to 0.8 and 0.9 cm, respectively, the effect being bacteriostatic for both EOs tested.

The results obtained on *P. syringae* showed that for EO₁, during the first 24 h of incubation, the results were very good for $C_1 = 100\%$ (0.7 cm) and $C_2 = 75\%$ (0.6 cm), while for $C_3 = 50\%$ and $C_4 = 25\%$ the density of bacterial cells was only reduced on the agar medium. After 3 days of incubation in the presence of EO₁, at $C_4 = 25\%$, the bacteria uniformly colonized the substrate. At the other concentrations tested, although the trace

left by the basil oil spot could be distinguished, the bacteria grew rapidly on the surface of the agar; however, at a lower density than in the rest of the plate. The situation is similar, for undiluted EO₂ (C₁ = 100%) the growth of bacteria was inhibited by 0.8 cm, while for C₂ = 75%, C₃ = 50%, and C₄ = 25% the density of bacterial cells was only reduced on the agar medium in the dissemination areas of the oil spot. After 3 days of incubation in the presence of EO₂, the bacterium grew on the surface of the agar and in the area of the essential oil spot, but at a lower density than in the rest of the plate. In conclusion, more effective bacterial activity of the two EOs have been determined for the two high concentrations (C₁ = 100% and C₂ = 75%), reducing bacterial growth, the effect being bacteriostatic.

3.4. Antifungal Activity of the EOs

The two EOs obtained by hydrodistillation, were tested against the pathogenic fungi, *Rhizoctonia solani* (DSM63002), *Fusarium oxysporum* (ZUM 2407), and *Botrytis cinerea* (naturally isolated). For the case of *B. cinerea*, the three pure compounds (linalool, estragole, and eugenol), were also tested.

Tests performed against *R. solani* showed that after 3 days of incubation, the pathogen developed a slimy exudate around the fungal colonies in the volatile oil treated plates. After 3 days of incubation, the untreated control revealed a complete colonization of the plates with mycelia.

Both basil EOs showed a high antifungal activity (Figure 6). The pathogen started growing in this environment, after 5 days of incubation, only in the plates treated with C₄ = 25% oil in the emulsion, where colonies had a radial growth of less than 0.4 cm in length. After 7 days of incubation, the radial growth of the fungi was 0.8 ± 0.2 cm in length in the plates treated with C₄ = 25% of basil oils, or less than 0.2 cm long in the plates treated with C₃ = 50% oil in the emulsion. After 10 days of incubation, EO₁ expressed 100% efficacy in *R. solani* growth inhibition, when tested as an emulsion of 75% and 100% oil concentration. At a ratio of 50% oil in the emulsion, the EO₁ expressed 94.2% efficacy, while at a ratio of 25% oil concentration, the EO₁ had 70.1% efficacy in inhibition of fungal growth. The EO₂ expressed 98.1% efficacy in fungal growth inhibition, when applied undiluted, and 75.2% efficacy at a ratio of 25% oil concentration in the emulsion.

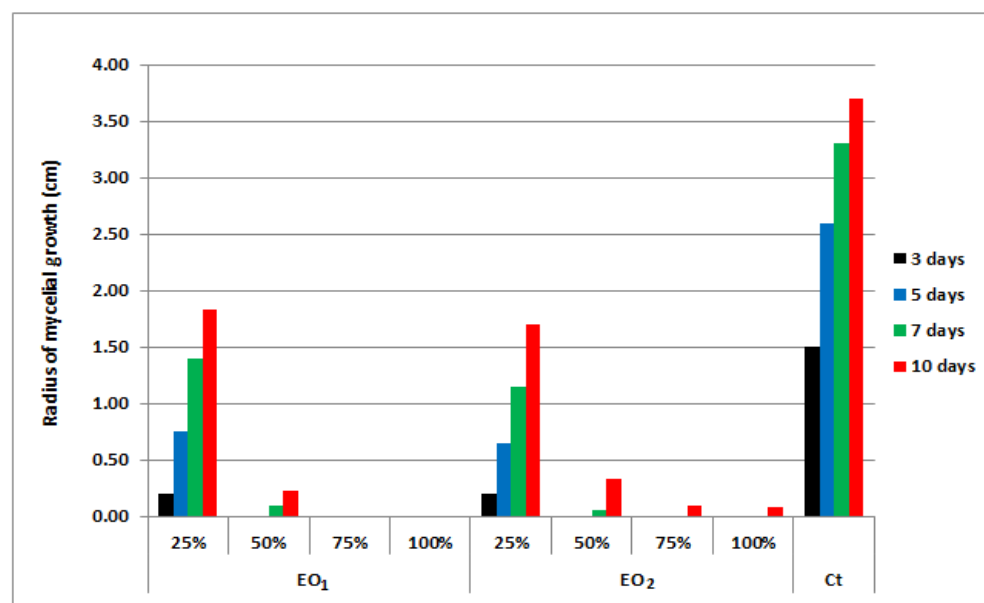


Figure 6. Basil essential oils (EOs) evaluation against the pathogen *R. solani* on CGA medium, tested at different concentrations.

Optical microscopy studies showed several fungal-cell structure alterations in the presence of EOs (Figure 7). Compared to the mycelial growth in the absence of EOs, it is observed that in the presence of EO₁ the density of the mycelial hyphae reduced their slightly zigzag growth (Figure 7a), respectively the occasional lysis of the mycelial filaments (Figure 7b).

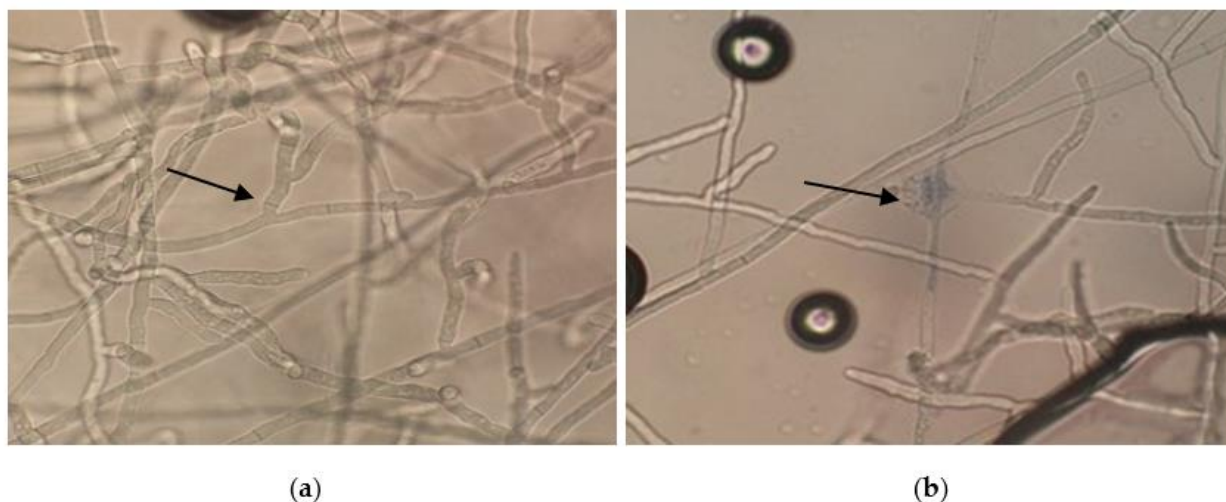


Figure 7. Microscopic aspects of *R. solani* cultures developed in the presence of EO₁, C₄ = 25%; (a) zigzag growth of mycelial hyphae; (b) lysis of mycelial hyphae.

Electron microscopy photos were illustrated only for the most unfavorable scenario (C₄ = 25% concentration) to emphasize the clear effect of EO₁ in breaking the pathogen cells.

The tests performed against *F. oxysporum* (after 10 days of incubation at 27 °C), cultivated in the presence of undiluted EO₁ and EO₂, had mycelial growth inhibited performance by 96.7% and respectively 98.2%, compared to the untreated control. At C₂ = 75%, the two essential oils presented an inhibitory efficacy of 82.4% in the case of EO₁ and respectively 83% in the case of EO₂ (Figure 8). When tested at C₃ = 50%, the two EOs showed a weak inhibition efficacy (below 30%), while at C₄ = 25% the inhibition of mycelial growth after 10 days of incubation was similar to the untreated control sample.

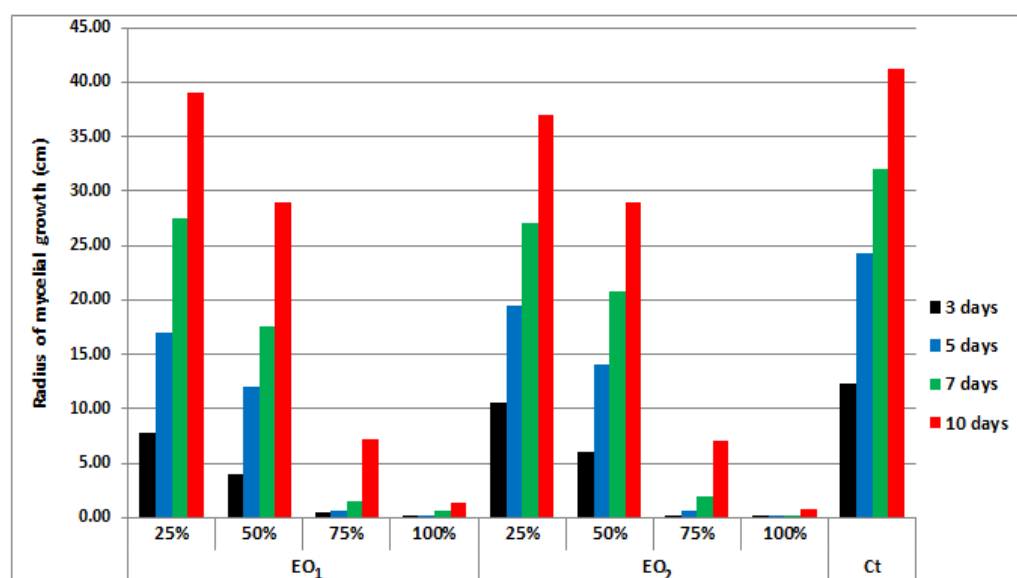


Figure 8. Basil essential oils (EOs) evaluation against the pathogen *F. oxysporum* on CGA medium, tested at different concentrations.

The microscopic evaluation of the mycelial growth of *F. oxysporum*, inhibited with EO₂ did not reveal any notable changes in morphology, only a weaker branching of the mycelium (in the control culture the mycelium is denser), as depicted in Figure 9.



Figure 9. Reduction in the density of *F. oxysporum* mycelium in the presence of EO₂ (a), compared to control sample (b).

Studies carried out against *Botrytis cinerea* showed that basil EOs were more effective in fungal inhibition. In the case of yellow and purple basil, the EOs applied in C₃ = 50% or higher, revealed a decrease in fungal growth rate, and complete fungal inhibition of *B. cinerea* was maintained for at least 10 days of incubation (Figure 10). Optical microscopy studies showed fungal cells lysis and cytoplasm leaching when the mycelia were closely exposed to EO spots (Figure 11).

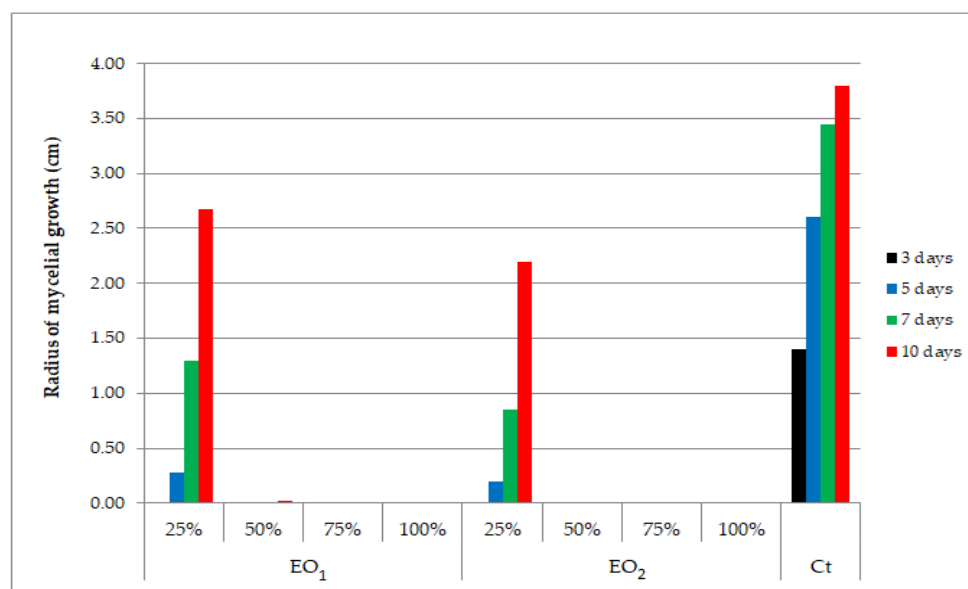


Figure 10. Basil essential oils (EOs) evaluation against the pathogen *B. cinerea* on CGA medium, tested at different concentrations.



Figure 11. Fungal cell lysis of *Botrytis cinerea* when exposed to EO₂ basil at C₄ = 25% in the emulsion (the arrow indicates cytoplasm leaks).

Among the three volatile compounds tested (Table 6), eugenol completely inhibited fungal growth in all tested concentrations (25% and higher). As the complete inhibition was maintained not only in the first 10 days of cultivation, but also after a prolonged incubation, it can be concluded that eugenol has a fungicidal effect on the gray mold. Complete fungal growth inhibition was obtained also when using linalool in 50% and higher concentrations. Although, at C₄ = 25% and 40 µL dose/plate, linalool revealed only 12.17% fungal inhibition efficacy. However, estragole revealed a poor antifungal effect, of 25.99% inhibition efficacy, even when tested undiluted, in 40 µL dose/plate.

Table 6. Fungal growth inhibition efficacy of compounds against phytopathogenic *Botrytis cinerea*.

Compound	Conc * %	Mycelial Growth Rate (cm)				E ** %
		3 Days	5 Days	7 Days	10 Days	
Control sample	-	1.40	2.60	3.45	3.80	-
	100	0	0	0	0	100
Linalool	50	0	0	0	0	100
	25	0	0.45	1.57	3.34	12.17
	100	0	0.40	1.41	2.81	25.99
Estragole	50	0	1.11	2.05	3.53	13.82
	25	0.56	1.72	2.67	3.75	1.32
	100	0	0	0	0	100
Eugenol	50	0	0	0	0	100
	25	0	0	0	0	100

* Concentration in tested emulsion; ** The effectiveness of the performed treatments.

4. Discussion

4.1. Chemical Composition of the Essential Oils (EOs) and Floral Waters (FWs), Obtained from the Two New Varieties of Basil

The main classes of compounds identified for 'Aromat de Buzau' in the EO were oxygenated monoterpenes (76.37%) followed by sesquiterpenes (19.01%). In the EO obtained from the variety 'Serafim', the concentrations varied significantly oxygenated monoterpenes with lower rates (56.95%), sesquiterpenes (25.83%), and hydrogenated monoterpenes (11.90%). Linalool is the major compound for both EOs, varying quantitatively, from 39.29% for EO₁ to 55.51% for EO₂. The next component identified according to the percentage quantity was estragole, that showed large variations, from 31.48% for EO₁ to 9.78% for EO₂. The eugenol compound was identified in percentage of 5.42% for EO₁ and 7.63% for

EO₂. The differences in concentrations recorded when dosing the three compounds and the percentage increase in other minor compounds can potentiate the antioxidant activity of EOs [42]. In many cases, the major compound cannot be confirmed as the only one responsible for the biological activity of the EOs, since this phenomenon is the result of the interaction between the different compounds.

Similar research [42,43] indicate that different *Ocimum* species (including violaceus) have different chemical compositions and component percentages according to their geographic origins, cultivation methods, cultivars, etc. As an example, cultivars of basil violaceus from northern Brazil contain linalool (39.3%) and mururol (11.0%), while the species from Bangladesh [44] contain mainly methyl-cinnamate (59.95%), linalool (16.40%), and cadinal (4.37%). Purple basil cultivars found in Iran [45] include methyl chavicol (52.40%), linalool (20.10%), and epi-cadinol (5.90%). In research on *O. basilicum* var. *purpureum* carried out in Nigeria [46], GC-MS analysis revealed the presence of 25 chemicals representing 99.7% of the of the total oil composition. The most abundant compounds were methyleugenol (15.5%), 2-phenyl-1-hexanol (14.0%), 1-(4,5-dimethyl-2-nitrophenyl)-1-H-tetrazole (14.0%), 2-methyl-3,5-dodecadiene (14.0%), o-nitrocumene (14.0%), and patchoulol (6.7%).

In a study conducted on EOs obtained from two varieties of basil cultivated in Yemen (var. *basilicum* and var. *purpurascens*), [47] twenty-three compounds were identified in violet variety and thirty-one compounds in green variety. Linalool (44.37%; 46.24%), estragole (20.05%; 13.26%), trans-methyl cinnamate (15.05%; 0.45%), 1,8-cineole (9.28%; 3.28%), and epi- α -cadinol (1.38%; 3.10%) were the main compounds common to both EOs. In the study conducted in Serbia [48] on EOs obtained from three sweet basil cultivars, the main classes of compounds identified were sesquiterpenes (38.39% and 37.95%), oxygenated monoterpenes (25.44% and 28.04%), and phenylpropanoids (17.43% and 15.71%). The main constituents of the EOs were linalool (13.68% and 15.38%), eugenol (10.83% and 8.97%), and α -bergamotene (8.12% and 9.25%).

In a study carried out in 2009 [49] on sweet basil grown in Romania, between 11 and 19 compounds have been identified, of which linalool was the main compound with 46.95% of the total identified compounds. Comparing the composition of the analyzed EOs and FWs, both quantitative and qualitative differences were identified. The results obtained were generally different from those in the specialized literature, regarding the main compounds [49,50]. The observed differences could be the result of different environmental factors, genetics, the different chemotypes used, but also a number of other factors that can influence the composition of the oil.

The results obtained for both EOs and FWs showed a variation in both the number and the amount of compounds (linalool, estragole, and eugenol) showing important quantitative variations, both for EOs and FWs. The differences are caused by the variability of the two cultivars and the climatic conditions of 2018 (high temperatures during the vegetation period of the plants, water shortage in the summer months).

4.2. Antioxidant Potential Assessment for Basil EOs and FWs

The highest antioxidant potential obtained by the *DPPH method* was of the EO obtained from Red-violet basil, the 'Serafim' variety ($0.85 \pm 0.01 \text{ g L}^{-1}$), compared to the EO obtained from the Yellow basil, the 'Aromat de Buzau' variety ($15.47 \pm 0.50 \text{ g L}^{-1}$). Therefore, the lower the IC₅₀ value, the higher the antioxidant capacity of the analyzed sample.

The obtained values were found to be similar to the data previously reported in various publications. D. Beatović reports in the study carried out on various varieties of basil, IC₅₀ values between 0.03 and $6.8 \mu\text{g mL}^{-1}$ [51]. The IC₅₀ values obtained for the EOs extracted from the new varieties of basil demonstrate a high antioxidant capacity. The values obtained are higher than those reported in other studies evaluating *Ocimum basilicum* L., with IC₅₀ values ranging from 3.6–8.1 mg/mL [30] and IC₅₀ = $1.09 \pm 0.066 \text{ mg/mL}$ [52].

In the case of ABTS testing method, the highest antioxidant capacity was also recorded for EO₂ with a value of 0.20 ± 0.00 mM Trolox g⁻¹. In addition, EO₁ registered a good antioxidant activity by this method, with a value of 0.08 ± 0.00 mM Trolox g⁻¹.

In the case of testing the antioxidant activity of the two EOs by the FRAP method, it was seen that the EO₂ had the highest value (28.45 ± 0.46 mM Trolox g⁻¹) compared to EO₁ (22.46 ± 0.72 mM Trolox g⁻¹).

The variation of climatic conditions during the vegetation period of the plants significantly influenced the chemical composition of the studied EOs and FWs, and implicitly the decrease or increase in the antioxidant activity. However, combinations of compounds provide information regarding the interactions between them and help in developing more effective formulations. Different interactions between major compounds can lead to synergistic, additive, or antagonistic effects. The activity that results from these combinations may be higher/lower or equal to the results observed by comparison with a pure compound [53].

In some recent studies [14], volatile compounds have been shown to possess important antioxidant activity. Ref. [16] showed that eugenol (major compound in basil EOs) had the highest antioxidant potential. However, despite the influence of this compound, the strongest antioxidant activity of basil EOs is related to the synergy between other minor compounds. Ref. [54] investigated the antioxidant activity determined by the DPPH method and showed that a series of free volatile compounds shows good antioxidant properties, comparable to those of the essential oil, or the synthetic antioxidant BTH (butylated hydroxytoluene), but less than pure eugenol. The antioxidant activity of eugenol has been reported in previous studies, however, it is related to a limit value [55]. The Food and Drug Administration (FDA) states that the use of eugenol in food is considered safe only if administered within certain established limits, indicating that exceeding it can cause health problems and even be fatal [56].

4.3. Antibacterial Potential Assessment of Basil EOs

The evaluations conducted against the three pathogenic bacteria showed that *P. carotovorum* was more susceptible to be controlled by the tested EOs than *P. marginalis* and *P. syringae*.

EOs contain various active compounds, which can disturb multiple targets in bacterial cells [57]. One of the most important parts is the cytoplasmic membrane. Some compounds found in the volatile oils are increasing cell membrane permeability, which leads to the cell viability loss. This is mostly associated with ionic homeostasis and electron chain transport [58]. For example, carvacrol, thymol, and eugenol decrease the intracellular potassium level, and increase the extracellular level [59]. Another characteristic of the EOs is their lipophilic nature, and ability to access the periplasm of bacterial cells. The interaction with polysaccharides, fatty acids, and phospholipids makes bacterial membranes more permeable for the volatile oils, in order that the loss of ions and cell content leads to cell death [60]. Other important mechanisms of action include denaturation of cytoplasmic proteins and inactivation of cellular enzymes, which lead to bacterial cells death [61]. In general, alcohol, aldehydes, and phenolic constituents are responsible for the cytotoxicity of EOs [62]. These properties are important for EO applications against various viruses, bacteria, and fungi [57,63].

4.4. Antifungal Potential Assessment of Basil EOs

The preliminary tests carried out on the three phytopathogenic fungi, showed that for *F. oxysporum* there is a high probability to be controlled by the tested EOs. Regarding *R. solani* and *B. cinerea*, the effect of the two EOs is fungicidal starting from C₃ = 50%.

Similar studies [64] show that the germination percentage of *F. oxysporum* and *P. infestans* decreases with the increasing concentration of EOs composed of *C. citrinus*, *C. citratus*, *E. tereticornis*, and *O. gratissimum*. In this study, it is shown that the inhibition percentage of mycelial growth of the two fungi increases with the concentration used. The increase in the inhibitory effect of some EOs extracted from basil (*Ocimum basilicum* L.) in controlling the

fungus *F. oxysporum* also clearly validated the present paper main findings from the data presented by [65]. Similar conclusions were obtained by [66], for controlling the fungus *F. oxysporum*. In the study carried out by [67], a series of different basil EOs were evaluated against the phytopathogenic fungi *F. oxysporum* sp. *cicer* and *A. porri*. The results showed that basil EOs with the maximum inhibition rate were 17.25 mm.

The results confirm the output of other studies [68,69], which demonstrated that in *O. basilicum* chemotypes rich in estragole (1000 ppm) and linalool (300 ppm), the mycelial growth of *B. fabae* is reduced to 78–49%.

Several mechanisms have been stated regarding fungal growth inhibition due to the volatile compounds. Ref. [70] indicated that mycelia growth inhibition is caused by the monoterpenes present in the essential oils. These components could increase the amount of lipid peroxides inside the cells, such as hydroxyl, alkoxyl, and alcoperoxyl radicals, thus causing cell death. It has been shown that in the presence of EOs, the fungal cell wall is thinner, mycelia are distorted, cytoplasm is lost from the cells, and cell lysis occurs [71]. Although EOs obtained from plants have antimicrobial activity, the mechanisms of action are not completely elucidated. The antimicrobial activity could be caused by a major oil compound or due to a synergistic effect between major and other minor compounds [72,73].

4.5. Statistical Analysis of Antifungal Activity Results

The analysis corresponds to the populations formed by the values of the growth radius of the colony, measured during the experiment. The series of four repetitions performed for each reading over time, and for each of the two essential oils (with four concentrations) tested and the control variant, were studied by elementary statistical estimators: arithmetic means, median, quartiles.

Figure 12a,b shows that the weighted average efficiency over time increases with the concentration of EO₁ used to control the phytopathogenic fungus *F. oxysporum*. The shape of the curve in Figure 12 suggests that from C = 80%, the increases are small and negligible, compared to the increases corresponding to concentrations between 25% and 80%.

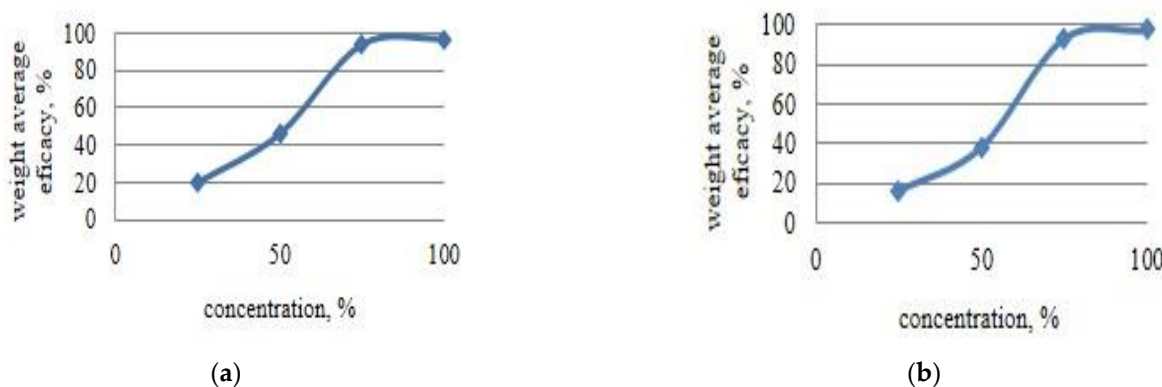


Figure 12. The dependence of the average temporal efficacy on the concentration, for EO₁ (a) and EO₂ (b) against *F. oxysporum*.

In conclusion, intensity of the process of controlling the phytopathogenic fungus *F. oxysporum* using two essential oils obtained from new varieties of medicinal plants, depends on two essential variables: the time and the concentration used in the control process.

The growth variation of *B. cinerea* and the effectiveness of the treatments carried out with the two basil EOs are shown in Figure 13a,b, where the average growths of the phytopathogen are represented as a function of four concentrations and the control sample. Each marked point on the figure corresponds to an average value for the four repetitions of the experiment. The variation of the effectiveness of the performed treatments (calculated according to Equation (3), depends on the time (period of 3–10 days).

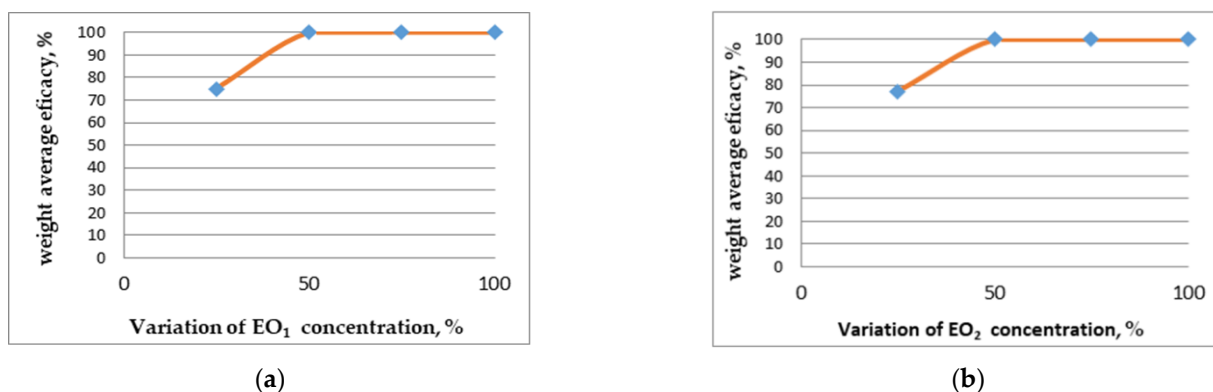


Figure 13. The dependence of the average temporal efficacy on the concentration, for EO₁ (a) and EO₂ (b) against *B. cinerea*.

Figure 13a,b shows that the time-weighted average efficiency increases with the concentration of the two EOs used to control the phytopathogenic fungus *B. cinerea*. The shape of the two curves in the figure suggests that at $C > 60\%$, the action of both EOs becomes fungicidal.

In addition, for *B. cinerea*, different concentrations for the pure compounds (linalool, estragole, eugenol) were comparatively tested, and the results are shown in Figure 14.

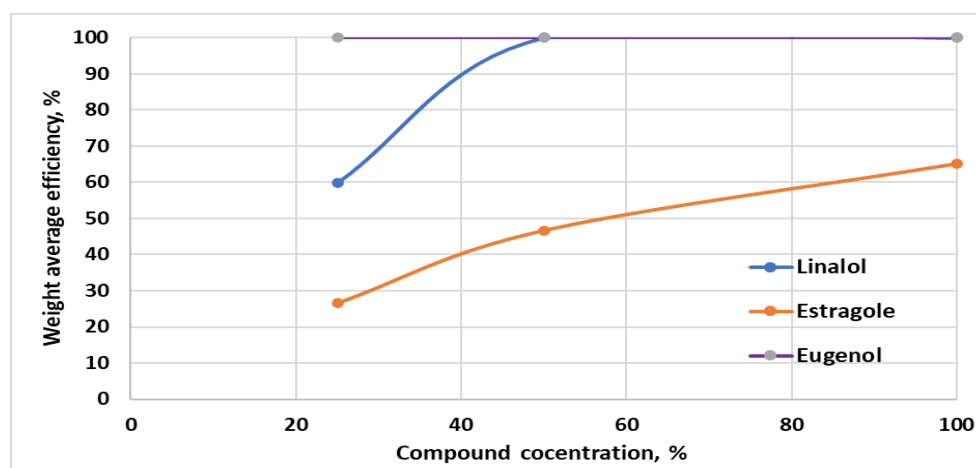


Figure 14. The dependence of the average temporal efficacy on the concentration, for linalool, estragole, and eugenol, on the *B. cinerea*.

The three chemicals (linalool, estragole, and eugenol) increase in effectiveness with concentration, as in the case of the two EOs. In terms of fungistatic potential, among the three tested compounds, linalool is the second most effective, considering that for concentrations exceeding 50% the effectiveness is 100% over the entire period of the experiments.

For estragole, the effectiveness increases with the concentration, and over time, it decreases with the fungus resuming its growth. As a fungistatic potential, for control, its action appears to be exclusively fungistatic.

The strongest antifungal effect on the phytopathogenic fungus *B. cinerea* is shown by eugenol solutions. Tested solutions maintain their constant 100% efficacy for all concentrations and throughout the testing period. The behavior of eugenol makes this substance the most effective of the three tested, and shows a fungicidal effect.

5. Conclusions

The novelty of this study is represented by the new two varieties of basil ('*Aromat de Buzău*' and '*Serafim*') which belong to different varieties (Yellow basil and respectively Red-violet), which were evaluated for the first time regarding their chemical composition, antioxidant capacity, and antimicrobial effects.

The two varieties are notable for their high content in linalool, estragole, and eugenol. Therefore, the variety '*Aromat de Buzău*' can be classified in the linalool and estragole-linalool chemotypes, while the variety '*Serafim*' can be classified in the linalool and linalool-eugenol chemotypes. Moreover, the complexity of compounds and classes of compounds identified in the two EOs and FWs is correlated with their high antioxidant activity. The two EOs show significant antimicrobial activity, with possible fungicidal effect on *Rizoctonia solani* and *Botrytis cinerea* strains at concentrations higher than 50%. It should be mentioned that the fungicidal effect on *Botrytis cinerea* for linalool starts at C > 50% and eugenol starts at C > 25%.

The results obtained are promising, and the deepening of these studies is needed, in order to use the two EOs (single or in combination) in the practice of ecological agriculture, and formulate effective products in the fight against specific pathogens.

Author Contributions: Conceptualization, C.S.; methodology, F.B.; software, C.S. and F.N.; validation, V.N.V., F.B. and F.I.-R.; formal analysis, C.S.; investigation, C.P., F.G., F.N. and O.A.B.-S.; resources, A.M.; writing—original draft preparation, C.S.; writing—review and editing, F.N.; visualization, V.N.V.; supervision, F.I.-R.; project administration, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by the NUCLEU Programme, carried out with the support of ANCSI, Project PN 5N/07.02.2019 "Research on the superior valorization of some new plant species cultivated in Romania". This work was supported by the Ministry of Research, Innovation and Digitalization through Program 1-Development of the national research-development system, Subprogram 1.2.—Institutional performance-Projects for financing excellence in RDI, Contract no. 1PFE/30.12.2021. This paper was also supported by a grant offered by the Romanian Minister of Research as Intermediate Body for the Competitiveness Operational Program 2014–2020, call POC/78/1/2/, project number SMIS2014 + 136213, acronym METROFOOD-RO.

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

Abbreviations

Medicinal and aromatic plants (MAPs); essential oils (EOs); floral waters (FW_s); EO₁—Essential oil of Yellow basil '*Aromat de Buzău*' variety; EO₂—Essential oil of Purple basil '*Serafim*' variety; FW₁—Floral water of Yellow basil '*Aromat de Buzău*' variety; FW₂—Floral water of Purple basil '*Serafim*'.

References

1. Dresselhaus, T.; Hückelhoven, R. Biotic and abiotic stress responses in crop plants. *Agronomy* **2018**, *8*, 267. [\[CrossRef\]](#)
2. Salvi, P.; Agarrwal, R.; Kajal, Gandass, N.; Manna, M.; Kaur, H.; Deshmukh, R. Sugar transporters and their molecular tradeoffs during abiotic stress responses in plants. *Physiol. Plant* **2022**, *174*, e13652. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Mircea, C.; Nenciu, F.; Vlăduț, V.; Voicu, G.; Cujbescu, D.; Gageanu, I.; Vocea, I. Increasing the performance of cylindrical separators for cereal cleaning, by using an inner helical coil. *INMATEH Agric. Eng.* **2020**, *62*, 249–258. [\[CrossRef\]](#)
4. Nenciu, F.; Vocea, I.; Cocarta, D.M.; Vladut, V.N.; Matache, M.G.; Arsenoaia, V.-N. "Zero-Waste" Food Production System Supporting the Synergic Interaction between Aquaculture and Horticulture. *Sustainability* **2022**, *14*, 13396. [\[CrossRef\]](#)
5. Al-Ahmadi, M.S. Pesticides, anthropogenic activities, and the health of our environment safety. In *Pesticides-Use and Misuse and Their Impact in the Environment*; Larramendy, M., Soloneski, S., Eds.; IntechOpen: London, UK, 2019; pp. 1–23.

6. Duran-Lara, E.F.; Valderrama, A.; Marican, A. Natural organic compounds for application in organic farming. *Agriculture* **2020**, *10*, 41. [CrossRef]
7. Nenciu, F.; Oprescu, M.R.; Biris, S.-S. Improve the Constructive Design of a Furrow Diking Rotor Aimed at Increasing Water Consumption Efficiency in Sunflower Farming Systems. *Agriculture* **2022**, *12*, 846. [CrossRef]
8. Nenciu, F.; Paraschiv, M.; Kuncser, R.; Stan, C.; Cocarta, D.; Vladut, V.N. High-Grade Chemicals and Biofuels Produced from Marginal Lands Using an Integrated Approach of Alcoholic Fermentation and Pyrolysis of Sweet Sorghum Biomass Residues. *Sustainability* **2022**, *14*, 402. [CrossRef]
9. Simon, J.E.; Morales, M.R.; Phippen, W.P.; Vieira, R.F.; Hao, Z. *Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb*; Janick, J., Ed.; ASHS Press: Alexandria, Egypt, 1999.
10. Lee, S.J.; Umamo, K.; Shibamoto, T.; Lee, K.G. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food Chem.* **2005**, *91*, 131–137. [CrossRef]
11. Telci, I.; Bayram, E.; Yilmaz, G.; Avci, B. Variability in essential oil composition of Turkish basil (*Ocimum basilicum* L.). *Biochem. System. Ecol.* **2006**, *34*, 489–497. [CrossRef]
12. Technavio. Available online: <https://www.technavio.com/> (accessed on 27 August 2022).
13. Sestili, P.; Ismail, T.; Calcabrini, C.; Guescini, M.; Catanzaro, E.; Turrini, E.; Layla, A.; Akhtar, S.; Fimognari, C. The potential effects of *Ocimum basilicum* on health: A review of pharmacological and toxicological studies. *Expert Opin. Drug Metab. Toxicol.* **2018**, *14*, 679–692. [CrossRef]
14. Antonescu, A.-I.; Miere, F.; Fritea, L.; Ganea, M.; Zdrinca, M.; Dobjanski, L.; Antonescu, A.; Vicas, S.I.; Bodog, F.; Sindhu, R.K.; et al. Perspectives on the combined effects of *Ocimum basilicum* and *Trifolium pratense* extracts in terms of phytochemical profile and pharmacological effects. *Plants* **2021**, *10*, 1390. [CrossRef]
15. Srivastava, A.; Gupta, A.K.; Sarkar, S.; Lal, R.K.; Yadav, A.; Gupta, P.; Chanotiya, C.S. Genetic and chemotypic variability in basil (*Ocimum basilicum* L.) germplasm towards future exploitation. *Ind. Crops Prod.* **2018**, *112*, 815–820. [CrossRef]
16. De Araújo Couto, H.G.S.; Blank, A.F.; de Oliveira e Silva, A.M.; de Lima Nogueira, P.C.; de Fátima Arrigoni-Blank, M.; de Castro Nizio, D.A.; de Oliveira Pinto, J.A. Essential oils of Basil chemotypes: Major compounds, binary mixtures and antioxidant activity. *Food Chem.* **2019**, *293*, 446–454. [CrossRef]
17. Bayala, B.; Bassole, I.H.N.; Gnoula, C.; Nebie, R.; Yonli, A.; Morel, L.; Figueredo, G.; Nikiema, J.B.; Lobaccaro, J.M.A.; Simporé, J. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. *PLoS ONE* **2014**, *9*, e92122. [CrossRef]
18. Hanif, M.A.; Al-Maskari, M.Y.; Al-Maskari, A.; Al-Shukaili, A.; Al-Maskari, A.Y.; Al-Sabahi, J.N. Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. *J. Med. Plants Res.* **2011**, *5*, 751–757.
19. Bozin, B.; Mimica, D.; Simin, N.; Anackov, G. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J. Agric. Food Chem.* **2006**, *54*, 1822–1828. [CrossRef]
20. Abbas, A.; Anwar, F.; Ahmad, N. Variation in Physicochemical Composition and Biological Attributes of Common Basil Essential Oils Produced by Hydro-distillation and Super Critical Fluid Extraction. *J. Essent. Oil Bear. Plants* **2017**, *20*, 95–109. [CrossRef]
21. Pieroni, A.; Quave, C.L.; Giusti, M.E.; Papp, N. “We Are Italians!”: The Hybrid Ethnobotany of a Venetian Diaspora in Eastern Romania. *Hum. Ecol.* **2012**, *40*, 435–451. [CrossRef]
22. Dafni, A.; Petanidou, T.; Vallianatou, I.; Kozhuharova, E.; Blanche, C.; Pacini, E.; Peiman, M.; Stevanovic, Z.D.; Franchi, G.G.; Benitez, G. Myrtle, Basil, Rosemary, and Three-Lobed Sage as Ritual Plants in the Monotheistic Religions: An Historical–Ethnobotanical Comparison. *Econ. Bot.* **2020**, *74*, 330–355. [CrossRef]
23. Dajić Stevanović, Z.; Petrović, M.; Ačić, S. Ethnobotanical Knowledge and Traditional Use of Plants in Serbia in Relation to Sustainable Rural Development. In *Ethnobotany and Biocultural Diversities in the Balkans*; Pieroni, A., Quave, C., Eds.; Springer: New York, NY, USA, 2014. [CrossRef]
24. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
25. Boulos, L. *Medicinal Plants of North Africa*; Reference Publications, Inc.: Algonac, MI, USA, 1983.
26. Alexiou, M. *The Ritual Lament in Greek Tradition*, 2nd ed.; Rowman & Littlefield Publishers: Lanham, MD, USA, 2002.
27. Wright, P.J.; Hale, C.N. A field and storage rot of onion caused by *Pseudomonas marginalis*. *N. Zeal. J. Crop Hortic. Sci.* **1992**, *20*, 435–438. [CrossRef]
28. Kúdela, V.; Krejzar, V.; Pánková, I. *Pseudomonas corrugata* and *Pseudomonas marginalis* associated with the collapse of tomato plants in Rockwool slab hydroponic culture. *Plant Protect. Sci.* **2010**, *46*, 1–11. [CrossRef]
29. Krejzar, V.; Mertelík, J.; Pánková, I. *Pseudomonas marginalis* Associated with Soft Rot of *Zantedeschia* spp. *Plant Prot. Sci.* **2008**, *44*, 85–90. [CrossRef]
30. Jinhua, L.; Zhaoxiang, C.; Hetong, Y.; Guoquan, L.; Di, W. First report of *Pseudomonas marginalis* pv. *marginalis* as a cause of soft rot of potato in China. *Australas. Plant Dis. Notes* **2007**, *2*, 71–73.
31. Xin, X.F.; Kvitko, B.; He, S.Y. *Pseudomonas syringae*: What it takes to be a pathogen. *Nature reviews. Microbiology* **2018**, *16*, 316–328. [PubMed]
32. Boyraz, N.; Bastas, K.K.; Maden, S.; Yasar, A. Bacterial leaf and peduncle soft rot caused by *Pectobacterium carotovorum* on tulips in Konya, Turkey. *Phytoparas* **2006**, *34*, 272–280. [CrossRef]
33. Raymond, G.A.T.; Roland, T.V.F. *Diseases of Temperate Horticultural Plants*; Edit. CAB International: New York, NY, USA, 2014; p. 488.

34. Enciclopedia of Life. Available online: <https://eol.org/> (accessed on 19 September 2022).
35. Prabuseenivasan, S.; Jayakumar, M.; Ignacimuthu, S. In vitro antibacterial activity of some plant essential oils. *BMC Comp. Altern. Med.* **2006**, *6*, 39. [CrossRef]
36. Lahlali, R.; Hijri, M. Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS Microbiol. Lett.* **2010**, *311*, 52–159. [CrossRef]
37. MathSoft Incorporation. *Mathcad Reference Manual*; MathSoft Incorporation: Cambridge, MA, USA, 2000.
38. Santana-Rios, G.; Orner, G.A.; Amantana, A.; Provost, C.; Wu, S.Y.; Dashwood, R.H. Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2001**, *495*, 61–74. [CrossRef]
39. Hoet, S.; Stevigny, C.; Herent, M.F.; Quentin-Leclercq, J. Antitrypanosomal compounds from the leaf essential oil of *Strychnos spinosa*. *Planta Med.* **2006**, *72*, 480–482. [CrossRef]
40. Chen, Y.; Zhu, Z.; Guo, Q.; Zhang, L.; Zhang, X. Variation in concentrations of major bioactive compounds in *Prunella vulgaris* L. related to plant parts and phenological stages. *Biol. Res.* **2012**, *45*, 171–175. [CrossRef]
41. Silva, M.G.; Matos, F.J.; Machado, M.I.; Craveiro, A.A. Essential oils of *Ocimum basilicum* L., *Ocimum basilicum* var. *minimum* L. and *Ocimum basilicum* var. *purpurascens* Benth. grown in north-eastern Brazil. *Flavour Frag. J.* **2003**, *18*, 13–14. [CrossRef]
42. Abhay, K.P.; Pooja, S.; Nijendra, N.; Tripathi, I. Chemistry and bioactivities of essential oils of some *Ocimum* species: An overview. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 682–694.
43. Mohhiuddin, M.; Chowdhury, M.J.; Alam, M.K.; Hossain, M.K. Chemical composition of essential oil of four flavouring plants used by the tribal people of Bandarban hill district in Bangladesh. *Int. J. Med. Arom. Plants* **2012**, *2*, 106–113.
44. Sajjadi, S.E. Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. *DARU J. Pharm. Sci.* **2006**, *14*, 128–130.
45. Ololande, Z.S.; Fakankun, O.A.; Alao, F.O.; Udi, O.U. *Ocimum basilicum* var. *purpureum* floral essential oil: Phytochemicals, phenolic content, antioxidant, free radical scavenging and antimicrobial potentials. *Glob. J. Sci. Front. Res. B Chem.* **2014**, *14*, 30–37.
46. Ahmed, H.A.; Hussein, K.; Alsyari, H.A.; Ibrahim, M.H.; Altanobi, L. Chemotaxonomy and spectral analysis (GC/MS and FT-IR) of essential oil composition of two *Ocimum basilicum* L. varieties and their morphological characterization. *Jordan J. Chem.* **2017**, *12*, 147–160.
47. Ilić, A.S.; Antić, M.P.; Jelačić, S.C.; Šolević Knudsen, T.M. Chemical composition of the essential oils of three *Ocimum basilicum* L. cultivars from Serbia. *Not. Bot. Horti Agrobot.* **2019**, *47*, 347–351. [CrossRef]
48. Benedec, D.; Oniga, I.; Oprean, P.; Tamas, M. Chemical composition of the essential oils of *Ocimum basilicum* L. cultivated in Romania. *Farmacia* **2009**, *57*, 625–629.
49. Shatar, S.; Altantsetseg, S.H.; Sarnai, I.; Zoljargal, D.; Thang, T.D.; Nguyen, X.D. Chemical composition of the essential oil of *Ocimum basilicum* cultivated in Mongolian Desert-Gobi. *Chem. Nat. Compd.* **2007**, *43*, 726–727. [CrossRef]
50. Beatović, D.; Krstić-Milošević, D.; Trifunović, S.; Šiljegović, J.; Glamočlija, J.; Ristić, M.; Jelačić, S. Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oils of Twelve *Ocimum basilicum* L. Cultivars Grown in Serbia. *Rec. Nat. Prod.* **2015**, *9*, 62–75.
51. Chenni, M.; El-Abed, D.; Rakotomanomana, N.; Fernandez, X.; Chemat, F. Comparative study of essential oils extracted from Egyptian basil leaves (*Ocimum basilicum* L.) using Hydro-Distillation and Solvent-Free Microwave extraction. *Molecules* **2016**, *21*, 113. [CrossRef] [PubMed]
52. Katiki, L.M.; Barbieri, A.M.E.; Araujo, R.C.; Veríssimo, C.J.; Louvandini, H.; Ferreira, J.F.S. Synergistic interaction of ten essential oils against *Haemonchus contortus* in vitro. *Vet. Parasitol.* **2017**, *243*, 47–51. [CrossRef] [PubMed]
53. Politeo, O.; Jukic, M.; Milos, M. Chemical composition and Antioxidant capacity of free volatile aglycones from Basil (*Ocimum Basilicum* L.) compared with its essential oil. *Food Chem.* **2007**, *101*, 379–385. [CrossRef]
54. Goñi, M.L.; Gañán, N.A.; Strumia, M.C.; Martini, R.E. Eugenol-loaded LLDPE films with antioxidant activity by supercritical carbon dioxide impregnation. *J. Supercrit. Fluids* **2016**, *111*, 28–35. [CrossRef]
55. U.S. Food & Drug. Available online: <https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2022> (accessed on 29 September 2022).
56. Carson, C.F.; Hammer, K.A. Chemistry and bioactivity of essential oils. In *Lipids and Essential Oils as Antimicrobial Agents*; John Wiley & Sons: Chichester, UK, 2011; pp. 203–238.
57. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
58. O'Bryan, C.A.; Pendleton, S.J.; Crandall, P.G.; Ricke, S.C. Potential of plant essential oils and their components in animal agriculture - in vitro studies on antibacterial mode of action. *Front. Vet. Sci.* **2015**, *2*, 35. [CrossRef]
59. Saad, N.Y.; Muller, C.D.; Lobstein, A. Major bioactivities and mechanism of action of essential oils and their components. *Flavour Fragr. J.* **2013**, *28*, 269–352. [CrossRef]
60. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef]
61. Bruni, R.; Medici, A.; Andreotti, E.; Fantin, C.; Muzzoli, M.; Dehesa, M.; Romagnoli, C.; Sacchetti, G. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm (Lauraceae) flower calices. *Food Chem.* **2004**, *85*, 415–421. [CrossRef]
62. Shankar, J.R.; Mohan, S.K. A status review on the medicinal properties of essential oils. *Ind. Crops Prod.* **2014**, *62*, 250–264.

63. Dakole, C.H.; Nguefack, J.; Lekagne, J.B.D. Antifungal potential of essential oils, aqueous and ethanol extracts of thirteen plants against *Fusarium oxysporum* sp. *lycopersici* and *Phytophthora infestans* (Mont.) de Bary as major tomato pathogens in Cameroon. *Int. J. Curr. Sci.* **2016**, *19*, 128–145.
64. Kocic-Tanackov, S.; Dimic, G.; Levic, J.; Tanackov, I.; Tuco, D. Antifungal activities of basil (*Ocimum basilicum* L.) extract on *Fusarium* species. *Afr. J. Biotechnol.* **2011**, *10*, 10188–10195.
65. Nosrati, S.; Hosseini, S.A.E.; Sarpeleh, A.; Shahrababak, M.S.; Shahrababk, Y.S. Antifungal activity of Spearmint (*Metha Spicata* L.) essential oil on *Fusarium oxysporum* f. sp. *radicis-cucumerinum* the causal agent of stem and crown of greenhouse cucumber in Yazd, Iran. In Proceedings of the International Conference on Environmental and Agriculture Engineering IPCBEE, Chengdu, China, 29–31 July 2011; Volume 15, pp. 52–56.
66. Pawar, V.C.; Thaker, V.S. Evaluation of the anti-*Fusarium oxysporum* f. sp. *cicer* and *Alternaria porri* effects of some essential oils. *World J. Microbiol. Biotechnol.* **2007**, *23*, 1099–1106. [[CrossRef](#)]
67. Oxenham, S.K.; Svoboda, K.P.; Walters, D.R. Antifungal activity of the essential oil of Basil (*Ocimum basilicum*). *J. Phytopathol.* **2005**, *153*, 174–180. [[CrossRef](#)]
68. Gurav, T.P.; Dholakia, B.B.; Ashok, P.; Giri, A.P. A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. *Phytochem. Rev.* **2021**, *21*, 879–913. [[CrossRef](#)]
69. Lucini, E.I.; Zunino, M.P.; López, M.L.; Zygodlo, J.A. Effect of Monoterpenes on Lipid Composition and Sclerotial Development of *Sclerotium cepivorum* Berk. *J. Phytopathol.* **2006**, *154*, 441–446. [[CrossRef](#)]
70. Sharma, S.; Tripathi, A. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microb. Res.* **2008**, *163*, 337–344. [[CrossRef](#)]
71. Carovic-Stanko, K.; Orlic, S.; Politeom, O.; Strikic, F.; Kolak, I.; Milos, M.; Satovic, Z. Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chem.* **2010**, *119*, 196–201. [[CrossRef](#)]
72. Badaway, M.E.I.; Abdelgaleil, S.A.M. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. *Ind. Crops Prod.* **2014**, *52*, 776–782. [[CrossRef](#)]
73. Salvi, P.; Kumar, G.; Gandass, N.; Kajal; Verma, A.; Rajarammohan, S.; Rai, N.; Gautam, V. Antimicrobial potential of essential oils from aromatic plant *Ocimum* sp.; A comparative biochemical profiling and in-silico analysis. *Agronomy* **2022**, *12*, 627. [[CrossRef](#)]