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# Assessment of *Ocimum basilicum* Essential Oil Anti-Insect Activity and Antimicrobial Protection in Fruit and Vegetable Quality

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**Abstract:** Basil (*Ocimum basilicum*) is a commonly used herb; it also contains essential oils and other valuable compounds. The basil oil obtained has a pleasant aroma, but also a broad spectrum of biological activity. This work reports on the chemical composition, antioxidant, antimicrobial and anti-insect activity in vitro and in situ of *Ocimum basilicum* essential oil (OBEO) obtained by steam distillation of fresh flowering plants. Gas chromatography–mass spectrometry, DPPH, agar and disc diffusion and vapor phase methods were used to analyze the OBEO properties. The analysis of the chemical composition of OBEO showed that its main components were methyl chavicol (88.6%), 1,8-cineole (4.2%) and α-trans-bergamotene (1.7%). A strong antioxidant effect was demonstrated at the level of 77.3%. The analysis of antimicrobial properties showed that OBEO exerts variable strength of inhibiting activity against various groups of microorganisms. The growth inhibition zones ranged from 9.67 to 15.33 mm in Gram-positive ($G^+$) and Gram-negative ($G^-$) bacteria and from 5.33 to 7.33 mm in yeast. The lowest measured minimal inhibition concentration (MIC) was 3.21 μL/mL against Gram-negative *Azotobacter chrococcum* and Gram-positive *Micrococcus luteus*. The antimicrobial activity of in situ vapor phase of OBEO was also confirmed on apples, pears, potatoes and kohlrabi. The highest insecticidal activity against *Pyrrhocoris apterus*, observed at the concentration of 100%, caused the death of 80% of individuals. Due to its broad spectrum of activity, OBEO seems an ideal candidate for preserving fruit and vegetables.

**Keywords:** basil essential oil; antimicrobial activity; in vitro; in situ; bacteria; yeast; fruits; vegetables

## 1. Introduction

Conventional antimicrobials can cause potential ecotoxicological risks and be hazardous to a wide range of non-target organisms in systems because they impact basic biological processes that are not unique to microorganisms [1]. The growing number of organic farms producing both fruit and vegetables creates a demand for organic compounds to control plant pathogens. Adapted as a worldwide agricultural strategy, integrated pest management (IPM) relies on the reduced use of pesticides [2,3]. Among the new sources of compounds used in organic agriculture, plant-derived compounds deserve special attention. The widely reported biological activity of essential oils indicates that they can play a role of compounds that control pests and plant pathogens [4,5]. The insecticidal, bactericidal, fungicidal and viricidal activity of the essential oils is due to the presence of various functional groups, such as alcohols, aldehydes, phenols, terpenes, ketones and other antimicrobial compounds [6,7]. However, the use of excessively high doses of essential oils (EOs) may be toxic for plants [8,9].

One of the most frequently mentioned factors causing severe losses of fruit and vegetables after their harvest is contamination and growth of microorganisms. Basil (*Ocimum basilicum* L.) EO (OBEO) was analyzed as a possible treatment to eliminate synthetic preservatives and to reduce storage losses. OBEO proved highly active against *Colletotrichum gleosporioides* that causes diseases in mangoes [10]. In the case of grapes stored at 0 °C, OBEO inhibited the development of fungal decay for up to 60 days, but also limited weight loss and browning, without significantly affecting the taste of the fruit [11]. Other studies indicated the possibility of extending the shelf life of grapes by dipping them in a 20% eugenol solution and packing under a modified atmosphere [12]. Herath and Abeywickrama [13] showed that OBEO and eugenol significantly reduced the prevalence of banana crown rot fungi, *Colletotrichum musae* and *Fusarium proliferatum*. Another study showed that linalool vapors limited the rotting of peach fruits caused by *Rhizopus stolonifer*, *Sclerotinia sclerotiorum* and *Mucor* sp. [14]. The combination of linalool and eugenol in the proportions found naturally in OBEO resulted in a significant increase in antimicrobial activity.

Moghaddam et al. [15] described the antibacterial activity of OBEO, in particular of methyl chavicol, against this important phytopathogenic bacteria. The excessive use of synthetic plant protection products, such as fungicides, insecticides or pesticides, which are also toxic to other organisms, causes substantial environmental and toxicological problems. Therefore, it is important to replace the synthetic plant protection products with other, more environmentally friendly alternatives [5,16]. The use of biological plant protection is safer also because of its environmental impacts. Therefore, in recent years, there has been an increasing interest in the use of natural and plant-based pest control products such as EO or their derivatives. The most frequently mentioned arguments in their support, in contrast to the synthetic products are, much lower toxicity to mammals, but also rapid degradation in the environment [17,18]. Literature reports indicate the wide antimicrobial or insecticidal activity of EOs, which allows their application for crop plant protection [19–21].

Considering the above, the aim of this study was to characterize the chemical composition of the essential oil of *Ocimum basilicum* (OBEO) as well as its antioxidant, anti-insect and antimicrobial activities in an in vitro and in situ model of fruit and vegetables.

## 2. Results

### 2.1. Chemical Composition of OBEO

The chemical composition of OBEO was determined by GC/MS analyses. The identified volatile compounds and their relative content (percentages) are listed in Table 1. In total, 28 compounds were identified in OBEO; those accounted for 99.8% of the total volatiles. The obtained data revealed that the main component of the OBEO was methyl chavicol (88.6%), followed by 1,8-cineole (4.2%) and α-trans-bergamotene (1.7%).

**Table 1.** Chemical composition of essential oil from *Ocimum basilicum*.

| No | RI [a] | Compound [b] | % [c] |
|---|---|---|---|
| 1 | 938 | α-pinene | 1.0 |
| 2 | 948 | camphene | tr |
| 3 | 977 | sabinene | tr |
| 4 | 980 | β-pinene | 0.2 |
| 5 | 1023 | *p*-cimene | 0.7 |
| 6 | 1028 | α-limonene | 0.4 |
| 7 | 1033 | 1,8-cineole | 4.2 |
| 8 | 1047 | (*E*)-β-ocimene | 0.4 |
| 9 | 1060 | γ-terpinene | 0.4 |
| 10 | 1085 | fenchone | tr |
| 11 | 1088 | α-terpinolene | 1.2 |
| 12 | 1148 | camphor | 0.3 |
| 13 | 1195 | methyl chavicol | 88.6 |
| 14 | 1223 | endo-fenchyl acetate | tr |
| 15 | 1235 | exo-fenchyl acetate | tr |
| 16 | 1286 | bornyl acetate | tr |
| 17 | 1360 | eugenol | 0.3 |
| 18 | 1388 | β-elemene | tr |
| 19 | 1406 | methyl eugenol | 0.2 |
| 20 | 1422 | (*E*)-caryophyllene | tr |
| 21 | 1437 | α-trans-bergamotene | 1.7 |
| 22 | 1443 | (*Z*)-β-farnesene | tr |
| 26 | 1485 | α-amorphene | 0.2 |
| 27 | 1568 | (*E*)-*p*-methoxy-cinnamaldehyde | tr |
| 28 | 1583 | caryophyllene oxide | tr |
| | total | | 99.8 |

[a] Values of retention indices on HP-5MS column; [b] Identified compounds; [c] tr–compounds identified in amounts less than 0.1%.

## 2.2. Antioxidant Activity of OBEO

The antioxidant activity of OBEO assessed by the DPPH method was determined at $77.3 \pm 5.0\%$ of inhibition that corresponds to $434.21 \pm 28.9$ µg of TEAC/mL (trolox equivalent antioxidant capacity).

## 2.3. In Vitro Antimicrobial Activity of OBEO

In the analysis of OBEO antimicrobial activity, $G^-$ bacteria, $G^+$ bacteria, and yeasts were used. The OBEO showed a varying activity against the growth of the microorganisms used in the disc diffusion tests (Table 2). The $G^-$ bacteria with inhibition zones $14.33 \pm 0.58$ mm (*A. chrococcum*) and $15.33 \pm 0.55$ mm (*S. marcescens*) were more sensitive to OBEO than the $G^+$ bacteria with inhibition zones of $9.67 \pm 0.58$ mm (*M. luteus*) and $11.33 \pm 0.58$ mm (*P. megatherinum*). The yeasts were the least sensitive to OBEO, with the inhibition zones of $5.33 \pm 0.58$ mm (*C. glabrata*) and $7.33 \pm 0.58$ mm (*C. tropicalis*), respectively.

The minimal inhibition concentration (MIC) of OBEO is shown Table 3. The MIC test showed the strongest inhibition activity and the lowest MIC 50 (3.21 µL/mL) and MIC 90 (5.36 µL/mL) values for *A. chrococcum* and *M. luteus*. Conversely, the highest MIC 50 (6.15 µL/mL) and MIC 90 (8.25 µL/mL) values were recorded for *C. glabrata*.

## 2.4. In Situ Antimicrobial Activity of OBEO

The results of the antibacterial activity of the vapor phase of OBEO on apples are summarized in Table 4. Intensity of the bacterial inhibition by OBEO increased with the increasing concentration of OBEO in assays across all bacteria tested.

The results of the anti-yeasts activity of the vapor phase of OBEO on apples are summarized in Table 5. Intensity of the yest growth inhibition by OBEO increased with the increasing concentration of OBEO in assays across all bacteria tested. In agreement with

the MIC results, *C. glabrata* was more sensitive to the OBEO than *C. tropicalis*, in particular at the higher tested concentrations.

**Table 2.** In vitro analysis of the antimicrobial activity of the OBEO with disc diffusion method.

| Microorganisms | Inhibition Zone (mm) | Activity of Basil EO | ATB (mm) |
|---|---|---|---|
| Gram-negative bacteria | | | |
| *Azotobacter chrococcum* | 15.33 ± 0.58 | *** | 26.33 ± 1.53 |
| *Serratia marcescens* | 14.33 ± 0.58 | *** | 30.33 ± 0.58 |
| Gram-positive bacteria | | | |
| *Priestia megatherium* | 11.33 ± 0.58 | *** | 30.33 ± 0.58 |
| *Micrococcus luteus* | 9.67 ± 0.58 | ** | 27.33 ± 0.58 |
| Yeasts | | | |
| *Candida glabrata* | 5.33 ± 0.58 | ** | 28.33 ± 0.58 |
| *Candida tropicalis* | 7.33 ± 0.58 | ** | 29.33 ± 1.15 |

Means ± standard deviation (*n* = 3). ** Moderate inhibitory activity (zone > 10 mm). *** Very strong inhibitory activity (zone > 15 mm). ATB—antibiotics, positive control (Cefoxitin for G$^-$, Gentamicin for G$^+$, Fluconazole for yeast).

**Table 3.** Minimal inhibition concentration of OBEO.

| Microorganisms | MIC50 (µL/mL) | MIC90 (µL/mL) |
|---|---|---|
| Gram-negative bacteria | | |
| *Azotobacter chrococcum* | 3.21 | 5.36 |
| *Serratia marcescens* | 3.46 | 6.23 |
| Gram-positive bacteria | | |
| *Priestia megatherium* | 3.25 | 5.41 |
| *Micrococcus luteus* | 3.21 | 5.36 |
| Yeasts | | |
| *Candida glabrata* | 6.15 | 8.25 |
| *Candida tropicalis* | 3.56 | 5.67 |

**Table 4.** In situ analysis of the antibacterial activity of the vapor phase of OBEO on apples.

| Basil EO (µL/L) | Bacterial Growth Inhibition [%] Apples | | | |
|---|---|---|---|---|
| | *Azotobacter chrococcum* | *Priestia megatherium* | *Serratia marcescens* | *Micrococcus luteus* |
| 62.5 | 12.31 ± 1.97 [a] | 13.07 ± 2.44 [a] | 14.87 ± 2.29 [a] | 7.79 ± 2.55 [a] |
| 125 | 24.69 ± 2.45 [b,a] | 17.78 ± 2.61 [b] | 28.71 ± 3.46 [b] | 14.38 ± 2.89 [b] |
| 250 | 53.19 ± 2.57 [c,a,b] | 35.91 ± 1.51 [c,a,b] | 72.09 ± 3.07 [c,a,] | 26.00 ± 2.56 [c,a,b] |
| 500 | 92.35 ± 3.56 [d,a,b,c] | 80.81 ± 2.11 [d,a,b,c] | 99.41 ± 1.01 [d,a,b] | 74.13 ± 3.13 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

**Table 5.** In situ analysis of the anti-yeast activity of the vapor phase of OBEO on apples.

| Basil EO (µL/L) | Mycelial Growth Inhibition [%] Apples | |
|---|---|---|
| | *Candida glabrata* | *Candida tropicalis* |
| 62.5 | 13.56 ± 2.94 [a] | 13.61 ± 3.82 [a] |
| 125 | 33.30 ± 2.48 [b,a] | 16.58 ± 2.70 [b] |
| 250 | 45.72 ± 3.40 [c,a,b] | 28.88 ± 2.88 [c,a,b] |
| 500 | 74.42 ± 3.64 [d,a,b,c] | 27.56 ± 4.09 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the antibacterial activity of the vapor phase of OBEO on pears are summarized in Table 6. Intensity of the bacterial inhibition by OBEO increased with the increasing concentration of OBEO in assays across all bacteria tested. The best antibacterial activity was found against *S. marcescens* at 500 μL/L concentration. In contrast to the similar assays in apples, *P. megatherium* was inhibited by OBEO the lowest in the pear assays.

**Table 6.** In situ analysis of the antibacterial activity of the vapor phase of OBEO on pears.

| Basil EO (μL/L) | Bacterial Growth Inhibition [%] Pears | | | |
| --- | --- | --- | --- | --- |
| | *Azotobacter chrococcum* | *Priestia megatherium* | *Serratia marcescens* | *Micrococcus luteus* |
| 62.5 | 6.39 ± 1.66 [a] | 1.85 ± 1.47 [a] | 2.51 ± 0.99 [a] | 10.17 ± 2.20 [a] |
| 125 | 13.18 ± 1.16 [b,a] | 3.18 ± 1.29 [b] | 7.54 ± 2.87 [b] | 12.65 ± 2.50 [b] |
| 250 | 35.34 ± 3.93 [c,a,b] | 5.16 ± 2.39 [c] | 16.09 ± 3.98 [c,a,b] | 28.61 ± 3.11 [c,a,b] |
| 500 | 73.11 ± 2.02 [d,a,b,c] | 16.02 ± 2.54 [d,a,b,c] | 82.38 ± 3.72 [d,a,b,c] | 63.40 ± 2.78 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the anti-yeast activity of the vapor phase of OBEO on pears are summarized in Table 7. Similar to the MIC results, *C. glabrata* was more sensitive to the OBEO than *C. tropicalis*, in particular at the highest tested concentration.

**Table 7.** In situ analysis of the anti-yeast activity of the vapor phase of OBEO on pears.

| Basil EO (μL/L) | Mycelial Growth Inhibition [%] Pear | |
| --- | --- | --- |
| | *Candida glabrata* | *Candida tropicalis* |
| 62.5 | 4.61 ± 0.74 [a] | 4.79 ± 2.13 [a] |
| 125 | 12.35 ± 2.05 [b] | 17.38 ± 1.89 [b,a] |
| 250 | 30.37 ± 5.00 [c,a,b] | 23.19 ± 1.51 [c,a,b] |
| 500 | 75.38 ± 3.89 [d,a,b,c] | 45.25 ± 2.70 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the antibacterial activity of the gas phase of OBEO on potatoes are summarized in Table 8. Similar to the other inoculated plants, there was an increasing trend of bacterial inhibition with the increasing amounts of the applied OBEO. The best antibacterial activity of OBEO on potatoes was found against *M. luteus* at 500 μL/L concentration.

**Table 8.** In situ analysis of the antibacterial activity of the vapor phase of OBEO on potatoes.

| Basil EO (μL/L) | Bacterial Growth Inhibition [%] Potatoes | | | |
| --- | --- | --- | --- | --- |
| | *Azotobacter chrococcum* | *Priestia megatherium* | *Serratia marcescens* | *Micrococcus luteus* |
| 62.5 | 17.27 ± 2.01 [a] | 4.61 ± 0.74 [a] | 12.12 ± 2.12 [a] | 15.47 ± 3.03 [a] |
| 125 | 25.38 ± 2.82 [b] | 13.30 ± 2.01 [b,a] | 19.82 ± 1.43 [b,a] | 4.82 ± 2.16 [b,a] |
| 250 | 46.52 ± 2.70 [c,a,b] | 31.38 ± 2.86 [c,a,b] | 6.49 ± 0.69 [c,b] | 46.70 ± 1.29 [c,a,b] |
| 500 | 67.02 ± 4.55 [d,a,b,c] | 72.04 ± 3.13 [d,a,b,c] | 39.89 ± 4.13 [d,a,b,c] | 79.74 ± 1.57 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the anti-yeast activity of the gas phase of OBEO on potatoes are summarized in Table 9. Again, application of higher amounts of OBEO resulted in higher levels of yeast growth inhibition, and *C. glabrata* was inhibited marginally higher than *C. tropicalis*.

**Table 9.** In situ analysis of the anti-yeast activity of the vapor phase of OBEO on potatoes.

| Basil EO (µL/L) | Mycelial Growth Inhibition [%] Potatoes | |
| --- | --- | --- |
| | *Candida glabrata* | *Candida tropicalis* |
| 62.5 | 7.74 ± 1.78 [a] | 15.94 ± 2.43 [a] |
| 125 | 21.21 ± 2.60 [b,a] | 26.65 ± 3.13 [b,a] |
| 250 | 46.82 ± 3.26 [c,a,b] | 44.07 ± 2.16 [c,a,b] |
| 500 | 87.31 ± 2.71 [d,a,b,c] | 78.04 ± 3.95 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the antibacterial activity of the vapor phase of OBEO on kohlrabies are summarized in Table 10. In agreement with other tested plants, increasing the amounts of applied OBEO resulted in increased bacterial inhibition. The best antibacterial activity was found against *S. marcescens* at 500 µL/L concentration reaching 98.95%.

**Table 10.** In situ analysis of the antibacterial activity of the vapor phase of OBEO on kohlrabies.

| Basil EO (µL/L) | Bacterial Growth Inhibition [%] Kohlrabies | | | |
| --- | --- | --- | --- | --- |
| | *Azotobacter chrococcum* | *Priestia megatherium* | *Serratia marcescens* | *Micrococcus luteus* |
| 62.5 | 10.93 ± 1.28 [a] | 27.35 ± 1.60 [a] | 12.39 ± 2.19 [a] | 12.68 ± 2.62 [a] |
| 125 | 23.46 ± 2.57 [b,a] | 35.89 ± 2.41 [b,a] | 25.61 ± 3.10 [b,a] | 25.30 ± 3.17 [b,a] |
| 250 | 55.15 ± 2.47 [c,a,b] | 43.81 ± 2.56 [c,a,b] | 55.04 ± 3.12 [c,a,b] | 7.77 ± 2.03 [c,b] |
| 500 | 75.11 ± 2.90 [d,a,b,c] | 55.45 ± 4.59 [d,a,b,c] | 98.95 ± 0.97 [d,a,b,c] | 6.07 ± 2.17 [d,b] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

The results of the anti-yeast activity of the gas phase of OBEO on kohlrabies are summarized in Table 11. Congruently with other tested plants, the inhibitory activity was higher at the higher OBEO concentration applied. In contrast with other tested plat species, *C. tropicalis* showed marginally higher sensitivity to OBEO than *C. glabrata*.

**Table 11.** In situ analysis of the anti-yeast activity of the vapor phase of OBEO on kohlrabies.

| Basil EO (µL/L) | Mycelial Growth Inhibition [%] Kohlrabies | |
| --- | --- | --- |
| | *Candida glabrata* | *Candida tropicalis* |
| 62.5 | 6.44 ± 1.84 [a] | 8.50 ± 1.11 [a] |
| 125 | 13.27 ± 2.25 [b,a] | 18.05 ± 4.50 [b] |
| 250 | 31.59 ± 1.48 [c,a,b] | 38.55 ± 5.71 [c,a,b] |
| 500 | 67.70 ± 2.13 [d,a,b,c] | 74.91 ± 0.66 [d,a,b,c] |

Means ± standard deviation (*n* = 3). Individual letters (a–d) in upper case indicate the statistical difference at $p \leq 0.05$.

## 2.5. Anti-Insect Activity of OBEO

All tested concentrations of the OBEO vapor phase showed insecticidal activity against *Pyrrhocoris apterus* (Table 12). The highest insecticidal activity was observed at the concentration of 100%, causing the death of 80% of individuals. About a quarter of the OBEO concentration resulted in roughly half the mortality, whereas 6.25% of OBEO applied proved indistinguishable from the control treatment.

**Table 12.** Anti-Insect activity of OBEO.

| Concentration [%] | Number of Living Individuals | Number of Dead Individuals | Insecticidal Activity [%] |
|---|---|---|---|
| 100 | 4 | 26 | 80 |
| 50 | 9 | 21 | 70 |
| 25 | 15 | 15 | 50 |
| 12.5 | 27 | 3 | 10 |
| 6.25 | 30 | 0 | 0 |
| Control group | 30 | 0 | 0 |

## 3. Discussion

Research on basil oil has showed that it has antimicrobial and insecticidal activity as well as the high antioxidant activity, antiprotozoal activity and anticancer effects, and therefore, it has been used as the flavoring agent in gastronomy, for food preservation, safety and production. There were four chemotypes of basil identified [22]; the European chemotype contained linalool as the main component (Italy, France, Bulgaria, Egypt and South Africa), the tropical chemotype with the major component of methyl cinnamate (India, Pakistan, Guatemala), the Reunion chemotype with methyl chavicol (Madagascar, Thailand, Vietman) [23,24] and the North Africa and Russia chemotype with eugenol [23]. In other studies, the major component of basil essential oil was estragole (methyl chavicol) [25–30].

We observed a high antioxidant activity of OBEO in our study. Other previous research documented that *Ocimum* sp. contained a high content of antioxidant compounds which could contribute to increasing the OBEO antiradical activity [31,32] and positively affecting human health [33]. Basil is characterized by a strong antioxidant capacity so its use can help in prevention of heart diseases, and help decrease the inflammation and the incidence of cancers and diabetes [34]. A strong correlation was found between phenolic compounds and antioxidant capacity of medicinal plants [35,36]. Extracts of *Ocimum basilicum* consist in a substantial part of phenolic acids and thus show high antioxidant activity. The antioxidant activity of *O. basilicum* var. *cinammom* caused an antioxidant inhibition of 87.9% at a concentration of 10 ppm, an inhibition of 91.4% at 30 ppm and 92.1% up to 50 ppm. EOs of *O. basilicum* var. *album* showed a 61.8% antioxidant inhibition at 10 ppm and 90% inhibition at 30 ppm. Inhibition remained constant at 50 ppm [37].

We tested the effects of OBEO application under in vitro conditions against $G^-$, $G^+$ and yeasts in our study. Similar to our results, there is evidence of an antibacterial activity of EOs from various *Ocimum* species in vitro against *Staphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella sonnei*, *Shigella boydii*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* [38–40]. OBEO activity was also tested against pathogenic fungi such as: *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium italicum* and *Rhizopus stolonifera* by using a disc diffusion method, and by determination of minimum inhibitory concentration. High antifungal activity found in that study highlighted the potential of *Ocimum* species to be used as a preservative in food and pharmaceutical industries [41]. We found the highest antimicrobial activity using the disc diffusion method against *A. chrococcum* and these results were confirmed by the agar diffusion assays. Our results confirm many other studied of the antibacterial properties of *O. basilicum*. Chenni et al. [42] reported that the EOs extracted from *O. basilicum* leaves using steam distillation displayed suitable antibacterial activity on $G^-$ and $G^+$ bacteria including *E. coli*, *P. aeruginosa*, *Bacillus cereus* and *S. aureus*. The results of another study reported the minimum inhibitory concentration (MIC) of *O. basilicum* extracts against *B. cereus* (36–18 µg/mL), *S. aureus* (18 µg/mL) and *E. coli* (9–18 µg/mL) [43]. The antibacterial activity of *O. basilicum* EOs has also been studied in Vietnam and the results have been very promising. Vu et al. [44] used in their study the Thai basil grown and harvested in Tien Giang and found out that the EO can inhibit some bacteria that cause intestinal diseases, such as *E. coli*, *Salmonella typhimurium*, *Shigella*, *B. cereus*, methicillin-resistant *Staphylococcus aureus*

(MRSA), but not *P. aeruginosa*. The anti-yeast activity of the OBEO was previously tested on *Candida albicans* [45].

The postharvest research attracted a substantial scientific and industrial interest; it is focused on phytonutrients that primarily increase the nutritional value of fruit and vegetables in addition to the reduction of the losses during storage [46]. Basil EOs showed high activities against postharvest diseases caused by *Fusarium* fungi [47]. Previous research showed a significant inhibition of pathogenic molds *B. cinerea* and *Mucor piriformis* by sweet basil EOs documented in in vitro tests by EOs incorporation in media or in vapor [11]. The basil EOs showed an inhibitory effect against G⁻ bacteria *Shigella sonnei* and *Shigella flexneri* on lettuce. In another study, washing fresh lettuce with 0.1 or 1% (*v*/*v*) of estragole chemotype basil EOs showed similar effectiveness to natural microbial flora as treatment with 125 ppm chlorine [48].

The plant pathogenic fungus *Botrytis fabae* and the rust fungus *Uromyces fabae* were controlled in vivo using basil EOs, estragole and linalool; these treatments significantly reduced the infection of broad bean leaves [49]. Basil EOs at 0.08% mL/L showed some efficiency against the survival of *E. coli* and *Salmonella typhimurium* inoculated into fresh-cut lettuce and purslane samples during refrigerated storage [50]. The results of our study showed a strong activity of OBEO against G⁻, G⁺ and yeast under in situ study on apples, pears, potatoes and kohlrabies. The highest antimicrobial effect of OBEO was found against *Serratia marcescens* on fruits and vegetables samples. The physiological quality of indicators such as fruit firmness depends on the EO dose applied. Crop firmness is one of the most important indicators used for the quality assessment of fruits. Our previous study used a vapor phase at 62.5 to 500 μL/L [14]. The minimum fungicidal dose in vapor phase was 300 μL/L for basil EO on lemon fruits [51]. The fungicidal activity of basil EO in vapor phase ranged between 30 to 400 μL/L [14]. A number of samples assayed by vapor phase in other studies were tested at relatively high concentrations (e.g., between 800 and 1600 mg/L); such high concentrations are only suitable for a qualitative evaluation, and can lead to a misinterpretation for the practical application of the obtained results [52].

The loss of fruit firmness during storage points out to water losses and metabolic changes in fruits [53]. Use of various polysaccharide, protein or carboxymethyl cellulose (CMC) coating techniques or edible crop coatings with chitosan bilayers did not help form an effective water-vapor barriers [54]. Therefore, the EO vapor-phase treatment can be more efficient during the post-harvest treatment of fruits. The research on the application of cinnamon and eucalyptus EOs vapor treatments at 50 ppm showed positive effects on maintaining the firmness in tomato and cherry tomatoes [53]. Firmness, texture and resistance to microbial infection in shiitake mushrooms was improved after fumigation with cloves, thymus and cinnamaldehyde EOs vapors for 20 days at 4 °C storage [55]. The tested samples of mushrooms were better-preserved in comparison to the control samples, but there was also a higher concentration of EOs with an undesirable effect observed. Due to their high volatility, EOs belong to the natural method of crop preservation with a high potential of use mainly in the organically grown fruit and vegetables. Furthermore, EOs utilized in the vapor-phase could be effective against fungal pathogens [51].

We observed a strong anti-insect activity of OBEO in higher concentration against *P. apterus*. Basil plants are attractive for many insects, including butterflies, bees, fruit flies and many others [56]. The addition of basil EOs on sticky traps increased approximately three and fivefold the number of trapped pea leafminer (*Liriomyza huidobrensis*) and greenhouse whitefly (*Trialeurodes vaporariorum*), respectively [57]. Sweet basil EOs showed antifeedant and insecticidal activities against the cowpea aphid *Aphis craccivora*. The successful adoption of basil EOs in the protection of food commodities promises an eco-friendly option compatible with the international biosafety regulations and the demands of organic certifications.

In conclusion, basil EOs demonstrate the desirable antimicrobial and anti-insect effects in both in vitro and in situ studies. OBOE shows a great potential in organic product cultivation and food preservation [58]. However, a number of previous studies were done

under limiting conditions in laboratories so an in-field analysis of the OBOE efficacy is needed [59].

Currently, the number of commercially available biocontrol products using EOs as active components is limited due to their selective effectiveness [60]. The EOs efficacies vary among the pathogen species, sometimes even among strains of the same pathogen species. The EOs may display anti-yeast effects and phytotoxic effects at the same time [61]. It is still difficult to find a proper application concentration for each crop at each developmental stage to optimally suppress the pathogens without damaging the crop plants.

As consumers become increasingly more aware of the human and environmental health, they are willing to pay a higher price for the organic commodities. Therefore, theoretical and practical studies of EOs should continue and more focus is needed on the development, extension and market introduction of newly formulated EO products [62].

## 4. Materials and Methods

### 4.1. Sample

The test essential oil *Ocimum basilicum* (OBEO) was purchased from company Hanus s.r.o. (Nitra, Slovakia) prepared by steam distillation of a fresh flowering plants from Vietnam. EO was stored in a refrigerator (4 °C) protected from light, in glass vessels.

### 4.2. Chemical Composition of OBEO

GC/MS analyses of OBEO were performed using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA). A HP-5MS capillary column (30 m × 0.25 mm × 0.25 mm) was used. Temperature program was: 60 °C to 150 °C (increasing rate 3 °C/min) and 150 °C to 280 °C (increasing rate 5 °C/min). The total run time was 60 min. Helium 5.0 was used as the carrier gas with flow rate of 1 mL/min. The injection volume was 1 mL (essential oil sample was diluted in pentane), while the split/splitless injector temperature was set at 280 °C. With split ratio at 40.8:1 investigated samples were injected in the split mode. Electron-impact mass spectrometric data (EI-MS; 70 eV) were acquired in scan mode over the m/z range 35–550. MS ion source and MS quadrupole temperatures were 230 °C and 150 °C, respectively. Acquisition of data started after solvent delay time of 3 min. GC-FID analyses were performed on Agilent 6890N gas chromatograph coupled to FID detector. Column (HP-5MS) and chromatographic conditions were same as for GC-MS. FID detector temperature was set at 300 °C.

The individual volatile constituents of the injected essential oil sample were identified based of their retention indices and comparison with reference spectra (Wiley and NIST databases). The retention indices were experimentally determined using the standard method which included retention times of n-alkanes (C6–C34), injected under the same chromatographic conditions. The percentages of the identified compounds (amounts higher than 0.1%) were derived from their GC peak areas [63].

### 4.3. Antioxidant Activity of OBEO

The antioxidant activity was determined spectrophotometrically by the DPPH method [64]. The percentage of inhibition was calculated as (A0−AA)/A0 × 100, where A0 was the absorbance of blank measurement, and AA was absorbance of sample. The antioxidant activity was expressed as antioxidant activity of Trolox related to 1 mL of sample (μg of TEAC/mL). Measurements were done in triplicate.

### 4.4. Microorganisms Tested

Gram-negative (G⁻) bacteria (*Azotobacter chrococcum* CCM 1912, *Serratia marcescens* CCM 8587), Gram-positive (G⁺) bacteria (*Bacillus* (isolated from 2020 *Priestia megatherium* CCM 2007, *Micrococcus luteus* CCM 732) and yeasts (*Candida glabrata* CCM 8270, *C. tropicalis* CCM 8223,) were obtained from the Czech Collection of Microorganisms (CCM; Brno, Czech Republic).

### 4.5. In Vitro Antimicrobial Activity of OBEO

The disc diffusion method was used to assess the antimicrobial activity of OBEO. The inoculum of bacteria was grown at 37 °C for 24 h on tryptone soy agar (Oxoid, Basingstoke, UK), whereas the yeast inoculum was grown at 25 °C on Sabouraud dextrose agar (Oxoid, Basingstoke, UK). The obtained inoculum was then adjusted to the optical density of a 0.5 McFarland standard ($1.5 \times 10^8$ CFU/mL) then 100 µL was placed in a Petri dish (PD) with Mueller Hinton medium (Oxoid, Basingstoke, UK) for bacteria and Sabouraud dextrose agar for yeast. Then, sterile disks with a diameter of 6 mm were placed on the solidified medium, onto which 10 µL of OBEO was applied. The PD prepared in this way was incubated in the above-mentioned conditions for the next 24 h. Antibiotics were used as a positive control for bacteria: cefoxitin for Gram-negative and gentamicin for Gram-positive, and for yeasts, an antifungal agent was used—fluconazole. All substances were obtained from Oxoid (Basingstoke, UK). As a negative control, discs with 10 µL of 0.1% DMSO applied on them (Centralchem, Bratislava, SK) were used. After incubation, the zones of growth inhibition were determined: above 10 mm antimicrobial activity was determined as very strong, above 5 mm as mild and above 1 mm as weak [63].

### 4.6. Minimum Inhibitory Concentration (MIC)

The MICs of bacteria and yeasts were determined using the agar microdilution method. The inoculum was cultured for 24 h in Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) at 37 °C for bacteria and Sabouraud Dextrose Broth (SDB, Oxoid, Basingstoke, UK) at 25 °C for yeast. Then, 100 µL of nutrient medium and 50 µL of inoculum with an optical density of 0.5 McFarland standard were applied to a 96-well microtiter plate. Subsequently, OBEO was prepared by serial dilution to a concentration range of 400 µL/mL to 0.2 µL/mL in MHB/SDB and mixed thoroughly with bacterial inoculum in the wells. The prepared 96-well microtiter plates were measured at 570 nm with a Glomax spectrophotometer (Promega Inc., Madison, WI, USA) at 0 h. Subsequently, the bacterial samples were incubated at 37 °C for 24 h. Yeast samples were incubated at 25 °C for 24 h and measured again. MHB/SDB with essential oil was used as a negative control, and MHB/SDB with inoculum was used as a positive control for maximal growth. The analysis was performed in triplicate [65–67].

### 4.7. In Situ Antimicrobial Activity of OBEO

The evaluation of the antimicrobial activity of OBCE on fruit and vegetable models (apples, pears, potatoes and kohlrabi) was performed according to the described by Borotová et al. [68] procedure against four bacteria and two yeasts. Briefly, 5 mm thick slices of fruit and vegetables were placed on solidified Mueller Hinton agar for PD ($\varnothing$ = 60 mm) and a microbial inoculum (0.5 McFarland) was applied. Then, diluted OBEO (100 µL) in ethyl acetate at 4 dilution levels (500, 250, 125 and 62.5 µL/L) was applied to a disc of sterile filter paper. The hermetically sealed PD was incubated for 7 days at the temperature appropriate for the analyzed microorganisms. An equivalent volume of ethyl acetate was used as a negative control. The percentage of inhibitory activity was calculated in ImageJ by stereological method. Bulk density was calculated according to the formula Vv = P/p × 100 where P is stereological lattice of the colonies and p is the substrate. Growth inhibition was expressed as GI = [(C − T)/C] × 100, where C was the growth density of control group and T was the growth density in the group contained LCEO [69].

### 4.8. Anti-Insect Activity of OBEO

The insecticidal activity of OBEO was tested using *Pyrrhocoris apterus* in accordance with the previously described method [69]. Briefly, concentrations of 100%, 50%, 25%, 12.5% and 6.25% OBEO were tested, and a 0.1% polysorbate was used as the negative control. A total of 30 individuals of *P. apterus* were placed in PD with vents. One hundred µL of the appropriate concentration of OBEO was placed on a filter paper wheel, placed in the cover PD and the plates sealed with parafilm. After 24 h of exposure at room temperature, the

numbers of living and dead *P. apterus* were assessed, and then the percentage of insecticidal activity was calculated. Analyses were carried out in triplicate.

*4.9. Statistical Data Evaluation*

One-way analysis of variance (ANOVA) was performed using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA) followed by Tukey's test at $\alpha$ = 0.05. SAS$^{®}$ software version 8 was used for data processing. The results of the MIC50 value (concentrations that caused 50% of microbial growth) and MIC90 (90% inhibition of bacterial growth) were determined by logit analysis.

## 5. Conclusions

Our study details the chemical composition, antioxidant, antimicrobial (in vitro, in situ) and anti-insect properties of commercial OBEO. The results obtained in this study strongly underscore that basil EOs could be used as the candidate for the development of natural antibiotics and disinfectants to control microbes pathogenic to crop fruit and vegetables. Thus, the basil EOs could be used as a naturally sourced food preservative to reduce and substitute or avoid the use of chemical preservatives. The results of our study and others encourage and prompt larger-scale, in-field research on basil EOs application, in particular for organic farming.

**Author Contributions:** Conceptualization, M.K. (Miroslava Kačániová) and L.G.; data curation, M.K. (Miroslava Kačániová), L.G., P.B., S.K., N.L.V., M.V., P.H. and L.B., methodology, M.K. (Miroslava Kačániová), L.G., N.L.V., M.V., P.B., S.K., M.K. (Maciej Kluz) and E.Z.; supervision, M.K. (Miroslava Kačániová); writing—original draft, M.K. and P.Ł.K. All authors have carefully revised and approved the final version of the manuscript.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article.

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

## References

1. Zubrod, J.P.; Bundschuh, M.; Arts, G.; Brühl, C.A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J.J.; Rohr, J.; Scharmüller, A.; et al. Fungicides: An Overlooked Pesticide Class? *Environ. Sci. Technol.* **2019**, *53*, 3347–3365. [CrossRef] [PubMed]
2. Sawinska, Z.; Strzelinska, J.; Switek, S.; Glowicka-Woloszyn, R. Integrated pest management (IPM) still not sufficiently used in practice. *Phytopathology* **2018**, *108*, 240.
3. Sawinska, Z.; Świtek, S.; Głowicka-Wołoszyn, R.; Kowalczewski, P.Ł. Agricultural Practice in Poland Before and After Mandatory IPM Implementation by the European Union. *Sustainability* **2020**, *12*, 1107. [CrossRef]
4. Grzanka, M.; Sobiech, Ł.; Danielewicz, J.; Horoszkiewicz-Janka, J.; Skrzypczak, G.; Sawinska, Z.; Radzikowska, D.; Świtek, S. Impact of essential oils on the development of pathogens of the *Fusarium* genus and germination parameters of selected crops. *Open Chem.* **2021**, *19*, 884–893. [CrossRef]
5. Grzanka, M.; Sobiech, L.; Stuper-Szablewska, K.; Danielewicz, J.; Skrzypczak, G. Effect of selected essential oils on the efficacy of volunteer oilseed rape control and to phytotoxicity in maize plants. *Chil. J. Agric. Res.* **2022**, *82*, 88–96. [CrossRef]
6. Swamy, M.K.; Akhtar, M.S.; Sinniah, U.R. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evid.-Based Complement. Altern. Med.* **2016**, *2016*, 1–21. [CrossRef] [PubMed]
7. Sartorelli, P.; Marquioreto, A.D.; Amaral-Baroli, A.; Lima, M.E.L.; Moreno, P.R.H. Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. *Phyther. Res.* **2007**, *21*, 231–233. [CrossRef] [PubMed]
8. LOPEZ-REYES, J.G.; SPADARO, D.; PRELLE, A.; GARIBALDI, A.; GULLINO, M.L. Efficacy of Plant Essential Oils on Postharvest Control of Rots Caused by Fungi on Different Stone Fruits In Vivo. *J. Food Prot.* **2013**, *76*, 631–639. [CrossRef] [PubMed]

9.  Sinha, S.; Jothiramajayam, M.; Ghosh, M.; Mukherjee, A. Evaluation of toxicity of essential oils palmarosa, citronella, lemongrass and vetiver in human lymphocytes. *Food Chem. Toxicol.* **2014**, *68*, 71–77. [CrossRef]

10. Abd-Alla, M.A.; Haggag, W.M. Use of some plant essential oils as post-harvest botanical fungicides in the management of anthracnose disease of mango fruits (*Mangifera indica* L.) caused by *Colletotrichum gloeosporioides* (Penz). *Int. J. Agric. For.* **2013**, *3*, 1–6.

11. Abdollahi, A.; Hassani, A.; Ghosta, Y.; Bernousi, I.; Meshkatalsadat, M.H.; Shabani, R.; Ziaee, S.M. Evaluation of essential oils for maintaining postharvest quality of Thompson seedless table grape. *Nat. Prod. Res.* **2012**, *26*, 77–83. [CrossRef] [PubMed]

12. Cristina, C.; Annalisa, L.; Amalia, C.; Francesco, C.; Del Nobile, M.A. In vitro and in vivo application of active compounds with anti-yeast activity to improve the shelf life of ready-to-eat table grape. *World J. Microbiol. Biotechnol.* **2013**, *29*, 1075–1084. [CrossRef] [PubMed]

13. Herath, H.; Abeywickrama, K. In vitro application of selected essential oils and their major components in controlling fungal pathogens of crown rot in Embul banana (*Musa acuminata*—AAB). *Int. J. Food Sci. Technol.* **2008**, *43*, 440–447. [CrossRef]

14. Edris, A.E.; Farrag, E.S. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. *Nahrung/Food* **2003**, *47*, 117–121. [CrossRef] [PubMed]

15. Moghaddam, M.; Alymanesh, M.R.; Mehdizadeh, L.; Mirzaei, H.; Ghasemi Pirbalouti, A. Chemical composition and antibacterial activity of essential oil of *Ocimum ciliatum*, as a new source of methyl chavicol, against ten phytopathogens. *Ind. Crops Prod.* **2014**, *59*, 144–148. [CrossRef]

16. Dey, D.; Gupta, M.K. Use of essential oils for insect pest management-a review. *Innov. Farming* **2016**, *1*, 21–29.

17. Isman, M.B. Plant essential oils for pest and disease management. *Crop Prot.* **2000**, *19*, 603–608. [CrossRef]

18. Isman, M.B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochem. Rev.* **2020**, *19*, 235–241. [CrossRef]

19. Soonwera, M.; Sittichok, S. Adulticidal activities of *Cymbopogon citratus* (Stapf.) and *Eucalyptus globulus* (Labill.) essential oils and of their synergistic combinations against *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Musca domestica* (L.). *Environ. Sci. Pollut. Res.* **2020**, *27*, 20201–20214. [CrossRef]

20. Felšöciová, S.; Vukovic, N.; Jeżowski, P.; Kačániová, M. Antifungal activity of selected volatile essential oils against *Penicillium* sp. *Open Life Sci.* **2020**, *15*, 511–521. [CrossRef]

21. Leja, K.; Szudera-Kończal, K.; Świtała, E.; Juzwa, W.; Kowalczewski, P.Ł.; Czaczyk, K. The Influence of Selected Plant Essential Oils on Morphological and Physiological Characteristics in *Pseudomonas Orientalis*. *Foods* **2019**, *8*, 277. [CrossRef] [PubMed]

22. Telci, I.; Bayram, E.; Yılmaz, G.; Avcı, B. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). *Biochem. Syst. Ecol.* **2006**, *34*, 489–497. [CrossRef]

23. Vernin, G.; Metzger, J. Analysis of basil oils by GC/MS data bank. *Perfum. Flavorist* **1984**, *9*, 71–86.

24. Simon, J.E.; Quinn, J.; Murray, R.G. Basil: A source of essential oils. In *Advanced in New Crops*; Janick, J., Simon, J.E., Eds.; Timber Press: Portland, OR, USA, 1999; p. 484.

25. Mondawi, B.; Duprey, R.; Magboul, A.; Satti, A. Constituents of essential oil of *Ocimum basilicum* var. Thyrsiflorum. *Fitoterapia* **1984**, *55*, 60–61.

26. Tangpao, T.; Chung, H.-H.; Sommano, S. Aromatic Profiles of Essential Oils from Five Commonly Used Thai Basils. *Foods* **2018**, *7*, 175. [CrossRef]

27. Bernhardt, B.; Sipos, L.; Kókai, Z.; Gere, A.; Szabó, K.; Bernáth, J.; Sárosi, S. Comparison of different *Ocimum basilicum* L. gene bank accessions analyzed by GC–MS and sensory profile. *Ind. Crops Prod.* **2015**, *67*, 498–508. [CrossRef]

28. Nurzyñska-Wierdak, R. Morphological Variability and Essential Oil Composition of four *Ocimum basilicum* L. cultivars. *J. Essent. Oil Bear. Plants* **2014**, *17*, 112–119. [CrossRef]

29. Khatri, L.; Nasir, M.; Saleem, R.; Noor, F. Evaluation of pakistani sweet basil oil for commercial exploitation. *Pak. J. Sci. Ind. Res.* **1995**, *38*, 281–282.

30. Verma, R.S.; Bisht, P.S.; Padalia, R.C.; Saikia, D.; Chauhan, A. Chemical composition and antibacterial activity of essential oil from two *Ocimum* spp. grown in sub-tropical india during spring-summer cropping season. *Asian J. Tradit. Med.* **2011**, *6*, 211–217.

31. Kwee, E.M.; Niemeyer, E.D. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. *Food Chem.* **2011**, *128*, 1044–1050. [CrossRef]

32. Oza, M.J.; Kulkarni, Y.A. Trifolium pratense (Red Clover) Improve SIRT1 Expression and Glycogen Content in High Fat Diet-Streptozotocin Induced Type 2 Diabetes in Rats. *Chem. Biodivers.* **2020**, *17*, e2000019. [CrossRef] [PubMed]

33. Flanigan, P.M.; Niemeyer, E.D. Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (*Ocimum basilicum* L.). *Food Chem.* **2014**, *164*, 518–526. [CrossRef] [PubMed]

34. Mastaneh, M.; Ahamd, M.; Taher, N.; Mehrdad, H. Antioxidant effect of Purple basil (Lamiaceae) Phenolics. *Orient. J. Chem.* **2014**, *30*, 1965–1969. [CrossRef]

35. Bahcesular, B.; Yildirim, E.D.; Karaçocuk, M.; Kulak, M.; Karaman, S. Seed priming with melatonin effects on growth, essential oil compounds and antioxidant activity of basil (*Ocimum basilicum* L.) under salinity stress. *Ind. Crops Prod.* **2020**, *146*, 112165. [CrossRef]

36. Miere, F.; Vicas, S.I.; Timar, A.V.; Ganea, M.; Zdrinca, M.; Cavalu, S.; Fritea, L.; Vicas, L.; Muresan, M.; Pallag, A.; et al. Preparation and Characterization of Two Different Liposomal Formulations with Bioactive Natural Extract for Multiple Applications. *Processes* **2021**, *9*, 432. [CrossRef]

37. Vivas Castaño, A.M.; Beltrán Cifuentes, M.C.; Cañón Rincón, D.J. Antioxidant activity of two varieties of *Ocimum basilicum* L. for potential use in phytocosmetics. *Rev. Fac. Nac. Agron. Medellín* **2016**, *69*, 7965–7973. [CrossRef]

38. Antonescu, A.-I.; Miere, F.; Fritea, L.; Ganea, M.; Zdrinca, M.; Dobjanschi, 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]

39. Vlaisavljevic, S.; Kaurinovic, B.; Popovic, M.; Djurendic-Brenesel, M.; Vasiljevic, B.; Cvetkovic, D.; Vasiljevic, S. *Trifolium pratense* L. as a Potential Natural Antioxidant. *Molecules* **2014**, *19*, 713–725. [CrossRef]

40. Reis, A.; Boutet-Mercey, S.; Massot, S.; Ratet, P.; Zuanazzi, J.A.S. Isoflavone production in hairy root cultures and plantlets of *Trifolium pratense*. *Biotechnol. Lett.* **2019**, *41*, 427–442. [CrossRef]

41. Al-Maskri, A.Y.; Hanif, M.A.; Al-Maskari, M.Y.; Abraham, A.S.; Al-sabahi, J.N.; Al-Mantheri, O. Essential Oil from *Ocimum basilicum* (Omani Basil): A Desert Crop. *Nat. Prod. Commun.* **2011**, *6*, 1934578X1100601. [CrossRef]

42. 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]

43. Rattanachaikunsopon, P.; Phumkhachorn, P. Antimicrobial Activity of Basil (*Ocimum basilicum*) Oil against *Salmonella Enteritidis* in Vitro and in Food. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1200–1204. [CrossRef] [PubMed]

44. Vu, T.T.; Kim, H.; Tran, V.K.; Le Dang, Q.; Nguyen, H.T.; Kim, H.; Kim, I.S.; Choi, G.J.; Kim, J.-C. In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria. *BMC Complement. Altern. Med.* **2015**, *16*, 32. [CrossRef] [PubMed]

45. Harlow, B.E.; Flythe, M.D.; Kagan, I.A.; Goodman, J.P.; Klotz, J.L.; Aiken, G.E. Isoflavone supplementation, via red clover hay, alters the rumen microbial community and promotes weight gain of steers grazing mixed grass pastures. *PLoS ONE* **2020**, *15*, e0229200. [CrossRef]

46. Porat, R.; Lichter, A.; Terry, L.A.; Harker, R.; Buzby, J. Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biol. Technol.* **2018**, *139*, 135–149. [CrossRef]

47. Sunčica, K.T.; Gordana, D.; Jelena, L.; Ilija, T.; Danijela, T. Antifungal activities of basil (*Ocimum basilicum* L.) extract on Fusarium species. *Afr. J. Biotechnol.* **2011**, *10*, 10188–10195. [CrossRef]

48. Wan, J.; Wilcock, A.; Coventry, M.J. The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. *J. Appl. Microbiol.* **1998**, *84*, 152–158. [CrossRef]

49. 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]

50. Karagözlü, N.; Ergönül, B.; Özcan, D. Determination of antimicrobial effect of mint and basil essential oils on survival of *E. coli* O157:H7 and *S. typhimurium* in fresh-cut lettuce and purslane. *Food Control* **2011**, *22*, 1851–1855. [CrossRef]

51. Sumalan, R.M.; Kuganov, R.; Obistioiu, D.; Popescu, I.; Radulov, I.; Alexa, E.; Negrea, M.; Salimzoda, A.F.; Sumalan, R.L.; Cocan, I. Assessment of Mint, Basil, and Lavender Essential Oil Vapor-Phase in Antifungal Protection and Lemon Fruit Quality. *Molecules* **2020**, *25*, 1831. [CrossRef]

52. Houdkova, M.; Kokoska, L. Erratum: Volatile Antimicrobial Agents and In Vitro Methods for Evaluating Their Activity in the Vapour Phase: A Review. *Planta Med.* **2020**, *86*, e4. [CrossRef] [PubMed]

53. Tzortzakis, N.G. Maintaining postharvest quality of fresh produce with volatile compounds. *Innov. Food Sci. Emerg. Technol.* **2007**, *8*, 111–116. [CrossRef]

54. Arnon, H.; Zaitsev, Y.; Porat, R.; Poverenov, E. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biol. Technol.* **2014**, *87*, 21–26. [CrossRef]

55. Jiang, T.; Luo, Z.; Ying, T. Fumigation with essential oils improves sensory quality and enhanced antioxidant ability of shiitake mushroom (*Lentinus edodes*). *Food Chem.* **2015**, *172*, 692–698. [CrossRef] [PubMed]

56. Chang, C.L.; Cho, I.K.; Li, Q.X.; Manoukis, N.C.; Vargas, R.I. A potential field suppression system for *Bactrocera dorsalis* Hendel. *J. Asia. Pac. Entomol.* **2013**, *16*, 513–519. [CrossRef]

57. Górski, R. Effectiveness of natural essential oils in monitoring of the occurrence of pea leafminer (*Liriomyza huidobrensis* Blanchard) in gerbera crop. *J. Plant Prot. Res.* **2005**, *45*, 287–291.

58. Mutlu-Ingok, A.; Devecioglu, D.; Dikmetas, D.N.; Karbancioglu-Guler, F.; Capanoglu, E. Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules* **2020**, *25*, 4711. [CrossRef]

59. Dimoski, A.; Jankuloska, V.; Pavlovska, G.; Trajkovska-Petkoska, A. The impact of apple preparation on the content of chlorpyrifos pesticide residues in the final products. *Acta Hortic. Regiotect.* **2021**, *24*, 134–140. [CrossRef]

60. Chang, Y.; Harmon, P.F.; Treadwell, D.D.; Carrillo, D.; Sarkhosh, A.; Brecht, J.K. Biocontrol Potential of Essential Oils in Organic Horticulture Systems: From Farm to Fork. *Front. Nutr.* **2022**, *8*, 1275. [CrossRef]

61. Perez-Nadales, E.; Almeida Nogueira, M.F.; Baldin, C.; Castanheira, S.; El Ghalid, M.; Grund, E.; Lengeler, K.; Marchegiani, E.; Mehrotra, P.V.; Moretti, M.; et al. Fungal model systems and the elucidation of pathogenicity determinants. *Fungal Genet. Biol.* **2014**, *70*, 42–67. [CrossRef]

62. Petkoska, A.T.; Trajkovska-Broach, A. Sustainable food systems and healthy diets: The case of mediterranean diet. *Acta Hortic. Regiotect.* **2021**, *24*, 110–118. [CrossRef]

63. Kačániová, M.; Galovičová, L.; Borotová, P.; Valková, V.; Ďúranová, H.; Kowalczewski, P.Ł.; Said-Al Ahl, H.A.H.; Hikal, W.M.; Vukic, M.; Savitskaya, T.; et al. Chemical Composition, In Vitro and In Situ Antimicrobial and Antibiofilm Activities of *Syzygium aromaticum* (Clove) Essential Oil. *Plants* **2021**, *10*, 2185. [CrossRef] [PubMed]

64. Olszowy, M.; Dawidowicz, A.L. Essential oils as antioxidants: Their evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-carotene bleaching methods. *Mon. Chem.-Chem. Mon.* **2016**, *147*, 2083–2091. [CrossRef]

65. Galovičová, L.; Borotová, P.; Valková, V.; Vukovic, N.L.; Vukic, M.; Terentjeva, M.; Štefániková, J.; Ďúranová, H.; Kowalczewski, P.Ł.; Kačániová, M. *Thymus serpyllum* Essential Oil and Its Biological Activity as a Modern Food Preserver. *Plants* **2021**, *10*, 1416. [CrossRef]

66. *M07-A10 Methods for Dilution antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*, 10th ed.; CLSI: Wayne, PA, USA, 2015.

67. *M7-A6 Methods for Dilution antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*, 6th ed.; NCCLS: Wayne, PA, USA, 2018.

68. Borotová, P.; Galovičová, L.; Valková, V.; Ďúranová, H.; Vuković, N.; Vukić, M.; Babošová, M.; Kačániová, M. Biological activity of essential oil from *Foeniculum vulgare*. *Acta Hortic. Regiotect.* **2021**, *24*, 148–152. [CrossRef]

69. Kačániová, M.; Galovičová, L.; Valková, V.; Ďúranová, H.; Štefániková, J.; Čmiková, N.; Vukic, M.; Vukovic, N.L.; Kowalczewski, P.Ł. Chemical Composition, Antioxidant, In Vitro and In Situ Antimicrobial, Antibiofilm, and Anti-Insect Activity of *Cedar atlantica* Essential Oil. *Plants* **2022**, *11*, 358. [CrossRef]