Nematicidal Activity of Essential Oils on a Psychrophilic *Panagrolaimus* sp. (Nematoda: Panagrolaimidae)

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**Abstract:** Essential oils (EOs) have historically been used for centuries in folk medicine, and nowadays they seem to be a promising control strategy against wide spectra of pathogens, diseases, and parasites. Studies on free-living nematodes are scarce. The free-living microbivorous nematode *Panagrolaimus* sp. was chosen as the test organism. The nematode possesses extraordinary biological properties, such as resistance to extremely low temperatures and long-term survival under minimal metabolic activity. Fifty EOs from 22 plant families of gymnosperms and angiosperms were tested on *Panagrolaimus* sp. The aims of this study were to investigate the in vitro impact of EOs on the psychrophilic nematode *Panagrolaimus* sp. in a direct contact bioassay, to list the activity of EOs based on median lethal concentration (LC50), to determine the composition of the EOs with the best nematicidal activity, and to compare the activity of EOs on *Panagrolaimus* sp. versus plant parasitic nematodes. The results based on the LC50 values, calculated using Probit analysis, categorized the EOs into three categories: low, moderate and highly active. The members of the laurel family, i.e., *Cinnamomum cassia* and *C. burmannii*, exhibited the best nematicidal activity. Aldehydes were generally the major chemical components of the most active EOs and were the chemicals potentially responsible for the nematicidal activity.

**Keywords:** essential oils; *Panagrolaimus* sp.; LC50; aldehydes

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1. Introduction

Nematodes are mostly microscopic size invertebrates that inhabit terrestrial and aquatic areas. Beside their significant economic importance as human, animal and plant parasites, they can also be beneficial, free-living microbivorous organisms. It has been estimated that about 2.5 million tons of pesticides are used on crops each year [1]. Such a practice has resulted in the decline of many beneficial organisms, such as nitrogen-fixing soil bacteria [2], blue-green algae [3], mycorrhizal fungi [4], water fishes [5], aquatic mammals [6], and birds [7]. In addition, the pesticide residues in food and water are massive long-term threats for human health at a global level. According to the European legislation (Regulation (EC) no. 1107/2009), the application of non-chemical and natural alternatives should be the first choice in plant protection and integrated pest management. The Regulation requires that substances or products produced or placed on the market do not have any harmful effect on human
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or animal health or any unacceptable effects on the environment, such as an impact on non-target species and impact on biodiversity and the ecosystem.

The use of essential oils (EOs) is known from folk medicine centuries ago [8]. Nowadays, it seems to be a promising control strategy against different nematode plant and animal parasites (Bursaphelenchus xylophilus, Cooperia spp., Ditylenchus dipsaci, Haemonchus contortus, Meloidogyne chitwoodi, M. incognita, M. javanica, Oesophagostomum spp., Pratylenchus penetrans, Steinernema feltiae, Trichostrongylus spp., Tylenchulus semipenetrans) [9–23]. Microbivorous nematodes contribute to decomposition of organic matter and the release of nutrients for plant uptake [24], which makes them important components of the soil microfauna. A free-living, microbivorous nematode, Panagrolaimus sp. Fuchs, was chosen as a test organism. Panagrolaimus sp. is a non-target organism, easy to maintain, does not have a complex life cycle, in contrast to plant parasitic nematodes [25], and possesses some extraordinary biological properties. This nematode, known as the Antarctic nematode, is famous for its resistance to intracellular freezing and extremely cold environmental conditions [26]. Panagrolaimus aff. detritophagus is the first viable multicellular organism, isolated from 30,000–40,000-year-old permafrost deposits [27].

This study aims to: (i) investigate the in vitro impact of EOs on the psychrophilic nematode Panagrolaimus sp. in a direct contact bioassay, (ii) list the activity of the EOs based on median lethal concentration (LC50), (iii) determine the composition of the EOs with the best nematicidal activity, and (iv) compare the activity of EOs on the non-target panagrolaimid nematode versus plant parasitic nematodes.

2. Results

The nematicidal activity of the EOs on the Panagrolaimus sp. juveniles are presented in Table 1.

Table 1. LC50 (in µL/mL with 95% confidence limits and the slope values) of 50 plant essential oils investigated on Panagrolaimus sp. juveniles.

| Species Name         | Plant Part | Family       | LC50 (95% CL)       | Slope  |
|----------------------|------------|--------------|---------------------|--------|
| Pogostemon cablin    | leaves     | Lamiaceae    | 5.641 (3.18–17.10)  | 2.14   |
| Pinus pinaster       | needles    | Pinaceae     | 5.078 (3.38–9.65)   | 1.65   |
| Santalum album       | wood       | Santalaceae  | 4.781 (3.45–6.34)   | 2.12   |
| Azadirachta indica   | seeds      | Meliaceae    | 4.082 (2.52–10.01)  | 2.02   |
| Boswellia serrata    | resin      | Burseraceae  | 4.394 (3.44–5.53)   | 3.03   |
| Comminiphora myrrha  | resin      | Burseraceae  | 4.301 (2.81–6.98)   | 2.79   |
| Juniperus virginiana | wood       | Juniperaceae | 3.782 (2.43–5.48)   | 1.81   |
| Cupressus sempervirens| needles   | Cupressaceae | 3.360 (2.47–4.82)   | 1.85   |
| Abies sibirica       | needles    | Pinaceae     | 3.269 (2.14–5.41)   | 1.58   |
| Cedrus atlantica     | wood       | Pinaceae     | 2.943 (2.04–4.43)   | 1.62   |
| Juniperus communis   | berries    | Juniperaceae | 2.513 (1.64–3.83)   | 1.68   |
| Eucalyptus globulus   | leaves     | Myrtaceae    | 1.994 (1.47–2.56)   | 3.70   |
| Myrtus communis      | leaves     | Myrtaceae    | 1.933 (1.34–2.65)   | 2.27   |
| Piper nigrum         | peppercorns| Piperaceae   | 1.775 (1.37–2.25)   | 2.37   |
| Petroselinum crispum | seeds      | Apiaceae     | 1.704 (1.14–2.38)   | 4.08   |
| Zingiber officinale  | roots      | Zingiberaceae| 1.633 (1.28–2.09)   | 1.98   |
| Turnera diffusa      | leaves/flowers| Passifloraceae| 1.550 (1.14–2.04)   | 3.11   |
| Abies alba           | needles    | Pinaceae     | 1.444 (1.03–1.95)   | 1.87   |
| Taxandria fragrans   | leaves     | Myrtaceae    | 1.437 (0.99–1.93)   | 2.49   |
| Melaleuca alternifolia| leaves    | Myrtaceae    | 1.150 (0.76–1.57)   | 4.83   |
| Vanilla planifolia   | beans      | Orchidaceae  | 1.135 (0.83–1.50)   | 2.08   |
| Salvia rosmarinus    | leaves/flowers| Lamiaceae    | 1.128 (0.88–1.41)   | 3.04   |
| Curcuma longa        | rhizomes   | Zingiberaceae| 1.116 (0.70–1.62)   | 1.50   |
| Lavandula sp.        | leaves/flowers| Lamiaceae    | 0.810 (0.62–1.01)   | 3.49   |
| Laurus nobilis       | leaves     | Lauraceae    | 0.594 (0.31–0.92)   | 4.80   |
| Melaleuca quinquenervia| leaves     | Myrtaceae    | 0.593 (0.40–0.82)   | 1.76   |
| Origaniun vulgare     | leaves/flowers| Lamiaceae    | 0.508 (0.38–0.65)   | 3.33   |
Table 1. Cont.

| Species Name                     | Plant Part     | Family    | LC50 (95% CL)     | Slope  |
|----------------------------------|----------------|-----------|-------------------|--------|
| Mentha spicata                   | leaves/flowers | Lamiaceae | 0.505 (0.39–0.64) | 2.78   |
| Pimpinella anisum                | seeds          | Apiaceae  | 0.450 (0.26–0.63) | 4.87   |
| Salvia sclarea                   | leaves/flowers | Lamiaceae | 0.430 (0.31–0.57) | 1.81   |
| Anethum graveolens               | seeds          | Apiaceae  | 0.428 (0.32–0.56) | 3.00   |
| Mentha piperita                  | leaves/flowers | Lamiaceae | 0.405 (0.23–0.58) | 3.93   |
| Thymus vulgaris                  | leaves/flowers | Lamiaceae | 0.391 (0.29–0.50) | 2.22   |
| Gaultheria procumbens            | leaves         | Ericaceae | 0.367 (0.26–0.48) | 2.25   |
| Myristica fragrans               | seeds          | Myristicaceae | 0.345 (0.23–0.48) | 4.24   |
| Pelargonium asperum              | leaves         | Geraniaceae | 0.279 (0.20–0.37) | 3.03   |
| Cymbopogon martini              | grass blades   | Poaceae   | 0.275 (0.20–0.35) | 4.06   |
| Syzygium aromaticum             | buds           | Myrtaceae | 0.272 (0.20–0.34) | 3.91   |
| Ocimum basilicum                | leaves/flowers | Lamiaceae | 0.263 (0.19–0.35) | 2.65   |
| Uncaria tomentosa               | bark           | Rubiaceae | 0.222 (0.17–0.29) | 2.47   |
| Illicium verum                  | seeds          | Schisandraceae | 0.191 (0.14–0.25) | 2.94   |
| Cinnamomum verum                | leaves         | Lauraceae | 0.172 (0.12–0.23) | 5.00   |
| Cananga odorata                 | flowers        | Annonaceae | 0.145 (0.10–0.19) | 5.00   |
| Melissa officinalis             | leaves         | Lamiaceae | 0.124 (0.09–0.16) | 2.74   |
| Litsea citrata                  | fruits         | Lauraceae | 0.091 (0.06–0.12) | 3.14   |
| Foeniculum vulgare              | seeds          | Apiaceae  | 0.080 (0.05–0.11) | 3.68   |
| Cymbopogon flexuosus            | grass blades   | Poaceae   | 0.071 (0.05–0.09) | 2.92   |
| Coriandrum sativum              | leaves         | Apiaceae  | 0.044 (0.02–0.04) | 3.95   |
| Cinnamomum cassia               | bark           | Lauraceae | 0.034 (0.02–0.04) | 2.00   |
| Cinnamomum burmannii            | bark           | Lauraceae | 0.033 (0.02–0.04) | 2.73   |

The results based on the median lethal concentration (LC50) of 50 EOs are in range from 0.033 to 5.641 µL/mL. The list of all EOs could be divided into three groups. The first group is made up of those with LC50 values higher than 1 µL/mL, the next group with LC50 values in the range 0.1 to 1 µL/mL, and the last group with the LC50 values lower than 0.1 µL/mL. The lowest nematicidal impact is observed in the first group containing EOs from different plants with a significant content of gymnosperms, represented by the families Pinaceae and Cupressaceae. In the same group are some members of angiosperms, such as Burseraceae, Myrtaceae, etc. The second group with a moderate nematicidal effect on the panagrolaimid nematode had EOs originating mainly from the family Lamiaceae and some representatives from individual families. This study demonstrates, for the first time, the nematicidal activity of Turnera diffusa, Taxandria fragrans and Uncaria tomentosa EOs originating from the families Passifloraceae, Myrtaceae, and Rubiaceae, respectively. The best nematicidal activity among the three species was exhibited by Uncaria tomentosa EO, with an LC50 of 0.222 µL/mL. The highest nematicidal impact was observed with three representatives from the family Lauraceae, namely Litsea citrata, Cinnamomum cassia, and C. burmannii, two representatives from the family Apiaceae—i.e., Foeniculum vulgare and Coriandrum sativum—and the single species Cymbopogon flexuosus from the family Poaceae, with LC50 values ranging from 0.033 to 0.091 µL/mL. The best nematicidal effect on panagrolaimid nematodes was shown by Cinnamomum burmannii EO, extracted from the bark. The chemical composition of the EOs with the best nematicidal performance on Panagrolaimus sp., with the retention time (RT, in minutes) and the retention indices obtained experimentally and from the literature (RI_exp and RI_lit, respectively), are given in Tables 2–7.

According to the gas chromatography/mass spectrometry (GC/MS) result obtained, 24 compounds were identified, representing 99.2% of total Litsea. citrata EO composition. The main components belong to oxygen-containing monoterpenes (contributing 84.3%), with citral—i.e., geranial (43.4%) and neral (32.2%)—as their representatives present in the highest percentage. They are followed by monoterpene hydrocarbons with 12.2% and limonene as their representative with a contribution of 9.4%, and sesquiterpene hydrocarbons ((E)-caryophyllene, β-elemene and α-humulene) with a contribution of 1.9% to the total EO composition (Table 2).
Table 2. Chemical composition of *Litsea citrata* essential oil (EO).

| RT  | Compound               | Molecular Formula | $\text{RI}^{\text{exp}}$ | $\text{RI}^{\text{lit}}$ | %   |
|-----|------------------------|-------------------|--------------------------|--------------------------|-----|
| 18.23 | Geranial (=E-citral)   | C$_{10}$H$_{16}$O | 1274                     | 1267                     | 43.4|
| 16.94 | Nerol (=Z-citral)     | C$_{10}$H$_{16}$O | 1243                     | 1238                     | 32.2|
| 8.49  | Limonene               | C$_{10}$H$_{16}$  | 1028                     | 1029                     | 9.4 |
| 8.57  | 1,8-Cineole            | C$_{15}$H$_{24}$O| 1030                     | 1031                     | 1.9 |
| 24.27 | (E)-Caryophyllene     | C$_{15}$H$_{24}$  | 1419                     | 1419                     | 1.6 |
| 13.17 | Citronellal            | C$_{10}$H$_{16}$O | 1153                     | 1153                     | 1.4 |
| 11.03 | Linalool               | C$_{10}$H$_{16}$O | 1100                     | 1096                     | 1.2 |
| 5.70  | α-Pinene               | C$_{10}$H$_{16}$  | 933                      | 939                      | 1.1 |
| 14.40 | (E)-Isocitral          | C$_{10}$H$_{16}$O | 1183                     | 1180                     | 1.1 |
| 17.42 | Geraniol               | C$_{10}$H$_{16}$O | 1255                     | 1252                     | 0.9 |
| 6.87  | β-Pinene               | C$_{10}$H$_{16}$  | 977                      | 979                      | 0.8 |
| 7.10  | 6-Methyl-5-hepten-2-one| C$_{8}$H$_{14}$O  | 985                      | 985                      | 0.8 |
| 13.66 | (Z)-Isoeostil         | C$_{10}$H$_{16}$O | 1164                     | 1164                     | 0.8 |
| 6.76  | Sabinene               | C$_{10}$H$_{16}$  | 973                      | 975                      | 0.6 |
| 14.71 | α-Terpineol            | C$_{10}$H$_{16}$O | 1190                     | 1188                     | 0.6 |
| 16.31 | Nerol                  | C$_{10}$H$_{16}$O | 1229                     | 1229                     | 0.4 |
| 6.10  | Camphene               | C$_{10}$H$_{16}$  | 948                      | 954                      | 0.3 |
| 7.25  | Dehydro-1,8-cineole    | C$_{10}$H$_{16}$O | 991                      | 991                      | 0.2 |
| 23.10 | β-Elemene              | C$_{15}$H$_{24}$  | 1392                     | 1390                     | 0.2 |
| 12.84 |exo-Isocitral          | C$_{10}$H$_{16}$O | 1144                     | 1144                     | 0.1 |
| 14.17 | Terpinen-4-ol          | C$_{10}$H$_{16}$O | 1177                     | 1177                     | 0.1 |
| 25.65 | α-Humulene             | C$_{15}$H$_{24}$  | 1453                     | 1454                     | 0.1 |
| 5.51  | α-Thujene              | C$_{10}$H$_{16}$  | 926                      | 930                      | tr  |
| 8.35  | o-Cymene               | C$_{10}$H$_{14}$  | 1024                     | 1026                     | tr  |

Total identified 99.2

* RT—retention time, $^*$ $\text{RI}^{\text{exp}}$—retention index obtained experimentally, $^{**}$ $\text{RI}^{\text{lit}}$—retention index from the literature, tr—traces.

According to the results of GC/MS analysis of *Foeniculum. vulgare* EO, 18 compounds were identified, representing 99.1% of total EO composition. The main components were aromatic compounds (78.5%), with (E)-anethole as their representative present in the highest percentage (74.3%), followed by oxygen-containing monoterpenes (14.8%) and their representatives fenchone (2.1%) and carvone (2.1%), and monoterpene hydrocarbons (5.8%) with the highest amount of limonene (2.3%) (Table 3).

Table 3. Chemical composition of *Foeniculum vulgare* EO.

| RT  | Compound               | Molecular Formula | $\text{RI}^{\text{exp}}$ | $\text{RI}^{\text{lit}}$ | %   |
|-----|------------------------|-------------------|--------------------------|--------------------------|-----|
| 18.93 | (E)-Anethole           | C$_{10}$H$_{15}$O$_2$ | 1291                     | 1284                     | 74.3|
| 11.28 | α-Pinene oxide         | C$_{10}$H$_{16}$O | 1106                     | 1099                     | 9.0 |
| 15.06 | Methyl chavicol (=Estragol) | C$_{10}$H$_{12}$O | 1199                     | 1196                     | 2.9 |
| 5.71  | α-Pinene               | C$_{10}$H$_{16}$  | 933                      | 939                      | 2.3 |
| 8.48  | Limonene               | C$_{10}$H$_{16}$  | 1028                     | 1029                     | 2.3 |
| 10.62 | Fenchone               | C$_{10}$H$_{16}$O | 1088                     | 1086                     | 2.1 |
| 16.94 | Carvone                | C$_{10}$H$_{14}$O | 1244                     | 1243                     | 2.1 |
| 17.39 | p-Anis aldehyde        | C$_{8}$H$_{8}$O$_2$ | 1254                     | 1250                     | 1.3 |
| 8.33  | o-Cymene               | C$_{10}$H$_{14}$  | 1024                     | 1026                     | 0.7 |
| 12.81 | Camphor                | C$_{10}$H$_{16}$O | 1144                     | 1146                     | 0.6 |
| 8.57  | 1,8-Cineole            | C$_{10}$H$_{18}$O | 1030                     | 1031                     | 0.5 |
| 11.07 | Linalool               | C$_{10}$H$_{18}$O | 1001                     | 1096                     | 0.4 |
| 9.53  | γ-Terpineol            | C$_{10}$H$_{16}$  | 1058                     | 1059                     | 0.3 |
| 6.10  | Camphene               | C$_{10}$H$_{16}$  | 948                      | 954                      | 0.1 |
Table 3. Cont.

| RT  | Compound       | Molecular Formula | RI<sup>exp</sup> | RI<sup>lit</sup> | %   |
|-----|----------------|-------------------|------------------|-----------------|-----|
| 6.87| β-Pinene       | C<sub>10</sub>H<sub>16</sub> | 977              | 979             | 0.1 |
| 11.79| α-Campholenal | C<sub>10</sub>H<sub>16</sub>O | 1119             | 1126            | 0.1 |
| 6.76| Sabinene       | C<sub>10</sub>H<sub>16</sub> | 973              | 975             | tr  |
| 7.24| Myrcene        | C<sub>10</sub>H<sub>16</sub> | 991              | 990             | tr  |

Total identified 99.1

* RT—retention time, ** RI<sup>exp</sup>—retention index obtained experimentally, *** RI<sup>lit</sup>—retention index from the literature, tr—traces.

The results of the GC/MS analysis of the *Cymbopogon flexuosus* EO revealed 32 compounds, representing 97.3% of total EO composition. The main components were oxygen-containing monoterpenes (86.3%) with geranial and neral (citral), contributing 40.3% and 30.9%, respectively, geranyl acetate and geraniol (5.4% and 4.5%, respectively), followed by sesquiterpene hydrocarbons (4.0%) and their representative (E)-caryophyllene (2.1%) (Table 4).

Table 4. Chemical composition of *Cymbopogon flexuosus* EO.

| RT  | Compound       | Molecular Formula | RI<sup>exp</sup> | RI<sup>lit</sup> | %   |
|-----|----------------|-------------------|------------------|-----------------|-----|
| 18.24| Geranial (E-citral) | C<sub>10</sub>H<sub>16</sub>O | 1274             | 1267            | 40.3 |
| 16.94| Neral (Z-citral)   | C<sub>10</sub>H<sub>16</sub>O | 1244             | 1238            | 30.9 |
| 22.86| Geranyl acetate   | C<sub>12</sub>H<sub>20</sub>O<sub>2</sub> | 1385             | 1381            | 5.4  |
| 17.45| Geraniol         | C<sub>10</sub>H<sub>16</sub>O | 1255             | 1252            | 4.5  |
| 7.10 | 6-Methyl-5-hepten-2-one | C<sub>6</sub>H<sub>14</sub>O | 985              | 985             | 2.7  |
| 24.27| (E)-Caryophyllene | C<sub>15</sub>H<sub>24</sub> | 1419             | 1419            | 2.1  |
| 11.03| Linalool         | C<sub>10</sub>H<sub>16</sub>O | 1101             | 1096            | 1.4  |
| 28.08| γ-Cadinene       | C<sub>15</sub>H<sub>24</sub> | 1514             | 1513            | 1.4  |
| 14.38| (E)-Isocitral    | C<sub>10</sub>H<sub>16</sub>O | 1182             | 1180            | 1.3  |
| 6.10 | Camphene         | C<sub>10</sub>H<sub>16</sub> | 948              | 954             | 1.1  |
| 8.48 | Limonene         | C<sub>10</sub>H<sub>16</sub> | 1028             | 1029            | 1.1  |
| 13.64| (Z)-Isocitral    | C<sub>10</sub>H<sub>16</sub>O | 1164             | 1164            | 0.9  |
| 5.71 | α-Pinene         | C<sub>10</sub>H<sub>16</sub> | 933              | 939             | 0.3  |
| 7.24 | 1,8-Dehydro-cineole | C<sub>10</sub>H<sub>16</sub>O | 991              | 990             | 0.3  |
| 8.56 | 1,8-Cineole      | C<sub>10</sub>H<sub>16</sub>O | 1030             | 1031            | 0.3  |
| 13.03| trans-α-Nerodol  | C<sub>10</sub>H<sub>16</sub>O | 1149             | 1148            | 0.3  |
| 13.16| Citronellal      | C<sub>10</sub>H<sub>16</sub>O | 1152             | 1153            | 0.3  |
| 28.45| δ-Cadinene       | C<sub>15</sub>H<sub>24</sub> | 1523             | 1523            | 0.3  |
| 30.72| Caryophyllene oxide | C<sub>15</sub>H<sub>24</sub>O | 1582             | 1582            | 0.3  |
| 8.76 | (Z)-β-Ocimene    | C<sub>10</sub>H<sub>16</sub> | 1036             | 1037            | 0.2  |
| 25.64| α-Humulene       | C<sub>15</sub>H<sub>24</sub> | 1453             | 1454            | 0.2  |
| 5.43 | Tricyclene       | C<sub>10</sub>H<sub>16</sub> | 922              | 926             | 0.1  |
| 8.34 | α-Cymene         | C<sub>10</sub>H<sub>14</sub> | 1024             | 1026            | 0.1  |
| 9.14 | (E)-β-Ocimene    | C<sub>10</sub>H<sub>16</sub> | 1046             | 1050            | 0.1  |
| 10.62| Terpinolene      | C<sub>10</sub>H<sub>16</sub> | 1088             | 1088            | 0.1  |
| 12.84| exo-Isocitral    | C<sub>10</sub>H<sub>16</sub>O | 1144             | 1144            | 0.1  |
| 14.08| Rosefuran epoxide | C<sub>10</sub>H<sub>14</sub>O<sub>2</sub> | 1175             | 1177            | 0.1  |
| 14.72| α-Terpineol      | C<sub>10</sub>H<sub>16</sub>O | 1191             | 1188            | 0.1  |
| 16.32| Nerol            | C<sub>10</sub>H<sub>16</sub>O | 1229             | 1229            | 0.1  |
| 6.87 | β-Pinene         | C<sub>10</sub>H<sub>16</sub> | 977              | 979             | tr  |
| 7.60 | n-Octanal        | C<sub>8</sub>H<sub>16</sub>O | 1003             | 998             | tr  |

Total identified 97.3

* RT—retention time, ** RI<sup>exp</sup>—retention index obtained experimentally, *** RI<sup>lit</sup>—retention index from the literature, tr—traces.

GC/MS analysis of the *Coriandrum sativum* EO resulted in identifying 29 compounds, representing 97.5% of total EO composition. The main components were aldehydes (contributing 51.8%),
with (2E)-decenal as their representative, followed by aliphatic alcohols (among which (2E)-decen-1-ol was present in the highest percentage of 16.3%) and oxygen-containing monoterpenes (21.7%) with linalool as the most abundant compound (18.4%) (Table 5).

### Table 5. Chemical composition of *Coriandrum sativum* EO.

| RT  | Compound          | Molecular Formula | R1<sup>exp</sup> | R1<sup>lit</sup> | %    |
|-----|-------------------|-------------------|------------------|----------------|------|
| 17.78 | (2E)-Decenal | C<sub>10</sub>H<sub>18</sub>O | 1263             | 1263           | 28.2 |
| 18.10 | (2E)-Decen-1-ol | C<sub>10</sub>H<sub>20</sub>O | 1271             | 1271           | 16.3 |
| 11.08 | Linalool        | C<sub>10</sub>H<sub>18</sub>O | 1101             | 1096           | 18.4 |
| 26.20 | (2E)-Dodecaenal | C<sub>12</sub>H<sub>22</sub>O | 1467             | 1466           | 8.8  |
| 15.35 | n-Decanal       | C<sub>10</sub>H<sub>20</sub>O | 1206             | 1201           | 6.2  |
| 18.19 | n-Decanol       | C<sub>10</sub>H<sub>22</sub>O | 1273             | 1269           | 5.4  |
| 34.07 | n-Tetradecanol  | C<sub>14</sub>H<sub>30</sub>O | 1673             | 1672           | 4.0  |
| 21.97 | (2E)-Undecenal  | C<sub>11</sub>H<sub>20</sub>O | 1363             | 1360           | 1.7  |
| 12.80 | Camphor         | C<sub>10</sub>H<sub>18</sub>O | 1143             | 1146           | 1.2  |
| 23.85 | Dodecanal      | C<sub>12</sub>H<sub>24</sub>O | 1409             | 1408           | 1.1  |
| 8.34  | α-Cymene        | C<sub>10</sub>H<sub>14</sub>  | 1024             | 1026           | 0.9  |
| 5.71  | α-Pinene        | C<sub>10</sub>H<sub>16</sub>  | 933              | 939            | 0.7  |
| 15.00 | (4E)-Decenal    | C<sub>10</sub>H<sub>18</sub>O | 1198             | 1196           | 0.6  |
| 17.43 | Geraniol        | C<sub>10</sub>H<sub>18</sub>O | 1255             | 1252           | 0.6  |
| 10.03 | cis-Linalool oxide | C<sub>10</sub>H<sub>18</sub>O<sub>2</sub> | 1072         | 1072           | 0.4  |
| 10.62 | trans-Linalool oxide | C<sub>10</sub>H<sub>18</sub>O<sub>2</sub> | 1088     | 1086           | 0.4  |
| 19.62 | Undecanal       | C<sub>11</sub>H<sub>22</sub>O | 1307             | 1306           | 0.4  |
| 22.85 | Geranyl acetate | C<sub>12</sub>H<sub>20</sub>O<sub>2</sub> | 1384         | 1381           | 0.4  |
| 7.60  | n-Octanal       | C<sub>8</sub>H<sub>16</sub>O | 1003             | 998            | 0.3  |
| 8.48  | Limonene        | C<sub>10</sub>H<sub>16</sub>  | 1028             | 1029           | 0.3  |
| 31.86 | Tetradecanal    | C<sub>14</sub>H<sub>28</sub>O | 1613             | 1612           | 0.3  |
| 8.57  | 1,8-Cineole     | C<sub>10</sub>H<sub>18</sub>O | 1030             | 1031           | 0.2  |
| 9.53  | γ-Terpinene     | C<sub>10</sub>H<sub>16</sub>  | 1058             | 1059           | 0.2  |
| 14.85 | (4Z)-Decenal    | C<sub>10</sub>H<sub>18</sub>O | 1194             | 1194           | 0.2  |
| 6.10  | Camphene        | C<sub>10</sub>H<sub>16</sub>  | 948              | 954            | 0.1  |
| 6.88  | β-Pinene        | C<sub>10</sub>H<sub>16</sub>  | 977              | 979            | 0.1  |
| 14.71 | α-Terpineol     | C<sub>10</sub>H<sub>18</sub>O | 1190             | 1188           | 0.1  |
| 13.69 | Borneol         | C<sub>10</sub>H<sub>18</sub>O | 1165             | 1169           | tr   |
| 14.17 | Terpinen-4-ol  | C<sub>10</sub>H<sub>18</sub>O | 1177             | 1177           | tr   |

Total identified 97.5

* RT—retention time, ** R1<sup>exp</sup>—retention index obtained experimentally, *** R1<sup>lit</sup>—retention index from the literature, tr—traces.

The GC/MS analysis of *Cinnamomum cassia* EO revealed 32 compounds, representing 99.3% of total EO composition. The main components belong to the group of aromatic compounds, contributing 91.8%, followed by sesquiterpene hydrocarbons (6.7%). (E)-Cinnamaldehyde and eugenol acetate were identified as the representatives of aromatic compounds contributing 76.7% and 7.4%, respectively. On the other hand, δ-cadinene with a contribution of 6.2% to the total EO composition, was identified as a representative compound from the sesquiterpene hydrocarbons group (6.7%) (Table 6).

According to the GC/MS results, 43 compounds, representing 98.1% of total *C. burmanii* EO composition, were identified. The main components belong to aromatic compounds (contributing 84.5% in the total EO composition), with (E)-cinnamaldehyde as their representative (80.5%). They are followed by sesquiterpene hydrocarbons (7.0%) with δ-cadinene and α-copaene as their members present in the amounts of 1.7% and 1.5%, respectively and oxygenated monoterpenes (5.5%) with α-terpineol (1.9%) as their main representative (Table 7).
Table 6. Chemical composition of *Cinnamomum cassia* EO.

| RT * | Compound                  | Molecular Formula | R \textsuperscript{exp} ** | R \textsuperscript{lit} *** | %   |
|------|--------------------------|-------------------|----------------------------|-----------------------------|------|
| 24.54| (E)-Cinnamaldehyde       | C\textsubscript{6}H\textsubscript{8}O | 1272                       | 1267                        | 76.7 |
| 33.02| Eugenol acetate          | C\textsubscript{12}H\textsubscript{14}O\textsubscript{3} | 1535                       | 1521                        | 7.4  |
| 32.39| δ-Cadinene               | C\textsubscript{15}H\textsubscript{24} | 1517                       | 1522                        | 6.2  |
| 29.95| (E)-Cinnamyl acetate     | C\textsubscript{11}H\textsubscript{12}O\textsubscript{2} | 1448                       | 1443                        | 4.0  |
| 17.88| Phenethyl alcohol        | C\textsubscript{8}H\textsubscript{10}O | 1126                       | 1106                        | 0.8  |
| 11.96| Benzaldehyde             | C\textsubscript{6}H\textsubscript{6}O | 962                        | 952                         | 0.7  |
| 21.91| (Z)-Cinnamaldehyde       | C\textsubscript{6}H\textsubscript{10}O | 1220                       | 1217                        | 0.6  |
| 22.78| Carvone                  | C\textsubscript{10}H\textsubscript{14}O | 1243                       | 1239                        | 0.4  |
| 19.69| Hydrocinnamaldehyde      | C\textsubscript{6}H\textsubscript{10}O | 1163                       | 1163                        | 0.3  |
| 25.28| α-Methylcinnamaldehyde   | C\textsubscript{10}H\textsubscript{10}O | 1332                       | 1318                        | 0.3  |
| 30.51| Coumarine                | C\textsubscript{6}H\textsubscript{8}O\textsubscript{2} | 1456                       | 1432                        | 0.3  |
| 15.10| γ-Terpinene              | C\textsubscript{10}H\textsubscript{16} | 1056                       | 1054                        | 0.2  |
| 23.20| 2-Phenyl ethyl acetate   | C\textsubscript{10}H\textsubscript{12}O\textsubscript{2} | 1259                       | 1254                        | 0.2  |
| 27.57| α-Copaene                | C\textsubscript{15}H\textsubscript{24} | 1368                       | 1374                        | 0.2  |
| 10.93| α-Pinene                 | C\textsubscript{10}H\textsubscript{16} | 929                        | 932                         | 0.1  |
| 14.39| 1,8-Cineole              | C\textsubscript{10}H\textsubscript{18}O | 1027                       | 1026                        | 0.1  |
| 19.88| Borneol                  | C\textsubscript{10}H\textsubscript{18}O | 1168                       | 1165                        | 0.1  |
| 20.19| Terpinen-4-ol            | C\textsubscript{10}H\textsubscript{18}O | 1174                       | 1174                        | 0.1  |
| 26.98| Eugenol                  | C\textsubscript{10}H\textsubscript{12}O\textsubscript{2} | 1359                       | 1356                        | 0.1  |
| 30.99| γ-Muurolene              | C\textsubscript{15}H\textsubscript{24} | 1470                       | 1478                        | 0.1  |
| 31.11| β-Selinene               | C\textsubscript{15}H\textsubscript{24} | 1478                       | 1489                        | 0.1  |
| 32.51| trans-Cadina-1,4-diene   | C\textsubscript{15}H\textsubscript{24} | 1526                       | 1533                        | 0.1  |
| 25.90| 1-epi-Cubenol            | C\textsubscript{15}H\textsubscript{26}O | 1622                       | 1627                        | 0.1  |
| 12.48| β-Pinene                 | C\textsubscript{10}H\textsubscript{16} | 973                        | 974                         | tr   |
| 14.25| β-Phellandrene           | C\textsubscript{10}H\textsubscript{16} | 1025                       | 1025                        | tr   |
| 16.93| Terpinolene              | C\textsubscript{10}H\textsubscript{16} | 1087                       | 1086                        | tr   |
| 27.27| Cyclosativene            | C\textsubscript{15}H\textsubscript{24} | 1357                       | 1358                        | tr   |
| 28.10| Sativene                 | C\textsubscript{15}H\textsubscript{24} | 1382                       | 1374                        | tr   |
| 28.84| Isosativene              | C\textsubscript{15}H\textsubscript{24} | 1401                       | 1417                        | tr   |
| 29.14| (E)-Caryophyllene        | C\textsubscript{15}H\textsubscript{24} | 1413                       | 1417                        | tr   |
| 31.76| α-Muurolene              | C\textsubscript{15}H\textsubscript{24} | 1494                       | 1500                        | tr   |

Total identified: 99.3

* RT—retention time, ** R\textsuperscript{exp}—retention index obtained experimentally, *** R\textsuperscript{lit}—retention index from the literature, tr—traces.

Table 7. Chemical composition of *Cinnamomum burmannii* EO.

| RT * | Compound                  | Molecular Formula | R \textsuperscript{exp} ** | R \textsuperscript{lit} *** | %   |
|------|--------------------------|-------------------|----------------------------|-----------------------------|------|
| 24.51| (E)-Cinnamaldehyde       | C\textsubscript{6}H\textsubscript{8}O | 1272                       | 1267                        | 80.5 |
| 20.70| α-Terpinene              | C\textsubscript{10}H\textsubscript{18}O | 1189                       | 1186                        | 1.9  |
| 32.51| δ-Cadinene               | C\textsubscript{15}H\textsubscript{24} | 1517                       | 1522                        | 1.7  |
| 27.57| α-Copaene                | C\textsubscript{15}H\textsubscript{24} | 1368                       | 1374                        | 1.5  |
| 21.82| (Z)-Cinnamaldehyde       | C\textsubscript{6}H\textsubscript{8}O | 1220                       | 1217                        | 1.5  |
| 19.60| Hydrocinnamaldehyde      | C\textsubscript{6}H\textsubscript{10}O | 1163                       | 1163                        | 1.3  |
| 19.79| Borneol                  | C\textsubscript{10}H\textsubscript{18}O | 1168                       | 1165                        | 1.2  |
| 20.17| Terpinen-4-ol            | C\textsubscript{10}H\textsubscript{18}O | 1174                       | 1174                        | 1.2  |
| 31.74| α-Muurolene              | C\textsubscript{15}H\textsubscript{24} | 1494                       | 1500                        | 1.2  |
| 29.05| (E)-Caryophyllene        | C\textsubscript{15}H\textsubscript{24} | 1413                       | 1417                        | 0.8  |
| 17.10| Linalool                 | C\textsubscript{10}H\textsubscript{18}O | 1100                       | 1095                        | 0.5  |
| 11.87| Benzaldehyde             | C\textsubscript{6}H\textsubscript{8}O | 962                        | 952                         | 0.4  |
| 24.54| Safrole                  | C\textsubscript{10}H\textsubscript{10}O\textsubscript{2} | 1288                       | 1285                        | 0.4  |
| 28.76| Isosativene              | C\textsubscript{15}H\textsubscript{24} | 1401                       | 1417                        | 0.4  |
| 31.32| α-Selinene               | C\textsubscript{15}H\textsubscript{24} | 1487                       | 1498                        | 0.4  |
| 24.75| Tridecane                | C\textsubscript{13}H\textsubscript{28} | 1295                       | 1300                        | 0.3  |
| 14.29| β-Phellandrene           | C\textsubscript{10}H\textsubscript{16} | 1025                       | 1025                        | 0.2  |
Table 7. Cont.

| RT  | Compound          | Molecular Formula | RI<sub>exp</sub> | RI<sub>lit</sub> | %   |
|-----|-------------------|-------------------|-----------------|-----------------|-----|
| 15.84 | cis-Linalool oxide      | C<sub>10</sub>H<sub>18</sub>O<sub>2</sub>  | 1071           | 1067            | 0.2 |
| 20.54 | Cryptone           | C<sub>9</sub>H<sub>14</sub>O            | 1185           | 1183            | 0.2 |
| 28.03 | Sativene           | C<sub>15</sub>H<sub>24</sub>            | 1382           | 1374            | 0.2 |
| 30.92 | γ-Muurolene        | C<sub>15</sub>H<sub>24</sub>            | 1470           | 1478            | 0.2 |
| 31.15 | β-Selinene         | C<sub>15</sub>H<sub>24</sub>            | 1478           | 1489            | 0.2 |
| 30.21 | (E)-Cinnamyl acetate | C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>  | 1448           | 1443            | 0.2 |
| 36.26 | epi-α-Murolol       | C<sub>15</sub>H<sub>26</sub>O           | 1639           | 1640            | 0.2 |
| 13.37 | α-Phellandrene     | C<sub>10</sub>H<sub>16</sub>            | 1002           | 1002            | 0.1 |
| 14.42 | 1,8-Cineole        | C<sub>10</sub>H<sub>18</sub>O           | 1027           | 1026            | 0.1 |
| 14.96 | γ-Terpineene       | C<sub>10</sub>H<sub>16</sub>            | 1056           | 1054            | 0.1 |
| 16.62 | p-Cymenene         | C<sub>10</sub>H<sub>14</sub>            | 1087           | 1089            | 0.1 |
| 16.99 | Terpinolene        | C<sub>10</sub>H<sub>16</sub>            | 1087           | 1086            | 0.1 |
| 18.87 | trans-Limonene oxide | C<sub>10</sub>H<sub>16</sub>O         | 1137           | 1137            | 0.1 |
| 25.24 | α-Methylcinnamaldehyde | C<sub>10</sub>H<sub>10</sub>O         | 1332           | 1318            | 0.1 |
| 27.18 | Cyclosativene      | C<sub>15</sub>H<sub>24</sub>            | 1357           | 1358            | 0.1 |
| 28.25 | β-Elemene          | C<sub>15</sub>H<sub>24</sub>            | 1386           | 1389            | 0.1 |
| 28.54 | (Z)-Caryophyllene  | C<sub>15</sub>H<sub>24</sub>            | 1400           | 1408            | 0.1 |
| 29.82 | Humulene           | C<sub>15</sub>H<sub>24</sub>            | 1446           | 1452            | 0.1 |
| 30.51 | Coumarin           | C<sub>9</sub>H<sub>6</sub>O<sub>2</sub>  | 1456           | 1432            | 0.1 |
| 36.35 | α-Muurol (Torreyol) | C<sub>15</sub>H<sub>26</sub>O           | 1643           | 1644            | 0.1 |
| 12.83 | Myrcene            | C<sub>10</sub>H<sub>16</sub>            | 994            | 998             | tr  |
| 17.70 | Phenethyl alcohol  | C<sub>8</sub>H<sub>10</sub>O            | 1126           | 1106            | tr  |
| 19.45 | Isoborneol         | C<sub>10</sub>H<sub>18</sub>O           | 1154           | 1155            | tr  |
| 32.80 | trans-Cadina-1,4-diene | C<sub>15</sub>H<sub>24</sub>        | 1526           | 1533            | tr  |
| 33.15 | α-Calacorene       | C<sub>15</sub>H<sub>20</sub>            | 1538           | 1544            | tr  |
| 32.92 | Benzyl benzoate    | C<sub>14</sub>H<sub>12</sub>O<sub>2</sub> | 1772           | 1759            | tr  |

Total identified 98.1

* RT—retention time, ** RI<sub>exp</sub>—retention index obtained experimentally, *** RI<sub>lit</sub>—retention index from the literature, tr—traces.

3. Discussion

Essential oils with the highest nematicidal activity, demonstrated in this study, have been reported to be efficient against wide spectra of pathogens, diseases, and parasites.

The *Litsea citrata* EO showed antibacterial, antifungal, acaricidal, and nematicidal activities. The fruit essential oil of *Litsea cubeba* (syn. *Litsea citrata*) exhibited antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Klebsiella pneumoniae* [28]. As an antifungal agent it was effective against *Candida krusei* and *C. guilliermondii* but did not act against *C. albicans*, *C. tropicalis* and *C. parapsilosis* [29]. The *Litsea cubeba* EO had acaricidal activity against house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*, and stored food mites, *Tyrophagus putrescentiae* [30]. The LC50 values of ajowan, allspice and litsea were 0.431, 0.609 and 0.504 mg/mL, respectively, and exhibited good nematicidal activity against *B. xylophilus* [31].

Citral, i.e., geranial and neral, were the main compounds in the *Litsea citrata* EO in this study. Citral (3,7-dimethyl-2,6-octadienal) is the monoterpene aldehyde representing natural mixture of the two geometric isomers: geranial (trans-isomer) with a strong lemon odor and neral (cis-isomer) with a lemon odor that is less intense and sweeter than geranial [32].

The *Foeniculum vulgare* EO exhibited antifungal, antibacterial, antiviral, and nematicidal activities. In the inverted petriplate method, the volatile oil showed complete zone inhibition against *Aspergillus niger*, *A. flavus*, *Fusarium graminearum*, and *F. moniliforme* at a 6-µL dose [33]. Hot water extracts of fennel seeds was effective against *Enterococcus faecalis*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *S. typhi*, and *Shigella flexneri* [34]. The DNA virus *Herpes simplex* type-1 (HSV-1) and the RNA virus parainfluenza type-3 (PI-3) were inhibited by the
was inhibitory to \( \text{Pyricularia oryzae} \) [54].

Trans-anethole was the most abundant compound in the \( \text{Foeniculum vulgare} \) EO, reaching 74% of the total identified constituents. Propenylbenzenes, such as anethole, were reported to be mutagenic for \( \text{Salmonella} \) tester strains and also carcinogenic in the induction of hepatomas in B6C3F1 mice and skin papillomas in CD-1 mice [36].

The \( \text{Cymbopogon flexuosus} \) lemongrass EO was reported to have antibacterial, antifungal and anti-inflammatory activity. The EO from \( \text{C. flexuosus} \) exhibited an antimicrobial effect against \( \text{B. subtilis}, \text{Staphylococcus aureus}, \text{A. flavus} \) and \( \text{A. fumigatus} \) [37]. The lemongrass (\( \text{C. flexuosus} \)) inflorescence EO was inhibitory to \( \text{Pyricularia oryzae, Drechslera oryzae, A. niger} \) and \( \text{Penicillium italicum} \) [38]. Lemongrass EO, which has citral as its main component, has exhibited an anti-inflammatory effect in both animal and human cells [39]. In this study, the content of the lemongrass EO’s major compounds, geraniol and neral, was similar to their content in the \( \text{L. citrata} \) EO with slightly lower amounts—40.3% and 30.9%, respectively.

The \( \text{Coriandrum sativum} \) EO exhibited antifungal, antibacterial, insecticidal, and nematicidal activities. The \( \text{Coriandrum sativum} \) EO showed excellent antifungal activity against seedborne pathogens \( \text{P. oryzae}, \text{Bipolaris oryzae, Alternaria alternata, Tricoconis padwickii, Drechslera tetramera, D. halodes, Curvularia lunata, F. moniliforme, and F. oxysporum} \) [40]. The methanolic extract of \( \text{C. sativum} \) showed antibacterial activity against \( \text{E. coli, P. aeruginosa, S. aureus, and K. pneumoniae} \) [41]. The leaf oil had significant toxic effects against the larvae of \( \text{Aedes aegypti} \) with an LC50 value of 26.93 ppm and an LC90 value of 37.69 ppm, and the stem oil has toxic effects against the larvae of \( \text{A. aegypti} \) with an LC50 value of 29.39 ppm and an LC90 value of 39.95 ppm [42]. Among the 28 plant EOs tested for their nematicidal activities against the pine wood nematode, \( \text{B. xylophilus} \), the best nematicidal activity was achieved with the EO of coriander [43]. In this study, the major compound in the \( \text{C. sativum} \) EO was trans-2-decenal with 28.2%, aminated to the group of medium-chain aldehydes. Aliphatic aldehydes (mainly \( \text{C}_{10-16} \) aldehydes), with their unpleasant odor, are the main components of the volatile oil from the fresh herb [44]. Aldehydes present in the coriander EO are important biologically active substances due to their possible toxic activity against tropical mosquitoes transmitting dangerous illnesses [45].

The \( \text{Cinnamomum cassia} \) EO exhibited antimicrobial, antiviral, insecticidal, and nematicidal activities. The cassia EO acted as fungal growth inhibitor against \( \text{A. flavus} \) and \( \text{A. oryzae} \) [46] and as a bacterial inhibitor of \( \text{S. aureus} \) and \( \text{E. coli} \) [47]. The silver nanoparticles derived from cinnamon extract enhanced the antiviral activity and were found to be effective against highly pathogenic avian influenza virus subtype H7N3, when incubated with the virus prior to infection and introduced to cells after infection [48]. The chloroform extract from \( \text{C. cassia} \) was the most effective against \( \text{Dermestes maculatus} \) larvae, the pest of Egyptian mummies [8]. Cassia oil was efficient against \( \text{Sitophilus zamais} \) [49], and the booklice \( \text{Liposcelis bostrychophila} \) [50]. As judged by the 24-h LC50 values, two cassia oils (0.084–0.085 mg/mL) and four cinnamon oils (0.064–0.113 mg/mL) were toxic toward adult \( \text{B. xylophilus} \) [51].

As opposed to cassia, \( \text{Cinnamomum burmannii} \) EO has been less studied. The \( \text{Cinnamomum burmannii} \) EO exhibited significant antibacterial properties against five common foodborne pathogenic bacteria, namely, \( \text{B. cereus, L. monocytogenes, S. aureus, E. coli, and Salmonella anatum} \) [52].

The major component of cinnamon bark EO is (E)-cinnamaldehyde. In the contact with bacterial membrane, cinnamaldehyde causes the loss of membrane functionality or the loss of channel proteins in the membrane, resulting in death of bacterial cells [53]. Besides this, (E)-cinnamaldehyde was significantly more effective than its corresponding acid (cinnamic acid) and alcohol (cinnamyl alcohol) and could be used as a fumigant with contact action in the control of house dust mites, \( \text{D. farinae} \) and \( \text{D. pteronyssinus} \) [54].

It has been emphasized that the major components play important roles in the toxicity of EOs [31,42,55] and the majority of them belong to the class of terpenes. Terpenes are the largest class
of secondary metabolites and basically consist of five carbon isoprene units, which are assembled to each other (many isoprene units) by thousands of ways. Terpenes are simple hydrocarbons, while terpenoids (monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes) are a modified class of terpenes with different functional groups and an oxidized methyl group moved or removed at various positions [56].

Organic compounds that contain the group -CHO (the aldehyde group; i.e., a carbonyl group (C=O) with a hydrogen atom bound to the carbon atom) are known as aldehydes. In systematic chemical nomenclature, aldehyde names end with the suffix -al [57].

In this study, the major components and presumably the most active components (geranial, neral, trans-2-decenal, and trans-cinnamaldehyde) of Litsea citrata, Cymbopogon flexuosus, Coriandrum sativum, Cinnamomum cassia, and C. burmannii EOs are aldehydes. Aldehydes are highly reactive molecules that may have a variety of effects on biological systems. Although some aldehyde-mediated effects are beneficial, many effects are deleterious, including cytotoxicity, mutagenicity, and carcinogenicity [58], and generally, they are toxic to the human body [59] and evidently, to nematodes. Despite the potential risks of aldehyde exposure, the toxic mechanisms are only understood in general terms. Human exposure to aldehydes represents a significant toxicological concern and, therefore, understanding the corresponding molecular mechanism of toxicity is important for accurate risk assessment and remediation. In this perspective, it has been shown that environmental and endogenous aldehydes can be described by their relative softness and electrophilicity, which are important electronic determinants of the respective second order reaction rates with nucleophilic targets on macromolecules. These soft-soft and hard-hard adduct reactions appear to mediate toxicity by impairing the function of macromolecules (e.g., proteins, DNA, and RNA) that play critical roles in cytophysiological processes. However, more research is needed to broaden our understanding of how these specific covalent reactions disable macromolecular targets [60].

Comparing the results for the toxicity of EOs for nematodes with a different oral apparatus, they are mostly in agreement. However, some results for Panagrolaimus sp. deviate from those obtained for plant parasitic nematodes.

The Rosmarinus officinalis (syn. Salvia rosmarinus) EO at 2 µg/mL induced 100% mortality of Xiphinema index adults [61], while in this study, the same EO was characterized as having low toxicity and classified into the first group of EOs.

The LC50 of the M. spicata EO was 0.2 mg/mL, for M. javanica juveniles [62], while in this study the same oil was characterized as having low toxicity and classified into the first group of EOs.

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Variations in acute toxicity among EOs of the same plant species are greatly influenced by production, storage conditions, climatic or edaphic factors [64]. The chemical content varies even within the same crop. Significant variations were found in many EO components, both across years and throughout harvest dates within locations [65]. However, the different impact of the same EO on free-living versus plant parasitic nematodes may be due to different feeding behaviors, different dimensions, and different metabolic activities and demonstrate a possible direction in the search for active compounds that will be at the same time toxic to plant parasitic nematodes and not have unacceptable effects on the environment and non-target species.
4. Materials and Methods

4.1. Nematode Culture and Direct Contact Bioassay

A culture of *Panagrolaimus* sp. was grown monoxenically on previously frozen agricultural compost and extracted from it with a Baerman funnel [66] over 24 h. Using a compound microscope and a micropipette, juveniles were separated from adult nematodes, counted in aliquots of 50 in 20 µL of water suspension and the live specimens were used in the experiments. The 50 commercial plant EOs from 22 families were purchased from the market and used to investigate their in vitro nematicidal activity against the panagrolaimid nematode *Panagrolaimus* sp. (Table 1). Serial dilutions starting from 0.2 µL/mL, in a double decreasing range up to 0.00975 µL/mL of EOs, were made and stabilized with 0.1-µL/mL Break-Thru® 446 oil enhancer. The direct contact bioassay was performed in small glass petri dishes containing 2 mL of solution and 50 nematodes incubated at 18 °C in the dark. The experiments were performed in five replicates. The lethal effect was monitored after 24 h. An aqueous solution of the emulsifier without EO served as the control. Prior to the assessment of the EOs, the mortality of panagrolaimid nematodes in the aqueous solution was compared with the mortality of nematodes in 0.1-µL/mL emulsifier and no significant differences between the treatments were observed. The nematodes were considered dead if they did not react on touching with a small needle.

4.2. Chemical Analyses

The gas chromatography/mass spectrometry (GC/MS) analysis was performed on an Agilent 6890N network gas chromatograph attached to a mass spectrometer (Agilent 5975B) equipped with a fused silica capillary column (HP-5ms) with dimensions as follows: 30-m length, 0.25-mm internal diameter, 0.25-µm film thickness, coated with 5% diphenyl- and 95% dimethyl-polysiloxane. The samples were diluted in diethyl ether (1:10) and a volume of 1.0 µL was injected. The injector was set at 220 °C and performed in the split mode at a ratio of 1:20. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. The oven temperature increased from 60 to 246 °C at a rate of 3 °C/min. Temperatures of the mass selective detector (MSD) transfer line, ion source and quadruple mass analyzer were set at 280, 230 and 150 °C, respectively. The ionization voltage was 70 eV and the scan range was 35–400 m/z.

Compound identifications were based on comparisons of their mass spectra with the mass spectra obtained from the National Institute of Standards and Technology database and by comparisons of the retention indices with values reported in the literature (RI\textsubscript{lit}) [67]. A homologous series of n-alkanes (C\textsubscript{8}–C\textsubscript{34}) was run under the same operating conditions as the EO to determine the experimental retention indices (RI\textsubscript{exp}). The relative amounts of individual components (expressed in percentages) were calculated via peak area normalization, without the use of correction factors. Compounds present in traces (tr) with their amounts less than 0.05% are indicated (Tables 2–7).

4.3. Statistical Data Analysis

In order to evaluate the nematicidal activity of the EOs, median lethal concentration (LC50) was calculated using the Probit Analysis program [68]. The *Panagrolaimus* mortality was corrected using Abbott’s formula [69]. The nematicidal activity, i.e., acute toxicity of the examined EOs based on the median lethal concentration, was designated as high (LC50: <0.1 µL/mL), moderate (LC50: 0.1–1 µL/mL) and low (LC50: >1 µL/mL) (Table 1).

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