Abstract: Mosquito-borne infectious diseases are a persistent problem in tropical regions of the world, including Southeast Asia. Vector control has relied principally on synthetic insecticides, but these have detrimental environmental effects and there is an increasing demand for plant-based agents to control insect pests. Invasive weedy plant species may be able to serve as readily available sources of essential oils, some of which may be useful as larvicidal agents for control of mosquito populations. We hypothesize that members of the genus *Conyza* (Asteraceae) may produce essential oils that may have mosquito larvicidal properties. The essential oils from the aerial parts of *Conyza bonariensis*, *C. canadensis*, and *C. sumatrensis* were obtained by hydrodistillation, analyzed by gas chromatography–mass spectrometry, and screened for mosquito larvicidal activity against *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus*. The essential oils of *C. canadensis* and *C. sumatrensis*, both rich in limonene (41.5% and 25.5%, respectively), showed notable larvicidal activities against *Ae. aegypti* (24-h LC₅₀ = 9.80 and 21.7 µg/mL, respectively) and *Ae. albopictus* (24-h LC₅₀ = 18.0 and 19.1 µg/mL, respectively). These two *Conyza* species may, therefore, serve as sources for alternative, environmentally-benign larvicidal control agents.
1. Introduction

Mosquito-borne infectious diseases have been a continuous health problem in Southeast Asia, including Vietnam. Dengue fever and dengue hemorrhagic fever are particularly problematic and chikungunya fever is an emerging threat in the country [1,2]. *Aedes aegypti* (L.) (Diptera: Culicidae), the yellow fever mosquito, is a recognized vector of dengue fever virus, chikungunya fever virus, Zika virus, and yellow fever virus [3]. *Aedes albopictus* (Skuse) (Diptera: Culicidae), the Asian tiger mosquito, is a key vector of several pathogenic viruses, including yellow fever virus [4], dengue fever virus [5], chikungunya virus [6], and possibly Zika virus [7]. *Culex quinquefasciatus* Say (Diptera: Culicidae), the southern house mosquito, is a vector of lymphatic filariasis [8] as well as several arboviruses such as West Nile virus and St. Louis encephalitis virus [9] and possibly Zika virus [10].

Several members of the genus *Conyza* Less. (Asteraceae) have been introduced throughout the tropics and subtropics where they have become invasive weeds [11–13]. *Conyza bonariensis* (L.) Cronquist (syn. *Erigeron bonariensis* L.), flaxleaf fleabane, probably originated in South America [14], but has been introduced throughout Asia, Africa, Mexico and the southern United States, Europe, and Oceania [13,15]. *Conyza canadensis* (L.) Cronquist (syn. *Erigeron canadensis* L.), Canada fleabane, is native to North America, but is also now naturalized throughout Europe, Asia, and Oceania [13]. *Conyza sumatrensis* (Retz.) E. Walker (syn. *Erigeron sumatrensis* Retz.) is probably native to South America, but this species has also been naturalized in tropical and subtropical regions [16].

Non-native invasive plant species are generally detrimental to the local environments where they have been introduced. They can outcompete native plant species and reduce biodiversity [17], they can alter ecosystem functions [18], and can have substantial economic impacts [19]. Control methods for invasive plants have generally included application of herbicides, physical cutting, or burning [20]. However, harvesting invasive species for beneficial uses as a method for control of invasive species may provide economic incentives to offset eradication costs [21]. For example, *Melaleuca quinquenervia* trees in south Florida have been cut and chipped for landscape mulch and boiler fuel [22]; it has been suggested that mechanical harvesting of invasive cattail (*Typha* spp.), common reed (*Phragmites australis*), and reed canary grass (*Phalaris arundinacea*) from coastal wetlands of Lake Ontario can be used as an agricultural nutrient source or as a biofuel [23]. The leaf essential oil of *Solidago canadensis*, an invasive plant in Europe, has been evaluated as a potential insecticide and demonstrated moderate larvicial activity against *Cx. quinquefasciatus* [24].

The use of synthetic pesticides for mosquito control has had detrimental effects on the environment [25,26]. They tend to be persistent, toxic to non-target organisms, and insecticide resistance has been steadily increasing in mosquito species [27]. Essential oils have been suggested as viable, environmentally benign, and renewable alternatives to synthetic pesticides [28–32]. We have recently studied several introduced invasive plant species in Vietnam for potential use as mosquito vector control agents [33–35], and as part of our ongoing efforts in identifying readily-available essential oils for mosquito control, we have examined three *Conyza* species for larvicidal activity against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*, with the aim of identifying new mosquito-control essential oils and the components responsible for the activity.

2. Results and Discussion

2.1. Essential Oil Compositions

The essential oils from the aerial parts of *C. bonariensis*, *C. canadensis*, and *C. sumatrensis* were obtained by hydrodistillation in 1.10%, 1.37%, and 1.21% yield. The chemical compositions of the *Conyza* essential oils, determined using gas chromatography–mass spectrometry, are summarized in Table 1.
Conyza bonariensis essential oil was dominated by sesquiterpenoids, especially allo-aromadendrene (41.2%), β-caryophyllene (13.3%), and caryophyllene oxide (12.2%). Concentrations of monoterpenoids (1.8%) and diterpenoids (trace) were relatively small. The essential oils of C. canadensis and C. sumatrensis, on the other hand, were rich in limonene (41.5% and 25.5%, respectively). The aerial parts essential oil of C. sumatrensis also had a large concentration of (Z)-lachnophyllum ester (20.7%). There is wide variation in the essential oil compositions of Conyza species, both between species and within the same species (see Table 2). This is not surprising given the very different geographical locations of the collection sites for these samples.

### Table 1. Chemical compositions of the aerial parts essential oils of Conyza bonariensis, Conyza canadensis, and Conyza sumatrensis collected in Vietnam.

| RI<sub>calc</sub> | RI<sub>db</sub> | Compound                          | Relative Content % |
|------------------|--------------|-----------------------------------|--------------------|
|                  |              | C. bonariensis                      | C. canadensis |
|                  |              | C. sumatrensis                      | C. sumatrensis |
| 931              | 932          | α-Pinene                           | 0.5                | 0.5 | 0.2 |
| 948              | 950          | Camphene                           | tr                 | —   | —   |
| 967              | 972          | (3Z)-Octen-2-ol                    | —                  | —   | tr   |
| 971              | 972          | Sabinene                           | tr                 | 0.1 | 0.1 |
| 976              | 978          | β-Pinene                           | 0.8                | 8.8 | 3.0 |
| 982              | 984          | 6-Methylhept-5-en-2-one            | —                  | —   | tr   |
| 987              | 989          | Myrcene                            | tr                 | 1.2 | 1.0 |
| 1023             | 1025         | p-Cymene                           | tr                 | 0.3 | 0.1 |
| 1028             | 1030         | Limonene                           | 0.2                | 41.5| 25.5|
| 1030             | 1031         | β-Phellandrene                      | tr                 | —   | —   |
| 1034             | 1034         | (Z)-β-Ocimene                      | —                  | —   | tr   |
| 1044             | 1045         | (E)-β-Ocimene                      | tr                 | —   | 1.9 |
| 1049             | 1051         | 2,3,6-Trimethylhepta-1,5-diene      | tr                 | —   | —   |
| 1056             | 1057         | γ-Terpine                           | tr                 | —   | —   |
| 1088             | 1091         | p-Cymene                           | 0.1                | —   | —   |
| 1090             | 1091         | Rosefuran                          | —                  | —   | 0.1 |
| 1093             | 1097         | α-Pinene oxide                     | —                  | —   | 0.2 |
| 1097             | 1098         | Perillene                           | tr                 | 0.1 | —   |
| 1098             | 1101         | Linalool                           | 0.2                | —   | —   |
| 1101             | 1101         | 6-Methyl-3,5-heptadien-2-one       | —                  | —   | 0.1 |
| 1103             | 1104         | Nonanal                             | tr                 | —   | —   |
| 1112             | 1113         | 4,8-Dimethyl-1,3,7-triene           | —                  | —   | 0.2 |
| 1118             | 1119         | enol-Fenchol                       | tr                 | —   | —   |
| 1120             | 1121         | trans-p-Menth-2,8-dien-1-ol        | 0.9                | 0.2 |
| 1124             | 1131         | Cyclooctanone                       | 0.8                | —   | —   |
| 1129             | 1130         | 4-Acetyl-1-methylcyclohexene       | 0.1                | —   | —   |
| 1131             | 1132         | cis-Limonene oxide                 | 0.6                | 0.2 |
| 1134             | 1137         | cis-p-Menth-2,8-dien-1-ol          | 1.2                | 0.3 |
| 1135             | 1137         | trans-Limonene oxide               | 0.6                | —   |
| 1137             | 1137         | Nopinone                            | —                  | 0.4 |
| 1137             | 1139         | (E)-Myroxide                       | —                  | —   | 0.1 |
| 1139             | 1141         | trans-Pinocarveol                  | tr                 | 1.6 | 0.1 |
| 1150             | 1152         | Citronellal                         | —                  | 0.1 |
| 1160             | 1164         | Pinocarvone                         | —                  | 0.8 | tr   |
| 1170             | 1170         | Borneol                             | tr                 | —   |
| 1177             | 1179         | 2-Isopropenyl-5-methylhex-4-enal    | 0.3                | —   |
| 1182             | 1184         | p-Methylcacetophenone              | 0.3                | —   |
| 1185             | 1185         | Cryptone                            | —                  | 0.4 |
| 1185             | 1187         | trans-p-Menth-1(7),8-dien-2-ol     | 0.2                | —   |
| 1189             | 1190         | Methyl salicylate                   | tr                 | —   |
| 1193             | 1195         | α-Terpineol                         | 0.1                | —   | 0.1 |
| 1193             | 1196         | Myrtenal                            | —                  | 1.4 |
| 1194             | 1195         | Myrtenol                            | —                  | 1.2 |
| 1196             | 1197         | Methyl chavicol (=Estragol)         | —                  | 0.2 |
| 1198             | 1201         | cis-Piperitol                       | —                  | 0.8 | 0.1 |
| 1206             | 1207         | Oct-3E-enyl acetate                 | —                  | —   | 0.1 |
| 1217             | 1218         | trans-Carveol                       | —                  | 3.8 | 0.2 |
| R_{calc} \ a | R_{db} \ b | Compound | Relative Content % |
|---------|----------|-----------|------------------|
|         |          |           | C. bonariensis   | C. canadensis | C. sumatrensis |
| 1227    | 1228     | cis-p-Mentha-1(7),8-dien-2-ol | — | 0.1 | — |
| 1230    | 1232     | cis-Carveol | — | 1.1 | 0.1 |
| 1242    | 1242     | Carvone | — | 3.8 | 0.2 |
| 1247    | 1249     | Linalyl acetate | tr | — | — |
| 1266    | 1270     | iso-Piperitenone | — | 0.6 | — |
| 1273    | 1277     | Perilla aldehyde | — | 0.5 | — |
| 1287    | 1287     | Limonene dioxide | — | 0.7 | — |
| 1296    | 1299     | Perilla alcohol | — | 0.4 | — |
| 1303    | —        | Unidentified | — | 1.1 | — |
| 1316    | 1324     | Limonene hydroperoxide | — | 1.1 | — |
| 1343    | 1346     | Limonene-1,2-diol | — | 2.6 | — |
| 1344    | 1349     | 7-epi-Silphiperfol-5-ene | — | — | 0.3 |
| 1345    | 1349     | α-Cubebeene | 0.2 | — | — |
| 1355    | 1340     | p-Mentha-6,8-diene-2-hydroperoxide | — | 1.2 | — |
| 1357    | 1371     | α-Ylangene | tr | — | — |
| 1374    | 1375     | α-Copaene | 4.5 | — | 0.1 |
| 1376    | 1380     | Daucene | — | 0.4 | — |
| 1377    | 1374     | Isoledene | — | — | 0.3 |
| 1379    | 1382     | Modheph-2-ene | — | — | 0.4 |
| 1381    | 1382     | β-Bourbonene | tr | — | — |
| 1385    | 1387     | β-Cubebeene | 0.4 | — | 0.1 |
| 1386    | 1385     | α-Isocomene | — | — | 0.1 |
| 1387    | 1390     | β-Elemene | 0.3 | — | 0.4 |
| 1392    | 1394     | Sativene | — | — | 0.1 |
| 1398    | 1405     | (Z)-Caryophyllene | 0.2 | — | — |
| 1404    | 1406     | α-Gurjunene | 0.1 | — | — |
| 1408    | 1411     | β-Isocomene | — | — | 0.1 |
| 1418    | 1417     | (E)-Caryophyllene | 13.3 | — | 5.5 |
| 1427    | 1430     | β-Copaene | 0.2 | — | 0.2 |
| 1430    | 1433     | trans-α-Bergamotene | — | — | 1.1 |
| 1432    | 1440     | 6,9-Guaiadiene | — | — | 0.2 |
| 1436    | 1436     | α-Guaiene | 1.8 | — | — |
| 1438    | 1438     | Aromadendrene | 0.2 | — | 0.1 |
| 1445    | 1449     | (E)-Lachnophyllum acid | — | — | 0.2 |
| 1451    | 1452     | (E)-β-Farnesene | — | — | 6.7 |
| 1453    | 1454     | α-Humulene | 5.4 | 0.3 | 0.7 |
| 1457    | 1463     | cis-Cadina-1(6),4-diene | — | — | 0.4 |
| 1460    | 1458     | allo-Aromadendrene | 41.2 | — | — |
| 1469    | —        | Unidentified | — | — | 1.3 |
| 1472    | 1472     | trans-Cadina-1(6),4-diene | 0.5 | — | 0.2 |
| 1476    | 1479     | α-Amorphene | 0.1 | — | — |
| 1478    | 1483     | Germacrene D | 0.3 | — | 2.1 |
| 1481    | 1483     | trans-β-Bergamotene | — | — | 0.2 |
| 1486    | 1489     | β-Selinene | 0.5 | — | — |
| 1488    | 1491     | Viridiflorene | 0.2 | — | — |
| 1492    | 1497     | Bicyclogermacrene | — | — | 0.3 |
| 1493    | 1497     | α-Selinene | 0.3 | — | — |
| 1495    | 1497     | α-Muurolene | 0.4 | — | 0.1 |
| 1498    | 1505     | α-Bulnesene | 1.8 | — | — |
| 1501    | 1505     | (E, E)-α-Farnesene | — | — | 0.1 |
| 1504    | 1514     | (Z)-Lachnophyllum acid | — | 0.2 | 0.8 |
| 1507    | 1510     | (E)-Lachnophyllum ester | — | — | 0.4 |
| 1510    | 1512     | γ-Cadine | 0.4 | — | 0.1 |
| 1515    | 1515     | (Z)-Lachnophyllum ester | — | 5.5 | 20.7 |
| 1515    | 1518     | δ-Cadine | 0.6 | — | — |
| 1518    | 1519     | trans-Calamenene | 0.3 | — | — |
| 1521    | 1523     | β-Sesquiphellandrene | — | — | 0.3 |
| 1531    | 1532     | Tridec-11-yn-1-ol | — | — | 0.3 |
| 1533    | 1538     | α-Cadinene | 0.1 | — | — |
| 1538    | 1541     | α-Calacorene | 0.1 | — | — |
| RI<sub>calc</sub><sup>a</sup> | RI<sub>db</sub><sup>b</sup> | Compound | Relative Content % | C. bonariensis | C. canadensis | C. sumatrensis |
|---|---|---|---|---|---|---|
| 1556 | 1557 | Germacrene B | — | — | 0.1 |
| 1558 | 1560 | (E)-Nerolidol | — | 0.2 | 1.8 |
| 1559 | 1564 | β-Calacorene | 0.1 | — | — |
| 1565 | 1566 | 1,5-Epoxyosalvial-4(14)-ene | — | — | 0.2 |
| 1566 | 1568 | Dendrolasin | — | — | 0.1 |
| 1567 | 1567 | Palustrol | 0.1 | — | — |
| 1574 | 1576 | Spathulenol | 1.3 | — | 5.2 |
| 1580 | 1577 | Caryophyllene oxide | 12.2 | 1.1 | 5.8 |
| 1582 | 1590 | Globulol | 0.4 | — | 0.5 |
| 1589 | 1593 | Salvial-4(14)-en-1-one | — | 0.1 | 0.2 |
| 1590 | 1594 | Viridiflorol | 0.8 | — | 0.3 |
| 1593 | 1599 | Cubean-11-ol | 0.2 | — | — |
| 1599 | 1601 | Carotol | — | — | 1.1 |
| 1601 | 1605 | Ledol | 0.6 | — | — |
| 1606 | 1611 | Humulene epoxide II | 2.2 | 2.9 | 0.4 |
| 1624 | 1628 | 1-epi-Cubanol | 0.2 | — | — |
| 1629 | 1629 | iso-Spathulenol | — | — | 0.6 |
| 1633 | 1635 | Caryophylla-4(12),8(13)-dien-5β-ol | 0.2 | — | — |
| 1635 | 1632 | Muurola-4,10(14)-dien-1β-ol | — | — | 0.7 |
| 1638 | 1643 | τ-Cadinol | 0.2 | — | 0.4 |
| 1640 | 1644 | τ-Muurolol | 0.1 | — | 0.3 |
| 1643 | 1643 | α-Muurolol | 0.2 | — | — |
| 1643 | 1644 | allo-Aromadendrene epoxide | — | 0.3 | — |
| 1652 | 1655 | α-Cadinol | 0.6 | 0.3 | 0.4 |
| 1655 | 1655 | Eudesma-4(15),7-dien-1α-ol | — | — | 0.1 |
| 1661 | 1664 | cis-Calamenen-10-ol | 0.1 | — | — |
| 1666 | 1666 | 14-Hydroxy-9-epi-(E)-caryophyllene | 0.1 | — | — |
| 1669 | 1677 | Cadalene | 0.1 | — | — |
| 1686 | 1685 | Eudesma-4(15),7-dien-1β-ol | — | 0.4 | 0.1 |
| 1698 | 1704 | cis-Thujopsenol | 0.1 | — | — |
| 1717 | — | Unidentified<sup>f</sup> | — | 1.0 | — |
| 1738 | 1740 | 8α,11-Elemodiol | 0.1 | — | — |
| 1751 | 1758 | Khusimol | 1.5 | — | — |
| 1790 | 1792 | 14-Hydroxy-δ-cadinene | — | — | 0.2 |
| 1800 | — | Unidentified<sup>g</sup> | 1.1 | — | — |
| 1833 | 1836 | Neophytadiene | — | — | 0.2 |
| 1857 | 1860 | Platambin | 0.1 | 0.5 | 0.1 |
| 1882 | 1884 | Corymbolone | 0.2 | — | — |
| 2103 | 2102 | Phytol | tr | 0.1 | — |
| | | Monoterpene hydrocarbons | 1.5 | 52.7 | 31.8 |
| | | Oxygenated monoterpenoids | 0.3 | 26.4 | 1.9 |
| | | Sesquiterpene hydrocarbons | 73.7 | 0.3 | 20.7 |
| | | Oxygenated sesquiterpenoids | 21.3 | 5.7 | 18.5 |
| | | Diterpenoids | trace | — | 0.4 |
| | | Others | trace | 7.2 | 22.9 |
| | | Total Identified | 96.8 | 92.3 | 96.1 |

<sup>a</sup> RI<sub>calc</sub> = Retention Index calculated with respect to a homologous series of n-alkanes on a ZB-5 column. <sup>b</sup> RI<sub>db</sub> = Retention Index from the databases [36–39]. <sup>c</sup> tr = trace (< 0.05%). <sup>d</sup> MS(EI): 150(3%), 135(51%), 121(29%), 119(38%), 109(42%), 107(66%), 93(07%), 91(89%), 81(50%), 79(100%), 69(82%), 67(37%), 55(65%), 53(40%), 43(75%), 41(85%). <sup>e</sup> MS(EI): 204(25%), 189(3%), 161(100%), 147(9%), 133(28%), 120(48%), 119(25%), 105(51%), 91(47%), 69(20%), 57(19%), 55(21%), 41(20%). <sup>f</sup> MS(EI): 175(3%), 135(11%), 111(48%), 93(20%), 83(19%), 67(19%), 55(26%), 43(100%), 41(20%). <sup>g</sup> MS(EI): 218(29%), 203(28%), 199(100%), 175(46%), 147(34%), 133(61%), 119(38%), 105(70%), 91(90%), 79(42%), 67(43%), 55(34%), 41(52%).
Table 2. Major components of Conyza bonariensis, Conyza canadensis, and Conyza sumatrensis essential oils from different geographical locations.

| Conyza Species (Collection Site) | Major Components (>5%)                                                                 | Ref. |
|----------------------------------|----------------------------------------------------------------------------------------|------|
| C. bonariensis aerial parts EO (Chapada dos Guimarães, Mato Grosso, Brazil) | limonene (6.9%), (E)-caryophyllene (14.4%), (E)-β-farnesene (23.3%), germacrene D (15.3%), bicyclogermacrene (8.3%), spathulenol (7.6%) | [40] |
| C. bonariensis aerial parts EO (Melgaço, Pará, Brazil) | limonene (22.9%), (E)-caryophyllene (13.3%), trans-α-bergamotene (5.3%), (E)-β-farnesene (20.1%), bicyclogermacrene (6.6%), spathulenol (6.3%) | [40] |
| C. bonariensis aerial parts EO (Peixe-Boi, Pará, Brazil) | (E)-caryophyllene (13.3%), trans-α-bergamotene (8.1%), (E)-β-farnesene (30.9%) | [40] |
| C. bonariensis aerial parts EO (alta Floresta, Mato Grosso, Brazil) | limonene (12.6%), (E)-caryophyllene (13.0%), (E)-β-farnesene (19.1%), germacrene D (13.2%), bicyclogermacrene (6.3%), spathulenol (5.7%) | [40] |
| C. bonariensis aerial parts EO (Macapá, Amapá, Brazil) | limonene (58.4%), (E)-β-farnesene (7.0%) | [40] |
| C. bonariensis aerial parts EO (Rio de Janeiro, Brazil) | limonene (45.0%), (E)-β-ocimene (13.0%), (E)-β-farnesene (6.6%), germacrene D (6.4%) | [41] |
| C. bonariensis leaf EO (Minas Gerais State, Brazil) | limonene (29.6%), trans-α-bergamotene (10.3%), matricaria methyl ester (8.3%), β-copaen-4α-ol (7.4%) | [42] |
| C. bonariensis aerial parts EO (Athens, Greece) | limonene (8.3%), (E)-β-ocimene (11.5%), (E)-β-farnesene (8.1%), (Z)-lachnophyllum ester (21.2%), matricaria ester (17.5%) | [43] |
| C. bonariensis aerial parts EO (Southwestern Misiones Province, Argentina) | limonene (13.5%), (E)-β-ocimene (13.3%), p-mentha-1,3,8-triene (5.2%), germacrene D (14.6%), bicyclogermacrene (6.6%) | [44] |
| C. bonariensis leaf EO (Monastir, Tunisia) | limonene (5.8%), terpinolene (5.3%), (E)-β-farnesene (7.5%), matricaria ester (17.8%), caryophyllene oxide (7.8%) | [45] |
| C. bonariensis aerial parts EO (Cagliari, Sardinia, Italy) | limonene (5.1%), carvacrol (9.8%), α-curcumene (10.2%), spathulenol (18.6%), caryophyllene oxide (18.7%), neophytadiene (6.1%) | [46] |
| C. bonariensis leaf EO (Mérida State, Venezuela) | limonene (5.1%), (Z)-β-ocimene (5.1%), (E)-β-ocimene (20.7%), (E)-β-farnesene (37.8%), α-farnesene (5.6%), β-sesquiphellandrene (9.8%) | [47] |
| C. bonariensis leaf EO (Kabianga, Kericho, Kenya) | β-pinene (5.4%), limonene (8.3%), 2,6,7,7a-tetrahydro-1,5-dimethyl-1H-indene-3-carboxaldehyde (49.1%) | [48] |
| C. bonariensis aerial parts EO (Parana State, Brazil) | limonene (66.3%), 2-heptyl acetate (6.9%) | [49] |
| C. bonariensis aerial parts EO (Quang Nam Province, Vietnam) | (E)-caryophyllene (13.3%), α-humulene (5.4%), allo-aromadendrene (41.2%), caryophyllene oxide (12.2%) | this work |
| C. canadensis aerial parts EO (Plovdiv, Bulgaria) | limonene (77.7–89.4%) | [50] |
| C. canadensis aerial parts EO (Łódź, Poland) | limonene (76.3%) | [51] |
| C. canadensis aerial parts EO (Alps, France) | limonene (83.2%) | [51] |
Table 2. Cont.

| Conyza Species (Collection Site) | Major Components (>5%)                                                                                                                                 | Ref. |
|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| *C. canadensis* aerial parts EO (Rome, Italy) | limonene (70.3%), (E)-β-ocimene (5.5%)                                                                                                                | [51] |
| *C. canadensis* aerial parts EO (Seville, Spain) | limonene (51.4%), (E)-β-ocimene (13.4%), *trans-α*-bergamotene (11.9%)                                                                                  | [51] |
| *C. canadensis* aerial parts EO (Belgium) | limonene (68.0%), (E)-β-ocimene (5.1%), *trans-α*-bergamotene (5.4%), germacrene D (7.3%) (*Z,Z*-matricaria ester (6.1%))         | [51] |
| *C. canadensis* aerial parts EO (Plovdiv, Bulgaria) | limonene (87.9%)                                                                                                                                       | [51] |
| *C. canadensis* aerial parts EO (Vilnius, Lithuania) | limonene (77.7%), *trans-α*-bergamotene (5.5%)                                                                                                          | [51] |
| *C. canadensis* aerial parts EO (Israel) | limonene (54.9%), (Z)-β-farnesene (6.3%) (*Z,Z*-matricaria ester (7.7%))                                                                                 | [51] |
| *C. canadensis* aerial parts EO (Kerman, Iran) | myrcene (8.9%), limonene (12.3%), (E)-β-farnesene (14.6%), *ar-curcumene* (7.8%), zingiberene (5.5%), spathulenol (14.1%), isospathulenol (7.7%), phytol (7.3%) | [52] |
| *C. canadensis* aerial parts EO (Athens, Greece) | β-pinene (9.5%), limonene (57.3%), matricaria ester (14.4%)                                                                                             | [43] |
| *C. canadensis* aerial parts EO (Korea) | limonene (68.3%), (E)-β-ocimene (15.9%)                                                                                                                | [53] |
| *C. canadensis* EO (China) | limonene (14.8%), *epi*-bicyclosesquiphellandrene (11.0%), C<sub>7</sub>H<sub>30</sub>B<sub>4</sub>Si (25.1%)<sup>c</sup>, 1-phenyl-1-nonyne (7.3%) | [54] |
| *C. canadensis* aerial parts EO (Szeged, Hungary) | limonene (79.2%)                                                                                                                                       | [55] |
| *C. canadensis* aerial parts EO (Manavgat, Antalya, Turkey) | β-pinene (9.7%), limonene (28.1%), spathulenol (16.3%)                                                                                                 | [56] |
| *C. canadensis* aerial parts EO (Da Nang City, Vietnam) | β-pinene (8.8%), limonene (41.5%), (Z)-lachnophyllum ester (5.5%)                                                                                      | this work |
| *C. sumatrensis* aerial parts EO (Rondôndia state, Brazil) | sabinene (5.3%), limonene (22.9%), (E)-β-oicimene (5.0%), (E)-β-farnesene (5.3%), (Z)-lachnophyllum ester (43.7%) | [57] |
| *C. sumatrensis* leaf EO (N’gorato village, Côte d’Ivoire) | limonene (13.0%), (E)-β-oicimene (6.5%), (E)caryophyllene (10.5%), (E)-β-farnesene (17.0%), (Z)-lachnophyllum ester (5.9%), germacrene D (13.6%), bicyclogermacrene (5.2%) | [58] |
| *C. sumatrensis* leaf EO (Monastir, Tunisia) | matricaria ester (7.5%), spathulenol (13.8%), caryophyllene oxide (20.5%)                                                                 | [59] |
| *C. sumatrensis* aerial parts EO (Da Nang City, Vietnam) | limonene (25.5%), (E)-caryophyllene (5.5%), (E)-β-farnesene (6.7%), (Z)-lachnophyllum ester (20.7%), spathulenol (5.2%), caryophyllene oxide (5.8%) | this work |

<sup>a</sup> The identification of this compound is uncertain; it is not found in the *Dictionary of Natural Products* [60].<sup>b</sup> This compound was listed as δ-3-carene, but the retention time is more consistent with (E)-β-oicimene rather than δ-3-carene. <sup>c</sup> The identification of this compound (2,3-µ-trimethylsilyl-C,C′-dimethyl-4,5-dicarba-nido-hexaborane) is not correct; the compound listed is not a natural product.
2.2. Mosquito Larvicidal Activity

The mosquito larvicidal activities of the Conyza essential oils are summarized in Table 3. The essential oil of C. canadensis showed the best larvicidal activity against both Ae. aegypti (24-h LC50 = 9.80 µg/mL) and Ae. albopictus (24-h LC50 = 18.0 µg/mL) and good larvicidal activity against Cx. quinquefasciatus (24-h LC50 = 39.4 µg/mL). Conyza sumatrensis essential oil also showed good larvicidal activity against the three mosquito species (24-h LC50 = 21.7, 19.1, and 26.7 µg/mL, respectively, for Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus). Conyza bonariensis essential oil was less active (24-h LC50 = 69.7, 81.1 and 130.0 µg/mL against Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus, respectively).

The larvicidal activities of Conyza essential oils roughly coincides with the concentration of limonene in the samples (41.5%, 25.5%, and 0.2%, respectively, for C. canadensis, C. sumatrensis, and C. bonariensis), and this relationship is borne out in a principle component analysis based on the major essential oil components (limonene, allo-aromadendrene, (Z)-lachnophyllum ester, caryophyllene oxide, β-caryophyllene, β-pinene, (E)-β-farnesene, spathulenol, and α-humulene, along with the 24-h larvicidal activities) (Figure 1). Limonene has shown excellent larvicidal activities against Ae. aegypti (24-h LC50 = 17.7 µg/mL) and Cx. quinquefasciatus (24-h LC50 = 31.6 µg/mL) (Table 3) as well as Ae. albopictus (LC50 = 10.8-41.8 µg/mL) [34]. Consistent with these results, Zeng and co-workers found the larvicidal activity of C. canadensis from China (14.8% limonene) to be 56.9 µg/mL and 32.1 µg/mL against Ae. albopictus and Cx. quinquefasciatus, respectively [54]. These workers also appreciated the remarkable larvicidal activity and noted that C. canadensis essential oil has a potential for further development. Furthermore, Citrus peel oils, rich in limonene, have also shown remarkable larvicidal activities against Ae. albopictus [61] and Cx. quinquefasciatus [62].

Table 3. Mosquito larvicidal activity and insecticidal activity of Conyza essential oils.

| Essential Oil or Major Compound | 24 h | 50 | 50 \(\chi^2\) | \(p\) | Slope |
|-------------------------------|------|----|---------|------|-------|
| **Aedes aegypti**              |      |    |         |      |       |
| C. bonariensis                | 69.71 (64.82–75.36) | 86.61 (82.13–97.54) | 9.39 | 0.009 | 9.45  |
| C. canadensis                 | 9.801 (8.730–10.986) | 23.27 (19.93–28.36) | 8.70 | 0.069 | 12.18 |
| C. sumatrensis                | 21.74 (20.16–23.36) | 31.02 (28.29–35.50) | 0.131| 0.988 | 7.98  |
| β-Pinene                      | 23.63 (22.16–25.33) | 32.12 (29.47–36.00) | 0.225| 0.994 | 7.69  |
| Limonene                      | 17.66 (16.45–18.97) | 23.62 (22.03–25.73) | 0.784| 0.941 | 8.23  |
| (E)-Caryophyllene             | 70.80 (65.49–76.69) | 107.2 (98.4–118.6)  | 4.08 | 0.395 | 12.75 |
| α-Humulene                    | 53.05 (48.69–58.08) | 82.78 (75.81–91.87) | 15.9 | 0.003 | 12.79 |
| Caryophyllene oxide           | 136.6 (129.2–143.9) | 180.2 (171.4–191.2) | 30.1 | 0.000 | 12.37 |
| Permethrin control            | 0.000643 (0.000551–0.00753) | 0.00246 (0.00192–0.00344) | 12.5 | 0.006 | 11.57 |
| **Aedes albopictus**           |      |    |         |      |       |
| C. bonariensis                | 81.13 (74.61–87.97)  | 127.1 (117.5–139.9) | 0.395| 0.821 | 11.44 |
| C. canadensis                 | 18.04 (16.71–19.52) | 26.20 (24.22–28.82) | 1.46 | 0.834 | 11.30 |
| C. sumatrensis                | 19.13 (17.73–20.66) | 27.49 (25.41–30.38) | 3.19 | 0.364 | 9.97  |
| Permethrin control            | 0.0024 (0.0021–0.0026) | 0.0042 (0.0038–0.0049) | 4.64 | 0.031 | 8.45  |
| **Cx. quinquefasciatus**      |      |    |         |      |       |
| C. bonariensis                | 130.0 (122.5–138.8) | 178.4 (165.6–197.2) | 0.675| 0.713 | 8.97  |
| C. canadensis                 | 39.37 (36.83–42.00) | 52.29 (49.04–56.56) | 0.493| 0.974 | 10.49 |
| C. sumatrensis                | 26.74 (24.80–29.20) | 36.83 (33.56–41.92) | 8.97 | 0.030 | 7.96  |
| β-Pinene                      | 30.46 (28.21–33.21) | 41.58 (38.10–46.58) | 0.399| 0.983 | 9.38  |
| Limonene                      | 31.63 (29.37–34.50) | 41.51 (38.03–46.78) | 0.874| 0.928 | 8.23  |
| (E)-Caryophyllene             | 165.4 (157.5–174.0) | 220.6 (207.8–238.5) | 10.0 | 0.040 | 9.91  |
| α-Humulene                    | 108.3 (101.4–115.5) | 158.2 (148.5–170.5) | 1.0  | 0.910 | 13.32 |
| Caryophyllene oxide           | 98.52 (90.70–108.68) | 144.5 (129.6–165.7) | 1.60 | 0.809 | 9.20  |
| Permethrin control            | 0.0165 (0.0149–0.0181) | 0.0305 (0.0266–0.0367) | 5.24 | 0.073 | 10.12 |
| **Diplonychus rusticus**      |      |    |         |      |       |
| C. canadensis                 | 135.7 (129.3–142.8) | 182.5 (172.6–195.5) | 7.78 | 0.051 | 12.35 |
| C. sumatrensis                | 111.0 (106.1–116.7) | 137.0 (129.5–147.6) | 16.1 | 0.001 | 9.85  |
Table 3. Cont.

| Essential Oil or Major Compound | 48 h LC₅₀ (95% Limits), µg/mL | 48 h LC₉₀ (95% Limits), µg/mL | χ² | p | Slope |
|---------------------------------|------------------------------|-------------------------------|----|---|-----|
| C. bonariensis                  | 63.85 (59.07–70.75)          | 81.84 (74.16–94.79)           | 3.43 | 0.180 | 6.89 |
| C. canadensis                   | 7.091 (6.099–8.141)          | 22.46 (18.63–28.59)           | 5.98 | 0.201 | 11.63 |
| C. sumatrensis                  | 22.52 (21.29–24.85)          | 31.37 (29.03–35.03)           | 0.323 | 0.988 | 9.08 |
| Limonene                        | 17.43 (16.24–18.74)          | 23.17 (21.38–25.28)           | 0.664 | 0.956 | 10.48 |
| (E)-Caryophyllene               | 65.92 (60.45–72.08)          | 106.4 (98.4–116.7)            | 14.2 | 0.007 | 13.10 |
| α-Humulene                      | 46.25 (42.27–50.94)          | 74.14 (67.47–82.99)           | 19.2 | 0.001 | 12.21 |
| Caryophyllene oxide             | 120.2 (112.7–127.5)          | 165.4 (156.4–176.6)           | 19.8 | 0.001 | 12.34 |
| Permethrin control              | 0.000575 (0.000483–0.00688)  | 0.00281 (0.00208–0.00423)     | 5.29 | 0.152 | 10.93 |

Aedes albopictus a

| C. bonariensis                  | 69.42 (63.20–75.93)          | 113.2 (103.8–125.8)           | 3.10 | 0.212 | 10.72 |
| C. canadensis                   | 15.12 (13.93–16.47)          | 22.67 (20.84–25.09)           | 7.23 | 0.124 | 12.22 |
| C. sumatrensis                  | 18.43 (17.05–19.93)          | 26.76 (24.71–29.58)           | 4.25 | 0.236 | 8.44 |

Culex quinquefasciatus

| C. bonariensis                  | 108.1 (101.4–115.1)          | 152.1 (142.4–165.1)           | 2.32 | 0.313 | 10.84 |
| C. canadensis                   | 29.81 (27.33–32.68)          | 47.06 (43.03–52.39)           | 14.5 | 0.006 | 12.17 |
| C. sumatrensis                  | 22.95 (21.22–25.08)          | 33.06 (30.07–37.60)           | 2.38 | 0.498 | 9.37 |
| β-Pinene                        | 28.36 (26.20–31.19)          | 39.01 (35.41–44.50)           | 2.41 | 0.661 | 8.39 |
| Limonene                        | 29.15 (26.89–31.98)          | 40.83 (37.19–46.07)           | 7.05 | 0.133 | 9.50 |
| (E)-Caryophyllene               | 138.5 (129.3–148.5)          | 215.3 (200.1–234.9)           | 13.5 | 0.009 | 13.11 |
| α-Humulene                      | 87.81 (81.14–94.89)          | 140.0 (127.4–152.7)           | 9.80 | 0.044 | 13.50 |
| Caryophyllene oxide             | 95.19 (86.49–106.26)         | 141.0 (127.6–160.8)           | 4.01 | 0.405 | 10.12 |

Diplonychus rusticus a

| C. canadensis                   | 124.0 (118.0–130.4)          | 165.0 (156.1–176.6)           | 1.17 | 0.760 | 12.17 |
| C. sumatrensis                  | 107.8 (103.1–113.4)          | 133.6 (126.1–144.4)           | 8.07 | 0.045 | 9.37 |

* Aedes albopictus and Diplonychus rusticus were obtained from the wild; the limited numbers of organisms available precluded screening of individual components on these two insect species.

Other components in the Conyza essential oils likely contribute to the mosquito larvicidal effects. Conyza bonariensis was rich in (E)-caryophyllene (13.3%) and caryophyllene oxide (12.2%), but both of these compounds have been found to have weak larvicidal activities against Ae. aegypti (24-h LC₅₀ = 70.8 and 137 µg/mL, respectively (Table 3). On the other hand, β-pinene, a major component of C. canadensis essential oil (8.8%), has shown larvicidal activity against Ae. aegypti (24-h LC₅₀ = 23.6 µg/mL), Cx. quinquefasciatus (24-h LC₅₀ = 30.5 µg/mL) (Table 3), and Ae. albopictus [61]. In addition, synergy between essential oil components may also be important [63,64]. Scalerandi and coworkers have found that the housefly (Musca domestica) metabolizes the major components in an essential oil, but leaves the minor components to act as toxicants [65].

In order to assess the potential detrimental impact of the Conyza essential oils on beneficial aquatic species, the insecticidal activity was assessed against the water bug, Diplonychus rusticus, an insect predator of mosquito larvae [66]. Both C. canadensis and C. sumatrensis essential oils were substantially less toxic to D. rusticus than they were to the mosquito larvae.
Figure 1. Principal component biplot of PC1 and PC2 scores and loadings demonstrating the relationships between Conyza essential oil major components and larvicidal activities.

3. Materials and Methods

3.1. Chemicals

Chemicals used for this study, dimethylsulfoxide (DMSO), β-pinene, limonene, (E)-caryophyllene, α-humulene, caryophyllene oxide, dichloromethane, and permethrin, were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further purification.

3.2. Plant Material

The three Conyza species were collected from Bach Ma National Park, Thue Thien Hue province (16° 11’ 34” N, 107° 51’ 12” E) in April 2020. The plants were identified by Dr. Do Ngoc Dai and Dr. Le Thi Huong. Voucher specimens, LTH129 (Conyza canadensis), LTH130 (Conyza sumatrensis), and LTH131 (Conyza bonariensis) have been deposited in the Pedagogical Institute of Science, Vinh University. Four-kg
samples of fresh aerial parts (leaves, stems, and flowers) of each of the plants were shredded and hydrodistilled for 4 h using a Clevenger-type apparatus.

3.3. Gas Chromatography–Mass Spectrometry

The Conyza essential oils were analyzed by GC-MS as previously described [67]: Shimadzu GCMS-QP2010 Ultra, electron impact (EI) mode, electron energy = 70 eV, scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, ZB-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness), He carrier gas, 552 kPa column head pressure, and 1.37 mL/min flow rate. Injector temperature was 250 °C and the ion source temperature was 200 °C. The GC oven temperature program was programmed for 50 °C initial temperature, temperature increased at a rate of 2 °C/min to 260 °C. A 5% w/v solution of the sample in CH₂Cl₂ was prepared and 0.1 µL was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the databases [36–39].

3.4. Mosquito Larvicidal Assay

Mosquito larvicidal activity was carried out on Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus as previously described [67]: For the assay, 1% stock solutions of each essential oil in dimethylsulfoxide (DMSO) were prepared, and aliquots of the stock solutions were placed in 500-mL beakers and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using DMSO was also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were carried out 25 ± 2°C. Each test was conducted with four replicates with three concentrations (50, 25, and 12.5, µg/mL for C. canadensis and C. sumatrensis; 150, 100, and 50 µg/mL for C. bonariensis). Permethrin was used as a positive control.

3.5. Non-Target Insecticidal Assay

The Diplonychus rusticus adults were collected in the field and maintained in glass tanks (60 cm long × 50 cm wide) containing water at 25 °C with a water depth of 20 cm. The essential oils were tested at concentrations of 200, 150, 100, 75, 50, and 25 µg/mL. Four replicates were performed for each concentration. Twenty D. rusticus adults were introduced into each solution. The non-target organism was observed for mortality after 24 h and 48 h exposure.

3.6. Data Analysis

The mortalities were recorded 24 h and 48 h after treatment. The data obtained were subjected to log-probit analysis [68] to obtain LC₅₀ values, LC₉₀ values, 95% confidence limits, and chi square values using Minitab® 18 (Minitab Inc., State College, PA, USA). For the principal component analysis (PCA), the 9 major components (limonene, allo-aromadendrene, (Z)-lachnophyllum ester, caryophyllene oxide, (E)-caryophyllene, β-pinene, (E)-β-farnesene, spathulenol, and α-humulene), and the 24-h larvicidal activities against Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus were taken as variables using a Pearson correlation matrix using XLSTAT Premium, version 2018.5 (Addinsoft, Paris, France). A total of 33 data (11 variables × 3 samples) were used for the PCA.

4. Conclusions

Invasive plant species are generally considered to be ecologically and detrimental with potential economic impacts, and the control or eradication of invasive plant species can be prohibitively costly. However, identification of beneficial uses of invasive plants could be economically advantageous and aid in the control of the species. Conyza spp., as well as Erechtites spp. [34], Crassocephalum crepidioides [35], and Severinia monophylla [33], are invasive weeds in Vietnam, and essential oils from these plants have demonstrated promising mosquito larvicidal activities. The plant materials are readily available and
harvesting of these weeds may provide economically valuable “cash crops” as well as serve as a means for ecological remediation. Note that *C. bonariensis* [69], *C. canadensis* [70], and *C. sumatrensis* [71] have all shown resistance to the commonly used herbicide glyphosate, so herbicidal control of these weeds is impractical as well as environmentally detrimental. Further research on potential formulations (e.g., nanoemulsions or essential oil-loaded nanoparticles) [72] for field use of these promising essential oils is warranted.

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Sample Availability: Samples of the Conyza essential oils are no longer available.