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# Chemical Composition, Insecticidal and Repellent Activities of Essential Oils from *Piper asperiusculum* and *Piper pertomentellum* against Red Flour Weevil

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**Abstract:** Many essential oils (EOs) from plants have potential applications as insecticides. In the present study, EOs from leaves and inflorescences of *Piper* cf. *asperiusculum* var. *glabricuale* Trel. & Yunck and *Piper pertomentellum* Trel. & Yunck were analyzed through gas chromatography coupled to mass spectrometry (GC-MS) and their insecticidal activity against the red flour weevil *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) was evaluated. The main components in *P. asperiusculum* were myristicin (15%–35%) and dillapiole (36-48%), whereas the main compounds in *P. pertomentellum* were limonene (4%–17%), germacrene D (10%–29%) and β-caryophyllene (6%–10%). Results showed that the EOs from fresh inflorescences of *P. pertomentellum* had higher fumigant toxicity against *T. castaneum* ($LC_{50}$ 63.2 µL/L air). All oils evaluated showed over 90% repellency at 0.063 µL/cm$^2$.

**Keywords:** *Piper* cf. *asperiusculum*; *Piper pertomentellum*; *Tribolium castaneum*; fumigant; repellent. 

## 1. Introduction

Essential oils (EOs) have long been considered promising alternatives to synthetic insecticides (phosphine, methyl bromide and dichlorvos) [1,2]. EOs contain secondary metabolites that can act as repellents, toxicants, antifeedants, oviposition inhibitors, growth inhibitors and attractants, showing potential as broad-spectrum insecticides [3,4]. In addition, EOs have low environmental impact and residuality owing to their volatility [5,6].

The insects of the genus *Tribolium*, popularly known as weevils, are pests with considerable worldwide economic importance because of the losses they cause in stored grains and *Tribolium castaneum* is the most widespread and destructive species [4,7]. Infestation by this pest results in weight loss; reduced germination; reduced levels of nutrients, flavor and odor in grains; and high temperature and humidity conditions that promote the proliferation of microorganisms [8-10].

Among the EOs with the greatest potential for the control of phytosanitary problems caused by insect pests of different orders, the genus *Piper* of the family Piperaceae is the most remarkable [11,12]. In Colombia, this genus is widely distributed and the efficacy of some of its species has been evaluated against stored produce pests, showing favorable results in all cases. *P. pseudo-lanceifolium*

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oil has shown strong repellency against *T. castaneum* adults [10]; meanwhile *P. aduncum*, *P. hispidinervum*, *P. guineense* and *P. marginatum* oils have shown fumigant and contact toxicity against *S. zeamais* [13].

Therefore, this study characterized the chemical composition of the EOs of two *Piper* species (*Piper cf. asperiusculum* var. *glabricuale* and *Piper pertomentellum*) and evaluated the EOs' fumigant toxicity and repellent activity against *T. castaneum* and the effects of drying on composition and bioactivity.

## 2. Materials and Methods

### *2.1. Plant Material*

The inflorescences and leaves of *P.* cf. *asperiusculum* var. *glabricuale* Trel & Yunck were collected from the La Laguna trail in San Mateo, Boyacá (72º33'18" W, 06º24'05" N) and the aerial parts of *P. pertomentellum* Trel & Yunck were collected from the El Rodeo trail in Guayabal de Síquima, Cundinamarca (72º33'18" W, 06º24'05" N). The voucher specimens are in the Herbario Nacional Colombiano, with numbers COL 579924 and COL 579920, respectively.

### *2.2. Extraction of EOs*

The aerial parts (leaves and inflorescences; fresh and dry) of the two plants collected were subjected to steam extraction for 2 h. The EOs were recovered through condensation with a Clevenger apparatus. After decantation, they were dried with anhydrous sodium sulfate and stored in amber-sealed glass bottles at 4 °C until use.

### *2.3. Chemical Composition of EOs*

#### *2.3.1. Sample Preparation*

The volume of each EO was increased from 25 µL to a final volume of 1 mL with *n*-hexane. The standard hydrocarbon solution was prepared by dissolving 25 µL of a homologous hydrocarbon solution (C8–C26) to a final volume of 1 mL with *n*-hexane.

#### *2.3.2. Analysis by GC-MS*

Chromatographic analysis was performed on an Agilent Technologies 7890 AGC gas chromatograph with a Hewlett Packard 5973 mass selective detector with a quadrupole analyzer in full scan mode at 4.57 scans per second. The mass spectrometer was operated at 70 eV and mass spectra were collected between 35 and 450 m/z. The temperatures of the ionization chamber, injector and transfer line were 185 °C, 250 °C and 280 °C, respectively. Separation was performed with two columns of orthogonal polarity (DB-5MS and HP-INNOWax).

The first analysis was performed with a DB-5MS capillary column ((5%-phenyl)-methylpolysiloxane; 60 m × 0.25 mm × 0.25 µm), with injection in split mode (20:1). The oven temperature was programmed from 150 °C (5 min) to 220 °C (5 min) at 2.5 °C/min and finally to 280 °C (4 min) at 9 °C/min for a total run time of 49 min. In the second analysis, an HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 µm) was used with split mode injection (20:1). The oven temperature was programmed from 45 °C (5 min) to 120 °C (3 min) at 3 °C/min and finally to 220 °C (5 min) at 4 °C/min for a total run time of 63 min. In both cases, the injection volume was 1 µL.

#### *2.3.3. Oil Component Determination*

The chemical constituents were determined by comparing the mass spectra and retention indices (RIs) obtained for each compound with those reported in the NIST 14.L, Wiley 8.1 and

Pherobase databases and those published in the literature [14]. The RIs were calculated using a homologous series of hydrocarbon standards from $C_8$ to $C_{26}$ and analyzed under the same operating conditions as those used for EOs [15].

### *2.4. Bioassays*

#### *2.4.1. Insects*

Adult *T. castaneum* were obtained from a stock colony maintained at the Química de Productos Naturales Vegetales Bioactivos (QuiProNaB) Research Group of the Department of Chemistry, Universidad Nacional de Colombia-Bogotá. Adults were maintained in a culture chamber in the dark at relative humidity (RH) of 65% ± 5% and temperature of 27 ± 1 °C). The diet of *T. castaneum* was based on a mixture of wheat flour and yeast (95/5 by weight) [16]. Adult insects between 6–10 days after emergence were used in different activity assays. The work with insects conducted in this study was approved by the Ethics Committee of the Faculty of Sciences at the Universidad Nacional de Colombia, under Resolution 077-2018.

#### *2.4.2. Fumigant Activity Test against T. castaneum*

Doses ranging from 0.5 µL to 11 µL (22.7–500 µL/L air) were used in determining the fumigant toxicity of EOs. The EOs were applied to 2 cm-diameter Wathman No. 1 filter paper discs placed on top of a 1.5 mL volume glass vial. The vial was then transferred to another larger vial (22 mL) with a screw cap containing 10 insects without sexing of the species to be tested. Nuvan 50 containing dichlorvos as the active ingredient (50 µL/L air) was used as positive control. The negative control was performed in the same manner but without the application of any substance. All tests were performed in triplicate under controlled temperature and humidity conditions (27 ± 1 °C and 65% ± 5% RH). Insect mortality was determined after 24 h. Insects were observed using a stereoscope and considered dead when no movement of the legs or antennae was observed after 15 s of stimulation with an entomological pin [17]. The percentage of insect mortality (%M) was calculated using the corrected formula of Abbott [18]: %Mortality = [(%Mt-%Mc)/100-%Mc]*100, where Mt is mortality on treatment and Mc is mortality on control.

#### *2.4.3. Repellent Activity Test on T. castaneum*

An area preference bioassay was used in evaluating repellency. Half of each 9 cm-diameter filter paper disc (Whatman No. 1) was treated with 500 µL of the acetone solution of each EO at concentrations of 0.0630, 0.0470 and 0.0252 µL/cm$^2$. The solvent was allowed to evaporate for 3 min in an extraction booth. Half of the filter paper treated with the samples and half treated with acetone only were placed in the bottom of a Petri dish. Then, 10 unsexed adults were placed in the center of each petri dish, which was closed and sealed with parafilm. The number of insects present on the two halves of the paper disks was recorded after 2 and 24 h of exposure. As a positive control, the commercial Stay Off repellent, whose active ingredient is a 15% formulation of IR3535 (ethyl-3-(*N*-acetyl-*N*-butylamine)-propionate), was evaluated with the same concentrations of EOs. Five replicates were prepared for each concentration evaluated. The test was conducted under controlled temperature and humidity conditions (27 ± 1 °C and 65% ± 5% RH). The repellency percentage (RP) was calculated using the formula RP = [(C-T)/(C+T)] × 100, where C is the number of insects in the untreated area and T is the number of insects in the treated area [19].

#### *2.3.4. Statistical Analysis*

EOs with fumigant potential were identified from the calculated mortalities at the maximum concentration (500 µL/L air). Analysis of variance (ANOVA) was then performed to determine whether the results obtained for the insecticidal activity tests were statistically different among the EOs with fumigant potential for each evaluated concentration. Statistical significance was set at P <

0.05. Tukey's honest significance test (HSD) was performed to evaluate pairwise differences among the EOs for the concentrations that showed significant differences from the ANOVA test. $LC_{50}$ was estimated to use the fumigant method and the probit model and compared in terms of their 95% confidence limit intervals among the EOs with fumigant potential. Finally, test results for the repellent activity are presented as mean ± standard deviation (SD).

## 3. Results and Discussion

### *3.1. Yield and Chemical Profile of the EOs*

The EOs from fresh and dried leaves and inflorescences of *P. pertomentellum* and *P.* cf. *asperiusculum* var. *glabricaule* were obtained through steam distillation. The extraction yields for each case are shown in Table 1. Herbal samples dried by different methods increased or decreased EO yields depending on the drying method, duration, temperature and the nature of each species [20, 21]. In the present study, shade drying was performed and the plant organs of the two species showed varying behavior. *P. asperiusculum* oils had high yields in the dry leaves and fresh inflorescences and *P. pertomentellum* oils had high yields in the fresh leaves and fresh inflorescences.

**Table 1.** Extraction yields of EOs obtained from *P. pertomentellum* and *P. asperiusculum*

| Essential oil | | Yield (w/w %) |
|---|---|---|
| **Specie** | **Plant part** | |
| *Piper cf. asperiusculum var. glabricaule* | Dry leaves | 0.98 |
| | Fresh leaves | 0.60 |
| | Dry inflorescences | 3.49 |
| | Fresh inflorescences | 3.71 |
| *Piper pertomentellum* | Dry leaves | 0.30 |
| | Fresh leaves | 0.94 |
| | Dry inflorescences | 0.36 |
| | Fresh inflorescences | 1.32 |

GC-MS analysis was performed using orthogonal polarity columns. The EOs were used in identifying 76 compounds, representing 65%–98% of the total composition (Table 2). The EOs of the leaves and inflorescences of *P. pertomentellum* were mainly composed of sesquiterpenes (32%–55%) and oxygenated sesquiterpenes (10%–23%). The main components were germacrene D for leaves and fresh inflorescences and caryophyllene oxide for dry inflorescences. Meanwhile, the oils of leaves and inflorescences of *P.* cf. *asperiusculum* contained mainly phenylpropanoid-type oxygenated compounds (48%–75%) and myristicin and dillapiole were the major components. The chemical composition of fresh inflorescences of both species had been studied previously [22] and a similar profile with few differences was found. The drying method has a significant effect on the oil content and composition of aromatic plants [20,23]. The percentage of oxygenated compounds in EOs obtained from dried plant material was higher than that in EOs obtained from fresh materials. This result may be attributed to oxidation during the drying and storage of material plants [24].

**Table 2.** Chemical composition of the EOs from *P. pertomentellum* and *P. asperiusculum*

| | | Retention Indices | | | | Relative percentage (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Compound** | **DB-5MS Experimental** | **DB-5MS Literature** | **HP-INNOWAX Experimental** | **HP-INNOWAX Literature** | *P. pertomentellum* Dry leaves | *P. pertomentellum* Fresh leaves | *P. pertomentellum* Dry inflorescences | *P. pertomentellum* Fresh inflorescences | *P. asperiusculum* Dry leaves | *P. asperiusculum* Fresh leaves | *P. asperiusculum* Dry leaves | *P. asperiusculum Fresh* inflorescences |
| 1 | α-thujene | 929 | 924-931 | - | - | - | - | - | - | - | 0.04 | 0.04 | 0.04 |
| 2 | α-pinene | 939 | 932-939 | - | - | 2.50 | 0.56 | 0.62 | 0.34 | 2.88 | 3.07 | 2.18 | 1.76 |
| 3 | camphene | 957 | 946-957 | - | - | 0.06 | - | - | - | 0.05 | 0.04 | 0.06 | 0.06 |
| 4 | sabinene | 977 | 960-980 | - | - | 0.23 | 0.09 | 0.13 | 0.11 | 0.06 | 0.09 | 0.08 | 0.07 |
| 5 | β-pinene | 985 | 980-990 | - | - | 5.55 | 1.41 | 1.62 | 0.52 | 2.81 | 3.40 | 1.47 | 1.22 |
| 6 | β-myrcene | 989 | 986-994 | 1168 | 1145-1187 | 0.29 | 0.17 | 0.10 | 0.19 | 0.62 | 1.02 | 0.66 | 0.65 |
| 7 | pseudolimonene | 1009 | 996-1005 | - | - | - | - | - | - | 0.02 | 0.02 | - | - |
| 8 | α-phellandrene | 1011 | 1005-1032 | 1170 | 1166-1205 | - | - | - | - | 0.12 | 0.19 | 0.52 | 0.64 |
| 9 | δ-3-carene | 1014 | 1008-1017 | 1153 | 1148-1180 | 0.04 | - | - | - | 0.03 | 0.08 | 0.03 | 0.03 |
| 10 | α-terpinene | 1021 | 1014-1020 | 1185 | 1178-1208 | - | - | - | - | 0.04 | 0.06 | 0.08 | 0.09 |
| 11 | o-cimene | 1029 | 1022-1045 | 1279 | 1276-1299 | 0.06 | 0.04 | 0.08 | 0.09 | - | - | - | - |
| 12 | limonene | 1035 | 1031-1039 | 1205 | 1199-1224 | 8.71 | 10.89 | 4.54 | 15.64 | 6.48 | 9.98 | 5.43 | 6.30 |
| 13 | eucalyptol | 1039 | 1031-1039 | 1214 | 1209-1237 | 0.39 | 0.27 | 0.33 | 0.47 | 6.44 | 5.87 | 5.87 | 7.03 |
| 14 | Trans-β-ocimene | 1047 | 1043-1097 | 1241 | 1242-1270 | 1.28 | 4.41 | 0.26 | 7.47 | 0.52 | 6.05 | 0.59 | 1.03 |
| 15 | γ-terpinene | 1063 | 1055-1074 | 1252 | 1338-1274 | - | - | - | - | 0.16 | 0.19 | 0.17 | 0.16 |
| 16 | α-terpinolene | 1090 | 1084-1096 | 1290 | 1275-1315 | - | - | - | - | 0.04 | 0.10 | 0.05 | 0.06 |
| 17 | rosefuran | 1092 | 1090-1116 | 1411 | 1400-1450 | 0.08 | 0.15 | 0.43 | 0.16 | - | - | - | - |
| 18 | linalool | 1100 | 1096-1101 | 1555 | 1557-1581 | 0.27 | 0.23 | 0.67 | 0.66 | 0.26 | 0.33 | 0.37 | 0.74 |
| 19 | nonanal | 1105 | 1098-1108 | - | - | - | - | 0.16 | - | - | - | - | - |
| 20 | allo-ocimene | 1129 | 1125-1134 | 1381 | 1371-1396 | 0.14 | 0.33 | - | 0.52 | - | 0.14 | - | - |
| 21 | E.E-cosmene | 1133 | 1120-1134 | - | - | - | - | - | 0.08 | - | - | - | - |
| 22 | 4-acetyl-1-methylcyclohexene | 1137 | 1130-1147 | - | - | 0.05 | - | - | - | - | - | - | - |
| 23 | E-myroxide | 1140 | 1123-1143 | - | - | 0.07 | 0.14 | 0.06 | 0.17 | - | - | - | - |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | camphor | 1159 | 1143-1192 | 1531 | 1498-1550 | - | - | - | - | - | 0.02 | 0.03 | 0.07 |
| 25 | rosefuran epoxy | 1171 | 1170-1174 | - | - | - | - | 0.10 | - | - | - | - | - |
| 26 | α-phellandrene-8-ol | 1178 | 1166-1178 | - | - | - | - | - | 0.11 | - | - | - | - |
| 27 | 4-terpineol | 1189 | 1177-1182 | 1612 | 1593-1642 | - | - | - | - | 0.07 | 0.07 | 0.06 | 0.11 |
| 28 | α-terpineol | 1202 | 1185-1207 | 1707 | 1711-1732 | - | - | - | - | 0.68 | 0.57 | 0.57 | 0.86 |
| 29 | estragole | 1203 | 1195-1208 | - | - | - | - | 0.09 | 0.12 | - | - | - | - |
| 30 | Myrtenal | 1205 | 1193-1209 | 1645 | 1619-1648 | 0.04 | - | 0.14 | - | - | - | - | - |
| 31 | linalyl acetate | 1249 | 1236-1254 | 1569 | 1551-1583 | 0.04 | 0.06 | 0.16 | 0.12 | - | - | - | - |
| 32 | carvone | 1252 | 1240-1265 | - | - | - | - | 0.03 | - | - | - | - | - |
| 33 | *p*-anisaldehyde | 1263 | 1249-1263 | 2053 | 2011-2053 | - | - | 0.05 | 0.13 | - | - | - | - |
| 34 | piperitone | 1264 | 1255-1269 | 1744 | 1730-1795 | - | - | - | - | 1.32 | 1.25 | 3.15 | 4.75 |
| 35 | anethole | 1293 | 1284-1303 | 1845 | 1803-1847 | 0.58 | 0.49 | 0.79 | 1.36 | - | 0.79 | 0.15 | 0.33 |
| 36 | δ-elemene | 1341 | 1337-1340 | - | - | 0.28 | 0.22 | 0.46 | 0.46 | - | - | - | - |
| 37 | terpinyl acetate | 1349 | 1317-1350 | 1708 | 1684-1700 | 0.04 | 0.08 | 0.06 | 0.12 | - | - | - | - |
| 38 | α-cubenene | 1354 | 1353-1372 | 1462 | 1459-1480 | 0.62 | 0.08 | 1.03 | 0.65 | 0.11 | 0.17 | 0.05 | 0.07 |
| 39 | cyclosativene | 1383 | 1370-1394 | 1489 | 1483-1522 | - | - | 0.13 | - | 0.21 | 0.19 | 0.07 | 0.08 |
| 40 | α-copaene | 1388 | 1379-1400 | 1499 | 1470-1527 | 0.86 | 0.39 | 2.07 | 0.78 | 1.71 | 1.87 | 0.92 | 1.07 |
| 41 | β-cubebene | 1399 | 1389-1400 | 1548 | 1519-1558 | - | - | - | - | 0.46 | 0.81 | 0.14 | 0.15 |
| 42 | β-elemene | 1400 | 1375-1393 | 1599 | 1570-1595 | 3.17 | 3.56 | 2.59 | 1.90 | - | - | - | - |
| 43 | α-gurjunene | 1420 | 1407-1419 | 1540 | 1529-1550 | 0.17 | 0.21 | - | - | - | - | - | - |
| 44 | α-ionone | 1427 | 1421-1434 | - | - | 0.06 | - | - | - | - | - | - | - |
| 45 | β-gurjunene | 1431 | 1423-1442 | - | - | 0.36 | 0.40 | 0.15 | 0.43 | - | - | - | - |
| 46 | β-caryophyllene | 1434 | 1438-1467 | 1609 | 1608-1657 | 6.32 | 5.64 | 11.54 | 6.93 | 3.50 | 6.41 | 0.48 | 0.50 |
| 47 | γ-elemene | 1439 | 1434-1451 | 1648 | 1641-1650 | - | 0.45 | - | 0.26 | - | - | - | - |
| 48 | aromadendrene | 1452 | 1436-1447 | 1618 | 1622-1635 | - | - | - | - | 0.22 | 0.32 | 0.02 | 0.03 |
| 49 | β-farnesene | 1452 | 1450-1458 | 1674 | 1663-1673 | 0.54 | 0.54 | 0.11 | 0.15 | - | - | - | - |
| 50 | Humulene | 1470 | 1457-1488 | 1682 | 1665-1688 | 0.97 | 1.01 | 1.80 | 0.98 | 0.22 | 0.43 | 0.03 | 0.04 |
| 51 | allo-aromadendrene | 1475 | 1458-1478 | 1657 | 1616-1662 | 0.52 | 0.41 | 0.36 | 0.23 | 0.15 | 0.20 | 0.03 | 0.04 |
| 52 | α-amorphene | 1484 | 1479-1506 | 1698 | 1691-1705 | - | - | - | - | 0.15 | 0.21 | - | - |
| 53 | γ-muurolene | 1486 | 1477-1488 | 1699 | 1681-1741 | 0.68 | 0.31 | 0.40 | 0.37 | - | - | - | - |
| 54 | germacrene-D | 1497 | 1480-1500 | 1724 | 1705-1772 | 17.63 | 24.41 | 7.33 | 28.29 | 0.26 | 2.33 | 0.23 | 0.47 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | viridiflorene | 1504 | 1493-1512 | - | - | - | - | - | - | 0.09 | 0.15 | - | - |
| 56 | α-bisabolene | 1506 | 1502-1510 | 1739 | 1730-1751 | 5.98 | 11.05 | - | 0.50 | - | - | - | - |
| 57 | bicyclogermacrene | 1510 | 1500-1520 | 1747 | 1740-1784 | 3.99 | 4.14 | 1.47 | 2.54 | 0.56 | 1.64 | 0.16 | 0.23 |
| 58 | β-bisabolene | 1514 | 1509-1513 | - | - | 1.34 | - | - | - | - | - | - | - |
| 59 | γ-cadinene | 1524 | 1512-1526 | 1773 | 1752-1819 | - | - | 0.32 | 0.39 | - | - | - | - |
| 60 | δ-cadinene | 1528 | 1524-1531 | 1769 | 1749-1808 | 1.88 | 1.59 | 2.18 | 1.65 | 0.67 | 0.92 | 0.16 | 0.24 |
| 61 | myristicin | 1530 | 1525-1566 | 2197 | 2225-2296 | - | - | - | - | 11.54 | 10.19 | 35.99 | 32.68 |
| 62 | calamenene | 1532 | 1521-1538 | 1848 | 1816-1853 | 0.68 | - | - | - | 0.20 | 0.18 | - | - |
| 63 | cadina 1,4-diene | 1543 | 1530-1542 | - | - | - | - | - | 0.10 | - | 0.04 | - | - |
| 64 | elemicin | 1545 | 1533-1558 | 2149 | 2214-2264 | - | - | - | - | 0.09 | 0.03 | 0.14 | 0.12 |
| 65 | α-cadinene | 1547 | 1534-1548 | - | - | - | - | 0.14 | 0.08 | - | - | - | - |
| 66 | α-calacorene | 1552 | 1517-1548 | 1936 | 1906-1916 | 0.14 | - | - | - | - | - | - | - |
| 67 | β-calacorene | 1553 | 1548-1564 | - | - | - | - | - | - | 0.34 | 0.19 | 0.09 | 0.03 |
| 68 | elemol | 1557 | 1547-1559 | 2095 | 2080-2094 | 0.20 | 0.13 | 0.25 | 0.21 | - | - | - | - |
| 69 | nerolidol | 1564 | 1545-1564 | 2049 | 2038-2052 | 1.38 | 1.22 | 2.48 | 1.07 | 0.11 | 0.14 | - | - |
| 70 | germacrene B | 1574 | 1562-1578 | - | - | 0.44 | 0.58 | 0.49 | 0.36 | - | - | - | - |
| 71 | spathulenol | 1591 | 1575-1640 | 2123 | 2129-2189 | 6.01 | 5.05 | 5.44 | 2.99 | 0.85 | - | - | - |
| 72 | caryophyllene oxide | 1598 | 1581-1606 | 2006 | 1962-2068 | 2.06 | 0.88 | 13.99 | 1.04 | 2.85 | 0.45 | - | - |
| 73 | dillapiole | 1625 | 1602-1644 | 2292 | 2305-2351 | 4.69 | 0.48 | - | - | 48.51 | 36.82 | 38.39 | 36.67 |
| 74 | T-muurolol | 1655 | 1643-1655 | - | - | - | - | - | - | 0.15 | - | - | - |
| 75 | α-cadinol | 1666 | 1627-1653 | 2177 | 2180-2211 | 0.89 | 0.28 | 0.56 | 0.78 | - | - | - | - |
| 76 | apiole | 1677 | 1679-1685 | - | - | - | - | - | - | 0.29 | 0.13 | 0.12 | 0.10 |
| | Non-oxygenated monoterpenes (%) | | | | | 18.86 | 17.90 | 7.35 | 24.96 | 13.83 | 24.47 | 11.36 | 12.11 |
| | Oxygenated monoterpenes (%) | | | | | 0.89 | 0.85 | 1.92 | 1.69 | 8.77 | 8.11 | 10.05 | 13.56 |
| | Phenylpropanoids (%) | | | | | 5.27 | 0.97 | 0.88 | 1.48 | 60.43 | 47.96 | 74.79 | 69.90 |
| | Non-oxygenated sesquiterpenes (%) | | | | | 46.57 | 54.99 | 32.57 | 47.05 | 8.85 | 16.06 | 2.38 | 2.95 |
| | Oxygenated sesquiterpenes (%) | | | | | 10.54 | 7.56 | 22.72 | 6.09 | 3.96 | 0.59 | - | - |
| | Other (%) | | | | | 0.15 | 0.08 | 0.27 | 0.25 | - | - | - | - |
| | **Total (%)** | | | | | **82.28** | **82.35** | **65.71** | **81.52** | **95.84** | **89.08** | **98.58** | **98.52** |

The volatile metabolites determined in the studied EOs have been reported in other *Piper* species. Germacrene D is one of the main compounds of several EOs, such as those from *P. psilorhachis* (18.4%), *P. sempervirens* (11.7%), *P. hispidum* (6.0%), *P. oradendron* (10.7%) and *P. umbellatum* (17.4%). However caryophyllene oxide has been reported in the oils extracted from *P. phytolacciolium* (12.0%), *P. amalago* (23.4%) and *P. retalhuleuense* (27.0%) [25]. Phenylpropanoids are the most characteristic metabolites of the *Piper* genus's chemotaxonomy. Myristicin and dillapiole have been reported in some species and *P. aduncum* (2.4%–73.0%), *P. hostmannianum* (20.3%–7.7%) and *P. permucronatum* (25.6%–54.7%) [26, 27]. This work represents the first report on the chemical composition of EOs from the leaves of *P. pertomentellum* and *P.* cf. *asperiusculum*.

The EOs of *Piper* species exhibit significant chemotaxonomic variability, which is influenced by geographic, seasonal and organ-specific factors. Numerous species, including *P. aduncum*, *P. nigrum*, *P. longum*, *P. marginatum*, *P. amalago* and *P. hispidum*, are known to exhibit distinct chemotypes. These chemotypes are defined by specific combinations of sesquiterpenes and phenylpropanoids that are influenced by genetic polymorphisms and environmental conditions. For example, *P. aduncum* shows multiple chemotypes dominated by either monoterpenoid, phenylpropanoids or sesquiterpenoid compounds, whereas *P. marginatum* shows considerable variability in phenylpropanoid content [26] [28]. This diversity reflects the metabolic plasticity and adaptability of the genus. Our results support the idea that phenylpropanoids and sesquiterpenes serve as reliable chemotaxonomic markers in *Piper*, with their consistent presence across species reinforcing their taxonomic utility. Furthermore, the observed environmental and organ-specific differences in EO composition highlight the influence of ecological factors on chemical diversity and chemotypic expression within the genus [29-31]. These results provide valuable insights into the evolutionary and ecological drivers of secondary metabolite production in *Piper*, thereby advancing our understanding of its chemotaxonomic relationships.

### *3.2. Fumigant Activity against T. castaneum*

Preliminary fumigant activity was evaluated at a concentration of 500 µL/L air and the resulting mortality rates are shown in Figure 1. Of the EOs evaluated, only three showed fumigant toxicity (M > 65%): fresh leaves of *P. asperiusculum* (FLPA), fresh leaves of *P. pertomentellum* (FLPP) and fresh inflorescences of *P. pertomentellum* (FIPP). A common feature of these active EOs is the presence of limonene at concentrations above 10%. This monoterpene has previously been reported to be toxic to both larvae and adults of *T. castaneum* [32].

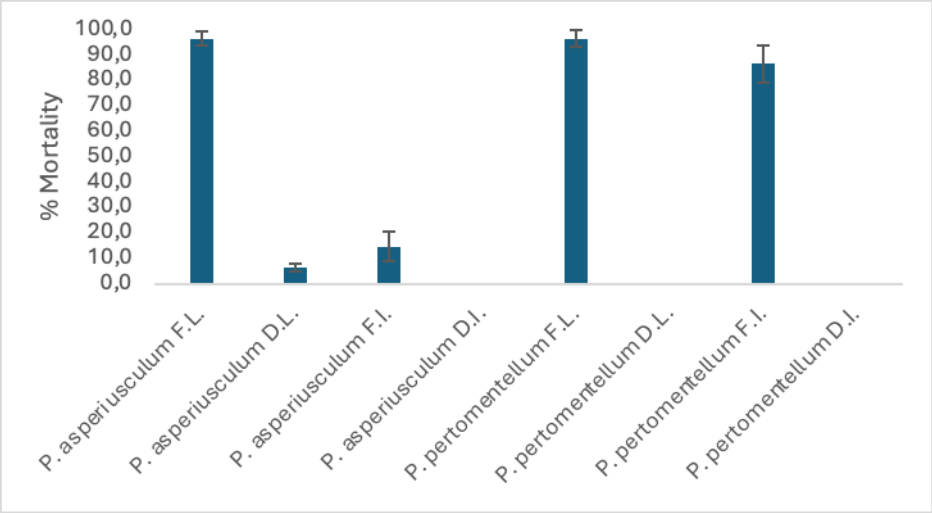


**Figure 1.** Results of the preliminary screening of fumigant activity of the 8 EOs from the two *Piper* species (**F.L.** Fresh leaves; **D.L.** Dry leaves; **F.I.** Fresh inflorescences; **D.I.** Dry inflorescences). Data is represented by the mean ± standard deviation of three independent replicates

To determine the $LC_{50}$ of the three active EOs, concentrations ranging from 500 to 50 µL/L air were evaluated. The ANOVA test results, shown in Table 3, indicate that at concentrations above 350 µL/L air, there were no significant differences in fumigant toxicity among the three active EOs ($P > 0.05$). However, at lower concentrations (250, 150 and 50 µL/L air), statistically significant differences were observed ($p < 0.05$) and Tukey's HSD tests were used to identify which substance pairs showed significant differences. As shown in Table 4, FIPP consistently had higher mortality rates than both FLPA and FLPP at these concentrations. In addition, FLPA and FLPP differed only at 150 µL/L air, where FLPP had a slightly higher mean mortality rate of 26.67% compared to FLPA.

**Table 3**. ANOVA tests results for differences in mortality for the three more active EOs: FLPP, FLPA, FIPP, for each concentration.

| **Concentration (**µL/L air) | | **Sum Sq** | **Mean Sq** | **F value** | **P value** |
|---|---|---|---|---|---|
| 500 | Treatment | 155.6 | 77.78 | 0.778 | 0.501 |
| | Residuals | 600.0 | 100.00 | | |
| 350 | Treatment | 800 | 400 | 4 | 0.0787 |
| | Residuals | 600 | 100 | | |
| 250 | Treatment | 3267 | 1633.3 | 18.38 | 0.0028** |
| | Residuals | 533 | 88.9 | | |
| 150 | Treatment | 12822 | 6411 | 144.2 | 8.46e-06*** |
| | Residuals | 267 | 44 | | |
| 50 | Treatment | 2222.2 | 1111.1 | 100 | 2.47e-05*** |
| | Residuals | 66.7 | 11.1 | | |

**Table 4**. Tukey's HSD results for pairwise differences in mortality for the three more active EOs: FLPP, FLPA, FIPP, for the concentrations that showed significant differences from the ANOVA tests

| **Concentration (**µL/L air) | | **Mean difference** | **95% CI** | **P value** |
|---|---|---|---|---|
| 250 | FLPP-FLPA | 6.67 | (-16.95, 30.29) | 0.68 |
| | FIPP-FLPA | 43.33 | (19.71, 66.95) | 0.0032** |
| | IFPP-FLPP | 36.67 | (13.05, 60.29) | 0.0074** |
| 150 | FLPP-FLPA | 26.67 | (9.97, 43.37) | 0.0065** |
| | FIPP-FLPA | 90 | (73.30, 106.70) | 7.00e-06*** |
| | FIPP-FLPP | 63.33 | (46.63, 80.03) | 6.00e-05*** |
| 50 | FLPP-FLPA | 0 | (-8.35, 8.35) | 1 |
| | FIPP-FLPA | 33.33 | (24.98, 41.68) | 4.50e-05*** |
| | FIPP-FLPP | 33.33 | (24.98, 41.68) | 4.50e-05*** |

The results presented in Table 5 show the lethal concentrations ($LC_{50}$) of three active EOs against *T. castaneum*, revealing distinct differences in their fumigant toxicity. The most active oil was FIPP, which exhibited the highest toxicity with an $LC_{50}$ of 63.2 µL/L air. This oil was significantly more potent than those from FLPA and FLPP oils, being more than three times as active. These results underscore the importance of the plant part used in oil extraction and indicate that it plays a critical role in determining the insecticidal efficacy of EOs.

**Table 5**. Lethal concentrations ($LC_{50}$) of the active EOs against *T. castaneum*

| **Essential oil** | **$LC_{50}$ (µL/L air) (CL 95%*)** |
|---|---|
| | ***T. castaneum*** |
| Fresh leaves *P. asperiusculum* (FLPA) | 264.1 (234.7 – 293.9) |
| Fresh leaves *P. pertomentellum* (FLPP) | 222.9 (186.9 – 257.9) |
| Fresh inflorescences *P. pertomentellum* (FIPP) | 63.2 (51.5 – 79.3) |
| Nuvan 50 ® | 2.1 (1.5 – 3.8) |

* 95% confidence limit interval.

Compared to the commercial insecticide Nuvan 50®, all three EOs show lower efficacy. However, FIPP comes closest to the efficacy of Nuvan 50®, indicating that the EOs, although less potent than the synthetic pesticide, still offer substantial insecticidal activity, particularly as natural alternatives. The confidence limits for the $LC_{50}$ values of the EOs demonstrate the reliability of the toxicity estimates, with narrower intervals for FIPP indicating a more consistent effect compared to the wider intervals observed for FLPA and FLPP.

Furthermore, the observed high limonene content (15.6%) in FIPP may contribute to its insecticidal activity, consistent with previous studies highlighting the role of specific volatile compounds in the efficacy of EOs. In addition, other species of the *Piper* genus have shown activity as inhibitors of vital or detoxifying enzymes in *T. castaneum*. For example, the EO from fresh fruits of *P. nigrum* has a median inhibitory concentration ($IC_{50}$) of 303.11 µL/L against acetylcholinesterase, while the EO from fresh inflorescences of *P. aduncum* has an $IC_{50}$ of 579.89 µL/L against glutathione S-transferase [33]. The EOs evaluated in this study may have similar mechanisms of action, reinforcing the potential of these natural products in pest management.

### *3.3. Repellent Activity on T. castaneum*

The repellent activity of the EOs tested on *T. castaneum* (Table 6) showed a clear dose-response relationship, with repellency percentages (RP) increasing with increasing concentrations. EOs from *P. asperiusculum* and *P. pertomentellum* showed high repellency at all concentrations, achieving RPs between 52% and 100%, often exceeding the positive control, IR3535. Notably, EOs from both fresh and dried leaves of *P. asperiusculum* and *P. pertomentellum* maintained over 90% repellency at most concentrations, even after 24 hours, indicating strong pest control potent.

The EOs from fresh leaves and inflorescences of *P. asperiusculum* were among the top performers, consistently achieving close to 100% repellency at all concentrations. Similarly, *P. pertomentellum* EO from dry leaves showed excellent repellency, reaching 100% repellency within 2 hours at the highest concentration and maintaining it at 24 hours. In contrast, EO from *P. pertomentellum* inflorescences exhibited lower repellency, especially at lower concentrations, highlighting the importance of the plant organ used for oil extraction in optimizing repellent efficacy.

In conclusion, the present research reported for the first time insecticidal activity EOs from *P. asperiusculum* and *P. pertomentellum* against *T. castaneum* and identifies them as promising natural alternatives for pest control. The EO from *P. pertomentellum* inflorescences showed the highest activity, significantly outperforming leaf oils, with sesquiterpenes and oxygenated sesquiterpenes as key components. In contrast, the EOs from *P. asperiusculum* were rich in phenylpropanoids, especially myristicin and dillapiole. While none of the EOs matched the potency of the commercial insecticide Nuvan 50®, the results highlight the importance of the plant part used for oil extraction, with inflorescences showing greater insecticidal potential.

**Table 6.** Results of repellent activity of *P. asperiusculum* and *P. pertomentellum* EOs on *T. castaneum*

| Essential oil | Concentration (µL/cm$^2$) | Repellence (%) | |
|---|---|---|---|
| | | 2 h | 24 h |
| Dry leaves *P. asperiusculum* | 0,063 | 100 ± 0 | 100 ± 0 |
| | 0,047 | 92 ± 11 | 92 ± 11 |
| | 0,025 | 92 ± 11 | 100 ± 0 |
| Fresh leaves *P. asperiusculum* | 0,063 | 100 ± 0 | 100 ± 0 |
| | 0,047 | 100 ± 0 | 100 ± 0 |
| | 0,025 | 80 ± 14 | 96 ± 9 |
| Fresh inflorescences *P. asperiusculum* | 0,063 | 96 ± 9 | 100 ± 0 |
| | 0,047 | 96 ± 9 | 100 ± 0 |
| | 0,025 | 100 ± 0 | 100 ± 0 |
| Dry inflorescences *P. asperiusculum* | 0,063 | 100 ± 0 | 100 ± 0 |
| | 0,047 | 96 ± 9 | 100 ± 0 |
| | 0,025 | 64 ± 17 | 72 ± 18 |
| Dry leaves *P. pertomentellum* | 0,063 | 100 ± 0 | 100 ± 0 |
| | 0,047 | 100 ± 0 | 100 ± 0 |
| | 0,025 | 92 ± 11 | 96 ± 9 |
| Fresh leaves *P. pertomentellum* | 0,063 | 96 ± 9 | 96 ± 9 |
| | 0,047 | 96 ± 9 | 92 ± 11 |
| | 0,025 | 84 ± 22 | 80 ± 20 |
| Fresh inflorescences *P. pertomentellum* | 0,063 | 88 ± 18 | 84 ± 22 |
| | 0,047 | 88 ± 18 | 80 ± 20 |
| | 0,025 | 52 ± 18 | 44 ± 9 |
| Positive control (Active compound: IR3535) | 0,063 | 92 ± 11 | 88 ± 27 |
| | 0,047 | 92 ± 11 | 84 ± 17 |
| | 0,025 | 84 ± 17 | 68 ± 23 |

Repellence values are expressed as mean ± standar deviation of five independent replicates

In addition, the strong repellent and fumigant effects of oils from both species make them valuable candidates for future integrated pest management strategies targeting stored product pests such as *T. castaneum*.

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