

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

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(Received August 06, 2024; Revised March 02, 2025; Accepted March 04, 2025)

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* (LC₅₀ 63.2 μ L/L air). All oils evaluated showed over 90% repellency at 0.063 μ L/cm².

Keywords: *Piper* cf. *asperiusculum*; *Piper pertomentellum*; *Tribolium castaneum*; fumigant; repellent. © 2025 ACG Publications. All rights reserved.

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 (72°33'18" W, 06°24'05" N) and the aerial parts of *P. pertomentellum* Trel & Yunck were collected from the El Rodeo trail in Guayabal de Siquima, Cundinamarca (72°33'18" W, 06°24'05" 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 C₈ to C₂₆ 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 Whatman 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/cm². 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 $RP = [(C-T)/(C+T)] \times 100$, 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 $P <$

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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. LC₅₀ 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 $\pm$ 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	
<i>Piper cf. asperiusculum</i> var. <i>glabricaule</i>	Dry leaves	0.98
	Fresh leaves	0.60
	Dry inflorescences	3.49
	Fresh inflorescences	3.71
<i>Piper pertomentellum</i>	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*

	Compound	Retention Indices				Relative percentage (%)							
		DB-5MS Experimental	DB-5MS Literature	HP-INNOWAX Experimental	HP-INNOWAX Literature	<i>P. pertomentellum</i> Dry leaves	<i>P. pertomentellum</i> Fresh leaves	<i>P. pertomentellum</i> Dry inflorescences	<i>P. pertomentellum</i> Fresh inflorescences	<i>P. asperiusculum</i> Dry leaves	<i>P. asperiusculum</i> Fresh leaves	<i>P. asperiusculum</i> Dry leaves	<i>P. asperiusculum</i> 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	-	-	-	-

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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	<i>p</i> -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

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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

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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 $\mu\text{L/L}$ 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].

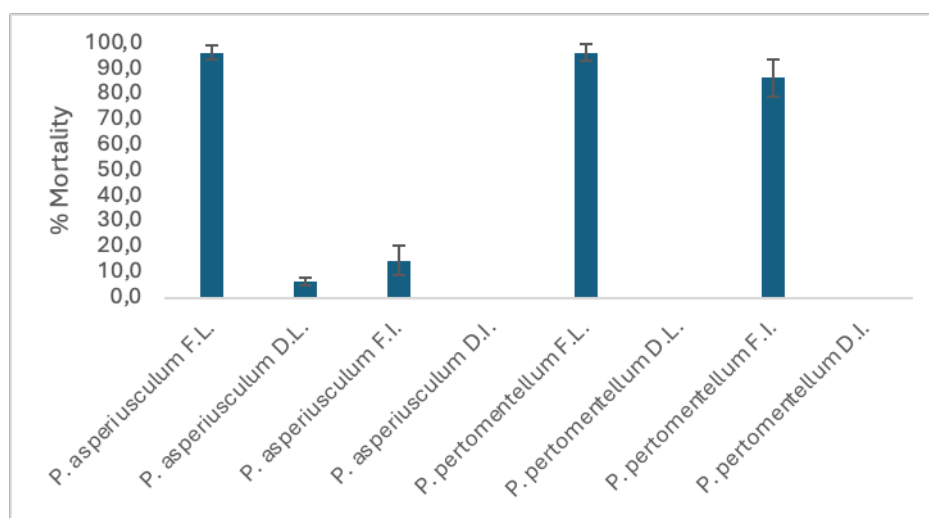


Figure 1. Results of the preliminary screening of fumigant activity of the 8 EOs from the two *Piper* species (F.L. Fresh leaves; D.L. Dry leaves; F.I. Fresh inflorescences; D.I. Dry inflorescences). Data is represented by the mean $\pm$ standard deviation of three independent replicates

To determine the LC_{50} of the three active EOs, concentrations ranging from 500 to 50 $\mu\text{L/L}$ air were evaluated. The ANOVA test results, shown in Table 3, indicate that at concentrations above 350 $\mu\text{L/L}$ 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 $\mu\text{L/L}$ 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 $\mu\text{L/L}$ 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 ($\mu\text{L/L}$ 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 ($\mu\text{L/L}$ 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 $\mu\text{L/L}$ 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 (LC₅₀) of the active EOs against *T. castaneum*

Essential oil	LC ₅₀ (μL/L air) (CL 95%*)
	<i>T. castaneum</i>
Fresh leaves <i>P. asperiusculum</i> (FLPA)	264.1 (234.7 – 293.9)
Fresh leaves <i>P. pertomentellum</i> (FLPP)	222.9 (186.9 – 257.9)
Fresh inflorescences <i>P. pertomentellum</i> (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 LC₅₀ 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 (IC₅₀) of 303.11 μL/L against acetylcholinesterase, while the EO from fresh inflorescences of *P. aduncum* has an IC₅₀ 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 ($\mu\text{L}/\text{cm}^2$)	Repellence (%)	
		2 h	24 h
Dry leaves <i>P. asperiusculum</i>	0,063	100 $\pm$ 0	100 $\pm$ 0
	0,047	92 $\pm$ 11	92 $\pm$ 11
	0,025	92 $\pm$ 11	100 $\pm$ 0
Fresh leaves <i>P. asperiusculum</i>	0,063	100 $\pm$ 0	100 $\pm$ 0
	0,047	100 $\pm$ 0	100 $\pm$ 0
	0,025	80 $\pm$ 14	96 $\pm$ 9
Fresh inflorescences <i>P. asperiusculum</i>	0,063	96 $\pm$ 9	100 $\pm$ 0
	0,047	96 $\pm$ 9	100 $\pm$ 0
	0,025	100 $\pm$ 0	100 $\pm$ 0
Dry inflorescences <i>P. asperiusculum</i>	0,063	100 $\pm$ 0	100 $\pm$ 0
	0,047	96 $\pm$ 9	100 $\pm$ 0
	0,025	64 $\pm$ 17	72 $\pm$ 18
Dry leaves <i>P. pertomentellum</i>	0,063	100 $\pm$ 0	100 $\pm$ 0
	0,047	100 $\pm$ 0	100 $\pm$ 0
	0,025	92 $\pm$ 11	96 $\pm$ 9
Fresh leaves <i>P. pertomentellum</i>	0,063	96 $\pm$ 9	96 $\pm$ 9
	0,047	96 $\pm$ 9	92 $\pm$ 11
	0,025	84 $\pm$ 22	80 $\pm$ 20
Fresh inflorescences <i>P. pertomentellum</i>	0,063	88 $\pm$ 18	84 $\pm$ 22
	0,047	88 $\pm$ 18	80 $\pm$ 20
	0,025	52 $\pm$ 18	44 $\pm$ 9
Positive control (Active compound: IR3535)	0,063	92 $\pm$ 11	88 $\pm$ 27
	0,047	92 $\pm$ 11	84 $\pm$ 17
	0,025	84 $\pm$ 17	68 $\pm$ 23

Repellence values are expressed as mean $\pm$ 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*.

Acknowledgments

This research was financed by the Universidad Nacional de Colombia, the Universidad Nacional Abierta y a Distancia and the Sistema General de Regalías de Colombia through project code BPIN 2020000100342, announcement “Convocatoria 8 del plan bienal de convocatorias publicas 2019-2020” and by the Pontificia Universidad Javeriana (project PPTA 7747). The authors thank the Ministry of Environment and Sustainable Development of Colombia for the amnesty established in Article 6 of Law 1955 of 2019.

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