ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 18:1 (2024) 114-124

records of natural products

Chemical Profiles and Bioactivities of the Essential Oils from Four Lauraceae Plants for Controlling *Tribolium castaneum* Herbst

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(Received September 18, 2023; Revised December 25, 2023; Accepted December 28, 2023)

Abstract: Essential oils (EOs) from Lauraceae plants have been extensively investigated in the control of stored-product insects. In this work, four Lauraceae species namely *Lindera communis* Hemsl., *Phoebe neurantha* (Hemsl.) Gamble, *Litsea rotundifolia* Hemsl. var. *oblongifolia* (Nees) Allen and *Litsea variabilis* Hemsl. var. *oblonga* Lec. were collected for extracting EOs by hydro-distillation and their chemical compositions were comparatively analyzed by GC/MS and GC/FID. Furthermore, contact toxicity and repellency of these EOs were evaluated against *Tribolium castaneum* Herbst, a universal model insect used in fundamental and applied research. Results indicated that EOs from *Lin. communis*, *P. neurantha*, *Lit. rotundifolia* and *Lit. variabilis* were mainly composed of sesquiterpenoids including (E)-*β*-famesene, *cis*-*α*-bisabolene, *α*-selinene, eremophilene, *β*-selinene, etc. In bioassays, all the EOs at maximum testing concentration of 78.63 nL/cm^2 were significantly repellent to *T. castaneum* adults at 2 and 4 h post-exposure, which were comparable to the positive control DEET. Among them, EOs from *P. neurantha*, *Lit. rotundifolia*, *Lin. communis* P and Q also had contact toxicity with LD_{50} values of 14.52, 17.58, 23.82 and 86.63 μg/adult respectively. It suggests that the four species of Lauraceae have promising potential to be developed into botanical repellents and contact toxicants against stored-product insects.

Keywords: *Lindera communis*; *Phoebe neurantha*; *Litsea rotundifolia* var. *oblongifolia*; *Litsea variabilis* var. *oblonga*; Biopesticides; Stored-product insects.

1. Introduction

Essential oils (EOs) derived from the secondary metabolism of aromatic plants are considered viable alternatives to synthetic pesticides for protecting stored grains [1]. Lauraceae plants especially in the genus *Cinnamomum*, *Litsea* and *Laurus* have been extensively investigated in the control of stored-product insects. Their EOs along with individual components could be promising repellents, feeding deterrents, reproductive inhibitors and insecticidal toxins via fumigation, contact and ingestion on storage pests [2]. In this work, four species in the Lauraceae family namely *Lindera communis* Hemsl., *Phoebe neurantha* (Hemsl.) Gamble, *Litsea rotundifolia* Hemsl. var. *oblongifolia* (Nees) Allen and *Litsea variabilis* Hemsl. var. *oblonga* Lec. were collected as research objects. Literatures about the chemical composition of their leaf oils are few available and meanwhile their bioactivities

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products January-February 2024 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.432.2309.2905
Available online: January 29, 2024

against stored-product insects have not been reported before. *Lit. rotundifolia* and *Lit. variabilis* are two species in the genus *Litsea*, mainly distributed in tropical and subtropical regions of India, South China and Japan [3]. *Lin. communis* is a member of the genus *Lindera*, predominant in tropical, subtropical and temperate zones of Asia and Midwestern America [4]. *Litsea* and *Lindera* plants are important sources of traditional medicines and spices for ages [3, 4], among which *Lit. cubeba* [5], *Lit. pungens* [6], *Lit. dilleniifolia* [7] and *Lin. aggregata* [8] have proved toxic and repellent to a wide range of stored-product insects including *Tribolium castaneum*, *Sitophilus zeamais*, *Lasioderma serricorne*, *Blattella germanica* and *Liposcelis bostrychophila*. *P. neurantha* belongs to the genus *Phoebe*, mostly growing in Indo-Malaysia, West Europe and Tropical America [9]. Its wood can release aroma to prevent pest intrusion and is globally used as superior furniture material [10]. Given facts above, the four species are thought valuable for current pest management research.

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is one of the most destructive and ubiquitous stored-product insects across the globe [11]. It is the representative pest species in flour mills and commonly known as the red flour beetle. Their damage to wheat flour results in substantial economic losses every year and the situation is particularly serious in developing countries [12]. It is an excellent model organism applied in different subject fields for its easy raising and experimental treating, short generation time, long lifespan, high reproductive capacity and efficient manipulation of gene expression. It has been widely used as the target insect for developing novel anti-insect agents in pest management [11]. Therefore, *T. castaneum* was adopted as the target insect in this work to evaluate bioactivities of EOs from four Lauraceae plants. The objective of this work involved investigating chemical profiles of EOs from *Lin. communis*, *P. neurantha*, *Lit. rotundifolia* and *Lit. variabilis*, as well as their contact toxicity and repellency against *T. castaneum* adults for the first time. It sought to excavate more Lauraceae plants with a great potential for combating stored-product pests.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction

Fresh leaves of four species in the Lauraceae family were collected at random from South China and identified by Prof. Q. R. Liu (Biology Department, College of Life Sciences, Beijing Normal University). Their voucher specimens were deposited at BNU Herbarium (NYBG Steere Herbarium). Among them, *Lin. communis* Hemsl. sampled from two cities of Yunnan Province (China). Specific sampling information was shown in Table 1.

Leaves were separately subjected to hydro-distillation for 5 h using a modified Clevenger-type apparatus to produce EOs. The distilled EOs were recorded for their volume to calculate the yield (v/w, mL/g). Then they were dehydrated by anhydrous sodium sulfate (Na_2SO_4), collected into sealed bottles and refrigerated at 4 °C for subsequent analysis.

Table 1. Sampling information of four Lauraceae plants

Species	Sampling time	Origin	Weight (kg)	Abbreviation	Specimen No.
Lindera communis Hemsl.	2018.06	Qujing, Yunnan Province	0.40	*Lin. communis* Q	BNU-201806002
	2021.05	Pu'er, Yunnan Province	0.75	*Lin. communis* P	BNU-202105001
Phoebe neurantha (Hemsl.) Gamble	2017.12	Quanzhou, Guangxi Province	1.30	*P. neurantha*	BNU-201712003
Litsea rotundifolia Hemsl. var. *oblongifolia* (Nees) Allen	2020.05	Guangzhou, Guangdong Province	1.14	*Lit. rotundifolia*	BNU-202005001
Litsea variabilis Hemsl. var. *oblonga* Lec.	2017.12	Fangchenggang, Guangxi Province	1.25	*Lit. variabilis*	BNU-201712002

2.2. Chemicals

n-Alkanes (C_6-C_{40}) was purchased from Sigma-Aldrich (St. Louis, USA). *n*-Hexane (A.R.) was purchased from Beijing Chemical Works (Beijing, China). *N, N*-diethyl-3-methylbenzamide (DEET) was purchased from the National Center of Pesticide Standards (Shenyang, China). Polytetrafluoroethylene (PTFE) was purchased from Huatong Ruichi Material Technology Co., LTD (Beijing, China).

2.3. GC/MS and GC/FID Analysis

The chemical composition of EOs was analyzed by GC/MS and GC/FID. GC analysis was carried out by a Thermo-Finnigan Trace-DSQ instrument (Thermo Fisher, USA) equipped with a HP-5MS fused silica column (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID). The GC oven temperature was initially held at 50℃ for 2 min and then programmed to 150 ℃ at 2 ℃/min, 250 ℃ at 10 ℃/min, and was finally held at 250 ℃ for 5 min. Helium was employed as carrier gas (1.0 mL/min). Injected volume was 1 μL (1% solution, v/v) and a split ratio of 1:20 was chosen. Mass spectra were recorded at 70 eV and scanned from 50 to 550 m/z. Relative retention indices (RI) of compounds on the HP-5MS column were calculated by a series of saturated *n*-alkanes (C_6-C_{40}) from the FID data. Individual components were ultimately identified by matching their mass spectra with those stored in the NIST library and comparing their calculated RI with reported RRI (RI in the range) in the NIST Chemistry WebBook, SRD 69.

2.4. Insect Culture

T. castaneum was reared on sterilized wheat flour mixed with active yeast (ratio: 10/1, w/w) in the laboratory. They were kept in dark incubators adjusted at 28-30 ℃ and 70-80 % relative humidity. Insect adults about 1-2 weeks' old were adopted for bioassays.

2.5. Biological Assays

Evaluation methods of candidates as toxicants and repellents against stored-product insects could trace back to the article from McDonald *et al* (1970) [13]. In this work, topical application and area preference method were used to access the contact toxicity and repellent activity of EOs against *T. castaneum* respectively.

2.5.1. Contact Toxicity

EOs were prepared as five to six concentration gradients by dilution with *n*-hexane. The solution with 0.5 μL was dropped onto the dorsal thorax of an insect using a pipette tip, so that it could result in five to six different dosages per insect when applied topically. *n*-Hexane was used as the negative control. Ten insects were set as a group and five groups were tested for each concentration. Insects treated were transferred to clean glass vials and maintained in incubators under the same rearing conditions in "*2.4. Insect Culture*". After 24 h, the number of dead insects in each group was counted. LD_{50} values were calculated by Probit analysis using SPSS V20.0 (IBM, USA).

2.5.2 Repellent Activity

EOs were diluted with *n*-hexane into testing solutions of five varying concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm^2). DEET was used as the positive control. A filter paper disc (Φ 9 cm) was cut in half. Two half discs were treated with 500 μL of diluted EOs and *n*-hexane respectively. They were stuck edge-to-edge lengthwise on the bottom of a Petri dish (Φ 9 cm) after the solvent evaporated. To avoid insects escaping from the paper disc, the Petri dish was coated with PTFE on the side. Twenty insects were exposed in a filter paper disk, which was considered as a group and five groups were tested for each concentration. The number of insects on the treated half (*Nt*) and

on the control half (*Nc*) was recorded at 2 and 4 h post-exposure. The percentage repellency (PR) was calculated by the following equation:

$$\text{PR } (\%) = \frac{Nc - Nt}{Nc + Nt} \times 100$$

For PR values, arcsine and square root transformation was performed, followed by one-way ANOVA analysis using SPSS V20.0 (IBM, USA). Means were compared by Tukey's HSD test at $p < 0.05$ to determine whether there were significant differences between treatments.

3. Results and Discussion

3.1. Chemical Composition and Chemotaxonomic Evaluation

Results of the chemical composition were listed in Table 2. The oil yield (v/w, mL/g) in descending order was 0.43 (*Lit. rotundifolia*), 0.28 (*Lin. communis* P), 0.20 (*Lin. communis* Q), 0.07 (*P. neurantha*) and 0.01% (*Lit. variabilis*) respectively. EOs are admittedly representative of aromatic plants in the Lauraceae family. Regardless of their compositions, presence or absence of themselves is regarded as a crucial taxonomic character [14]. Eighty-eight components accounting for 81.4-94.6% of EOs were identified here and sesquiterpenoids took up the largest proportion. It is in line with chemical characteristics of the Lauraceae family, that is, a predominance of sesquiterpenes in EOs [15]. As shown in Figure 1, *P. neurantha* in the genus *Phoebe* had slightly lower sesquiterpenoids and a relatively high content of monoterpenoids (23.7%) such as *α*-terpineol (8.1%) and 4-terpinenol (5.0%), which was distinguished from the other three species in the genus *Litsea* and *Lindera*. It suggested that *Phoebe* might have rather distant chemotaxonomic relationship to *Litsea* and *Lindera* in the Lauraceae family. Moreover, although numerous components including (E)-*β*-famesene, *cis*-*α*-bisabolene, *α*-selinene, eremophilene and *β*-selinene were found among three different genera in this context, none of them can serve as a taxonomic marker at the genus level since they are quite widespread constituents in plant EOs [16].

Lit. rotundifolia and *Lit. variabilis* were rich in sesquiterpenoids and had small amounts of monoterpenoids as well as aliphatics. However, the chemical composition showed variability between them. Sesquiterpenoids were present in up to 91.5% of *Lit. rotundifolia* with (E)-*β*-famesene (32.7%) and *cis*-*α*-bisabolene (21.2%) as the most abundant components, while *Lit. variabilis* displayed an abundance of isoaromadendrene (11.0%), elixene (10.1%) and globulol (9.4%). Besides the two species referred, *Litsea* plants have been widely analyzed for their EO composition. Several groups of components were identified including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated diterpenes, ketones, aldehydes, alcohols and alkanes. Although various species were known to exhibit diverse chemical profiles [17], some substances detected here like bulnesol, *β*-caryophyllene, *trans*-*α*-bergamotene, globulol and phytol were found in other *Litsea* species as well. For example, bulnesol in *Lit. resinosa* (Malaysia), *β*-caryophyllene in *Lit. deccanensi* (India), *trans*-*α*-bergamotene in *Lit. laevigata* (India), globulol in *Lit. ferestrata* and *Lit. gracilipes* (Malaysian) and phytol in *Lit. glutinosa* (Bangladesh) [18].

Both *Lin. communis* P and Q were composed of more than 70.0% sesquiterpenoids. Their remarkable difference was that the former demonstrated 19.8% monoterpenoids and its major components included *α*-cadinol (8.9%), germacrene D (8.6%), 3-carene (8.5%) and *τ*-muurolol (8.2%), while the latter had 12.6% aliphatics namely *α*-springene and other main components were (E)-*β*-famesene (38.8%), *α*-selinene (12.7%) and *β*-selinene (9.6%). An old article [19] reported that the leaf oil of *Lin. communis* collected from Kunming (Yunnan Province, China) mainly contained a pair of *cis-trans* isomers (E)-*β*-ocimene (69.3%) and (Z)-*β*-ocimene (4.5%). It followed that *Lin. communis* from different origins showed chemical diversity. EOs considerably differ in the qualitative and quantitative composition for many elements such as weather, soil, altitude, harvest time, extraction method, etc. Generally, different harvest dates could cause slight influence. Great variations were more likely due to the objective existence of chemotypes and the adaptation to surroundings of species itself [20]. The chemical composition within a species and consequent bioactivities could greatly vary depending on external factors, so plant cultivation and EO standardization play essential roles in product application [21].

Table 2. Chemical composition of the essential oils from four Lauraceae plants

Peak No.	Compound	RI exp. [a]	RRI. lit. [b]	Relative content (%)					Identification Method [c]
				Lin. communis		*P. neurantha*	*Lit. rotundifolia*	*Lit. variabilis*	
				Q	P				
	Yield (%, v/w, mL/g)			**0.20**	**0.28**	**0.07**	**0.43**	**0.01**	
	Monoterpene hydrocarbons								
1	*α*-Pinene	939	917-944 [d]	0.9	-	-	-	-	MS; RI
2	*β*-Pinene	978	949-987 [d]	0.8	-	-	-	-	MS; RI
3	*β*-Myrcene	990	955-998 [d]	3.0	-	-	-	-	MS; RI
4	3-Carene	1011	1001-1028 [d]	8.5	-	-	-	-	MS; RI
5	Sylvestrene	1022	1020-1027 [d,e]	1.0	-	-	-	-	MS; RI
6	Terpinolene	1093	1052-1097 [d]	0.1	-	-	-	-	MS; RI
7	Limonene	1035	998-1044 [d]	-	-	0.3	-	-	MS; RI
8	*β*-Ocimene	1039	1037-1044 [d]	1.9	1.4	-	-	-	MS; RI
9	1,5,5-Trimethyl-6-methylene-cyclohexene	1336	1338 [d]	-	-	1.4	-	-	MS; RI
	Oxygenated monoterpenes								
10	Eucalyptol	1046	1002-1046 [d]	-	-	0.9	-	-	MS; RI
11	Linalool oxide	1080	1060-1091 [d]	-	-	1.3	-	-	MS; RI
12	Linalool	1104	1062-1107 [d]	0.7	-	2.3	-	-	MS; RI
13	Fenchol	1121	1112-1121 [d]	-	-	0.4	-	-	MS; RI
14	Pinocarveol	1139	1138-1139 [d]	-	-	0.4	-	-	MS; RI
15	Camphor	1143	1100-1174 [d]	-	1.8	-	-	-	MS; RI
16	*cis*-Sabinol	1146	1143-1149 [d]	-	-	0.4	-	-	MS; RI
17	Borneol	1166	1134-1205 [d]	-	-	0.7	-	0.9	MS; RI
18	4-Terpinenol	1177	1140-1191 [d]	-	-	5.0	-	-	MS; RI
19	*α*-Terpineol	1190	1153-1224 [d]	0.2	-	8.1	-	1.6	MS; RI
20	*p*-Mentha-1,5-dien-7-ol	1199	1194 [d]	-	-	0.3	-	-	MS; RI
21	Phellandral	1277	1255-1281 [d]	-	-	0.8	-	-	MS; RI
22	Bornyl acetate	1281	1249-1295 [d]	2.1	-	-	-	-	MS; RI
23	*p*-Mentha-1,4-dien-7-ol	1329	1315-1332 [d]	-	-	0.9	-	-	MS; RI
24	*α*-Terpinyl acetate	1343	1330-1367 [d]	-	-	0.5	-	-	MS; RI
25	Geranic acid	1357	1355-1372 [d]	0.6	-	-	-	-	MS; RI
26	Geranyl acetate	1385	1357-1394 [d]	-	-	-	1.4	-	MS; RI
	Sesquiterpene hydrocarbons								
27	Elixene	1313	-	1.6	-	-	-	10.1	MS
28	*α*-Cubebene	1349	1314-1400 [d]	0.2	-	-	-	-	MS; RI
29	*α*-Copaene	1375	1340-1419 [d]	1.5	-	0.7	1.7	-	MS; RI
30	*β*-Cubebene	1388	1350-1419 [d]	0.7	-	-	-	-	MS; RI
31	*β*-Elemene	1394	1366-1421 [d]	-	1.1	2.2	-	-	MS; RI
32	*β*-Caryophyllene	1399	1390-1465 [d]	2.6	-	1.0	-	8.2	MS; RI
33	Aristolene	1421	1428 [d]	-	-	0.5	-	-	MS; RI
34	Himachala-2,4-diene	1427	1429 [d]	-	-	0.7	-	-	MS; RI

35	*trans*-*α*-Bergamotene	1433	1414-1441 [d]	-	6.6	4.2	0.6	-	MS; RI
36	Aromandendrene	1444	1436-1440 [d]	-	4.4	-	-	-	MS; RI
37	Cedrene	1447	1399-1449 [d]	0.3	-	-		-	MS; RI
38	*α*-Humulene	1452	1420-1491 [d]	-	2.2	-	-	-	MS; RI
39	Alloaromadendrene	1455	1416-1487 [d]	0.3	3.4	1.0		3.4	MS; RI
40	(E)-*β*-Famesene	1460	1415-1491 [d]	-	38.8	-	32.7	-	MS; RI
41	Muurola-4,11-diene	1469	1467 [d]	-	-	-	3.2		MS; RI
42	1,1,4,8-tetramethyl-*cis*,*cis*,4,7,10-cycloundecatriene	1472	-	-	-	-	-	7.5	MS
43	*γ*-Muurolene	1477	1445-1515 [d]	1.7	-	1.8	-	-	MS; RI
44	Germacrene D	1480	1436-1521 [d]	8.6	-	-	-	-	MS; RI
45	*γ*-Himachalene	1483	1481 [d]	-	-	1.1	-	-	MS; RI
46	*δ*-Selinene	1485	1483-1500 [d]	-	-	-	-	0.5	MS; RI
47	*β*-Selinene	1489	1464-1509 [d]	-	9.6	-	-	-	MS; RI
48	*α*-Selinene	1491	1470-1504 [d]	-	12.7	8.4	-		MS; RI
49	*β*-Guaiene	1493	1447-1500 [d]	-	-	1.6	-	3.3	MS; RI
50	Viridiflorene	1498	1485-1534 [d]	-	-	-	-	5.3	MS; RI
51	Bicyclogermacrene	1499	1483-1532 [d]	6.9	-	-	-	-	MS; RI
52	Eremophilene	1501	1486-1502 [d]	2.5	-	10.3	-	-	MS; RI
53	*α*-Muurolene	1505	1457-1540 [d]	0.3	-	-	-	-	MS; RI
54	*cis*-*α*-Bisabolene	1508	1498-1511 [d]	1.8	-	-	21.2	-	MS; RI
55	*γ*-Cadinene	1515	1470-1553 [d]	1.0	-	-	-	-	MS; RI
56	*β*-Bisabolene	1517	1478-1547 [d]	-	-	-	7.5	-	MS; RI
57	*δ*-Cadinene	1528	1484-1562 [d]	5.6	-	3.3	-	0.6	MS; RI
58	Germacrene B	1562	1535-1566 [d]	0.2	-	-	-	-	MS; RI
	Oxygenated sesquiterpenes								
59	*α*-Nerolidol	1535	1530-1583 [d]	5.3	-	-	7.4	1.5	MS; RI
60	Elemol	1537	1537-1565 [d]	-	-	-	-	1.2	MS; RI
61	Spathulenol	1576	1543-1624 [d]	4.2	-	2.6	-	-	MS; RI
62	Globulol	1581	1581-1598 [d]	2.3	-	-	-	9.4	MS; RI
63	Caryophyllene oxide	1583	1548-1625 [d]	0.8	-	4.2	-	-	MS; RI
64	Viridiflorol	1585	1558-1609 [d]	0.7	-	-	-	-	MS; RI
65	Isoaromadendrene epoxide	1600	1594 [d]	1.0	-	-	-	11.0	MS; RI
66	Epiglobulol	1608	1564-1629 [d]	-	-	-	-	1.5	MS; RI
67	Rosifoliol	1622	1598-1615 [d,f]	-	-	-	-	2.1	MS; RI
68	*τ*-Cadinol	1635	1608-1665 [d]	-	-	2.7	-	-	MS; RI
69	*τ*-Muurolol	1640	1598-1662 [d]	8.2	-	-	-	-	MS; RI
70	Cubenol	1643	1636-1650 [d]	4.0	-	1.2	-	1.2	MS; RI
71	Agarospirol	1646	1642-1646 [d]	-	-	3.4	-	-	MS; RI
72	*β*-Eudesmol	1649	1645-1667 [d]	0.6	-	8.1	-	3.5	MS; RI
73	*δ*-Cadinol	1652	1599-1653 [d]	-	-	-	-	1.1	MS; RI
74	*α*-Cadinol	1663	1617-1698 [d]	8.9	-	-	-	-	MS; RI
75	Bulnesol	1666	1665-1678 [d]	-	-	-	1.0	1.0	MS; RI
76	Cyperenone	1682	1680-1717 [d]	-	-	-	8.2	-	MS; RI
77	*α*-Bisabolol	1687	1666-1707 [d]	-	-	-	4.7	-	MS; RI

78	*cis*-Farnesol	1699	1686-1722 [d]	-	-	-	3.3	-	MS; RI
79	Nootkatone	1814	1794-1820 [d]	-	-	0.9	-	-	MS; RI
Oxygenated diterpenes									
80	Phytol	2116	2087-2148 [d]	-	-	0.6	-	3.1	MS; RI
Aromatics									
81	*β*-Cymene	1030	1022-1031 [d]	0.4	-	-	-	-	MS; RI
82	Benzyl alcohol	1034	1020-1060 [d]	-	-	0.6	-	-	MS; RI
83	Cuminaldehyde	1235	1226-1248 [d]	-	-	0.6	-	-	MS; RI
Aliphatics									
84	Sabina ketone	1160	1154-1160 [d]	-	-	1.0	-	-	MS; RI
85	*n*-Pentadecanal	1707	1702-1719 [d]	-	-	-	-	1.2	MS; RI
86	Farnesyl acetate	1843	1840-1854 [d]	-	-	-	-	0.5	MS; RI
87	*α*-Springene	1972	1969-2019 [d]	-	12.6	-	0.5	-	MS; RI
87	*n*-Hexadecanoic acid	1980	1929-2003 [d]	-	-	-	-	0.6	MS; RI
88	9-Octadecenal	1995	1999-2004 [d,g]	-	-	-	-	1.1	MS; RI
	Total			92.0	94.6	86.4	93.4	81.4	
	Monoterpenoids			19.8	3.2	23.7	1.4	2.5	
	Sesquiterpenoids			71.8	78.8	59.9	91.5	72.4	
	Aliphatics			-	12.6	1.0	0.5	3.4	
	Aromatics			0.40	-	1.2	-	-	

[a] RI exp.: calculated retention indices of individual components on a HP-5MS column using *n*-alkanes;
[b] RRI lit.: range of reported retention indices on a HP-5MS column;
[c] MS: mass spectra;
[d] RRI as seen in the NIST Chemistry WebBook, SRD 69 (https://webbook.nist.gov) [22];
[e-g] RI in references [23-25];

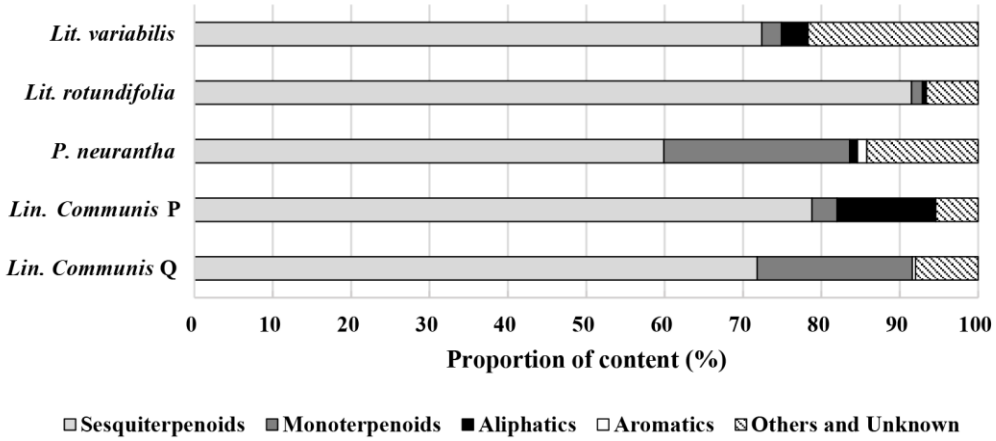

Figure 1. The proportion (%) of different types of compounds identified in the essential oils from four Lauraceae plants

3.2. Contact and Repellent Activities

Results of the contact toxicity were presented in Table 3. The amount of *Lit.* variabilis oil obtained was insufficient to complete the contact assay, so only data for *Lin. communis, P. neurantha* and *Lit. rotundifolia* were provided. Results indicated that *P. neurantha* (LD_{50} = 14.52 µg/adult), *Lit. rotundifolia* (LD_{50} = 17.58 µg/adult) and *Lin. communis* P (LD_{50} = 23.82 µg/adult) showed the similar level of contact toxicity with overlapped 95% confidence limits. The toxicity of *Lin. communis* P was approximately 3.6 times higher than that of *Lin. communis* Q (LD_{50} = 86.63 µg/adult).

Table 3. Contact toxicity of the essential oils from four Lauraceae plants against *T. castaneum* adults

Treatments		LD_{50} (μg/adult) [b]	95% LCL-UCL[c]	Slope ± SE	χ^2	P-value
Lin. communis	Q	86.63	71.78-103.01	3.00 ± 0.50	7.98	0.845
	P	23.82	20.86-26.39	3.88 ± 0.57	10.46	0.988
P. neurantha		14.52	11.49-17.37	2.40 ± 0.41	8.12	0.977
Lit. rotundifolia		17.58	14.39-22.51	1.87 ± 0.34	16.23	0.845
Pyrethrins [a]		0.26	0.22-0.30	3.34 ± 0.32	13.11	-

[a] Data from You *et al* (2014) [26].
[b] LD_{50}: median lethal dose.
[c] LCL-UCL: lower-upper confidence limit.

Results of percentage repellency (PR) were illustrated in Figure 2. All the EOs were highly repellent to *T. castaneum* adults at maximum concentration of 78.63 nL/cm^2 during 4 h of exposure, which were comparable to the positive control DEET. *P. neurantha* possessed potent repellency at 78.63 and 15.73 nL/cm^2 with PR values ranging from 88% to 58% at 2 and 4 h post-exposure, while it failed to act as a repellent at lower concentrations. At 2 h post-exposure, *Lit. rotundifolia* and *Lit. variabilis* at higher concentrations of 78.63 and 15.73 nL/cm^2 could result in 90-100% repellency. At 4 h post-exposure, they had PR values within 98-28% at the concentration range of 78.63-3.15 nL/cm^2 and there was no significant difference compared with DEET. Furthermore, the difference in repellency triggered by *Lin. communis* Q and P was insignificant under the same concentration condition.

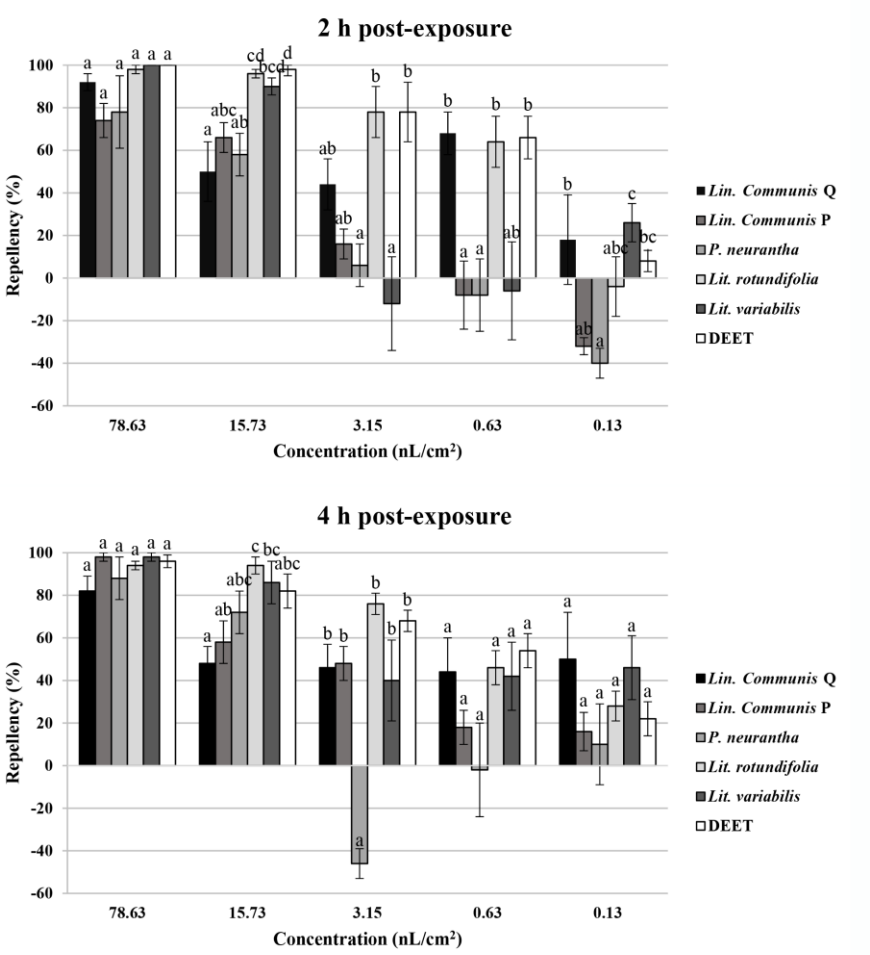

Figure 2. Percentage repellency (%) of essential oils from four Lauraceae plants against *T. castaneum* adults at 2 and 4 h post-exposure. Means at the same concentration column labeled by the same letter were believed to have no statistically significant difference in one-way ANOVA analysis (P > 0.05, Tukey's HSD tests).

EOs are potential candidates for sustainable and environment-friendly biopesticides for their bio activities, easy biodegradation into nontoxic compounds and low adverse effects on non-target organisms [27]. At the same time, EOs as complex mixtures of volatile chemicals could delay the development of pest resistance. In the past two decades, the number of published articles interested in the potential use of plant EOs for pest management has increased enormously [28]. Besides the four species investigated in this work, EOs from a variety of plants such as *Magnolia coriacea*, *M. macclurei* [29], *Calendula incana* subsp. *Maritima* [30] and *Cyperus rotundus* [31] were found to have plentiful sesquiterpenoids and demonstrate a broad-spectrum of bioactivities against storage pests under laboratory conditions. Major constituents tend to be responsible for the potency of EOs [32]. Certainly, some minor components sometimes also contribute to toxicity in a complex mixture even though they are present below their own effectiveness threshold [33]. Sesquiterpenoids like *β*-guaiene [29], *β*-caryophyllene, caryophyllene oxide, spathulenol, *β*-eudesmol and *α*-bisabolol [2] have been confirmed to show insecticidal and repellent activities against stored-product insects especially beetles before. But quite a few sesquiterpenoids identified here with high contents like (E)-*β*-famesene, *cis*-*α*-bisabolene, *α*-selinene and eremophilene remain future work for their anti-insect properties.

Most toxic reaction and behavioral interference of pests caused by low-molecular-weight terpenoids are a result of enzyme inhibition (AChE: Acetylcholinesterase; GST: Glutathione-S-transferase; ATP: Adenosine triphosphatase) or interaction with receptors in the nervous system of insects (octopamine; nicotinic acetylcholine receptors; GABA-gated chloride channels) [28]. For instance, eucalyptol, limonene, linalool, terpinen-4-ol, *α*-pinene, *α*-terpineol, *β*-caryophyllene and 3-carene could show AChE, GST or Na^+/K^+-ATPase inhibition against various beetle species. Among them, *α*-terpineol also exerted the octopaminergic effect [2]. On the other hand, volatile oils have an important impact on insect olfaction-related proteins such as odorant binding proteins (OBPs), chemosensory proteins (CSPs) and odorant receptors (ORs) capable of recognizing and binding odor molecules [34]. It was reported that *Artemisia vulgaris* EO could significantly induce the expression of *Tc*OBPs and *Tc*OBPC11 were possibly involved in the defense of *T. castaneum* against exogenous toxicants [35]. These proteins mentioned above are potential targets for screening novel agents in pest control. At present, most of research on Lauraceae plants are aimed at bioassays on target insects. To gain deep insights into their bioactivites, mechanisms of action need further investigation.

Acknowledgments

This work was partially supported by Suzhou Science and Technology Development Plan Program (Grant no. SKY2023181).

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References

[1] B. M. Leite, F. A. Cunha, B. G. Bertozzi, L. O. Rocha and L. C. Queiroz (2023). A review of insecticidal effect of essential oils on stored grain pests, *Food Sci. Technol*. **43**, e10602.
[2] S. Karaborklu and A. Ayvaz (2023). A comprehensive review of effective essential oil components in stored-product pest management, *J. Plant Dis. Prot.* **130**, 449-481.
[3] Y. Zhang, Z. Pan, K. Wu, G. Yan, H. Xu, Q. Gong and L. Ni (2023). Research progress on chemical constituents and biological activities of *Litsea cubeba* (Lour) Pers, *Rec. Nat. Prod.* **17**, 1577-594.

[4] Y. Cao, B. F. Xuan, B. Peng, C. Li, X. Y. Chai and P. F. Tu (2016). The genus *Lindera*: a source of structurally diverse molecules having pharmacological significance, *Phytochem. Rev.* **15**, 869-906.
[5] Z. L. Liu, M. Yu, X. M. Li, T. Wan and S. S. Chu (2011). Repellent activity of eight essential oils of Chinese medicinal herbs to *Blattella germanica* L, *Rec. Nat. Prod*. **5**, 176-183.
[6] Y. Wang, L. T. Zhang, Y. X. Feng, D. Zhang, S. S. Guo, X. Pang, Z. F. Geng, C. Xi and S. S. Du (2019). Comparative evaluation of the chemical composition and bioactivities of essential oils from four spice plants (Lauraceae) against stored-product insects, *Ind. Crop. Prod.* **140**, 111640.
[7] Y. Tian, H. Fan, Y. Wang, Y. Zheng, D. M. Hu and S. S. Du (2022). Insecticidal and repellent activities of volatile constituents from *Litsea dilleniifolia* P. Y. Pai et P. H. Huang against stored-product insects, *Rec. Nat. Prod.* **16**, 398-403.
[8] Z. L. Liu, S. S. Chu, C. H. Jiang, J. Hou, Q. Z. Liu and G. H. Jiang (2016). Composition and insecticidal activity of the essential oil of *Lindera aggregata* root tubers against *Sitophilus zeamais* and *Tribolium castaneum*, *J. Essent. Oil Bear. Plants.* **19**, 727-733.
[9] D. Kumar Semwal and R. Badoni Semwal (2013). Ethnobotany, pharmacology and phytochemistry of the genus *Phoebe* (Lauraceae), *Mini-Rev. Org. Chem.* **10**, 12-26.
[10] Q. F. Wang, B. Zeng, J. Shen and H. Y. Wang (2020). Effect of lacquer decoration on VOCs and odor release from *P. neurantha* (Hemsl.) Gamble, *Sci. Rep.* **10**, 9565.
[11] J. F. Campbell, C. G. Athanassiou, D. W. Hagstrum and K. Y. Zhu (2022). *Tribolium castaneum*: A model insect for fundamental and applied research, *Annu. Rev. Entomol.* **67**, 347-365.
[12] F. Q. Gao, Y. J. Qi, A. H. Hamadou, J. Y. Zhang, M. F. Manzoor, Q. Guo and B. Xu (2022). Enhancing wheat-flour safety by detecting and controlling red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *J. Consum. Prot. Food S.* **17**, 113-126.
[13] L. L. McDonald, R. H. Guy, R. D. Speirs (1970) Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. Marketing Research Report No. 882, Agricultural Research Service, U. S. Department of Agriculture, Washington, D.C., 1-8
[14] V. V. Bhargava, S. C. Patel, K. S. Desai (2013). Importance of terpenoids and essential oils in chemotaxonomic approach, *Int. J. Herb. Med.* **1**, 14-21.
[15] C. S. B. Damasceno, N. T. F. Higaki, J. F. G. Dias, M. D. Miguel and O. G. Miguel (2019). Chemical composition and biological activities of essential oils in the family Lauraceae: A systematic review of the literature, *Planta Med.* **85**, 1054-1072.
[16] C. Frezza, A. Venditti, D. De Vita, C. Toniolo, M. Franceschin, A. Ventrone, L. Tomassini, S. Foddai, M. Guiso, M. Nicoletti, A. Bianco and M. Serafini (2020). Phytochemistry, chemotaxonomy, and biological activities of the Araucariaceae family—A review, *Plants* **9**, 888.
[17] T. P. Gurav, B. B. Dholakia and A. P. Giri (2022). A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil, *Phytochem. Rev.* **21**, 879-913.
[18] M. A. M. Azhar and W. M. N. H. W. Salleh (2020). Chemical composition and biological activities of essential oils of the genus *Litsea* (Lauraceae)–A review, *Agriculturae Conspectus Scientificus* **85**, 97-103.
[19] J. K. Ding, X. J. Yu, Z. H. Ding, B. Q. Cheng, Y. F. Yi, W. Yu, N. Hayashi and H. Komae (1994). Essential oils of some Lauraceae species from the southwestern parts of China, *J. Essent. Oil Res.* **6**, 577-585.
[20] M. Aćimović, M. Rat, L. Pezo, B. Lončar, M. Pezo, A. Miljković and J. Lazarević (2022). Biological and chemical diversity of *Angelica archangelica* L.—case study of essential oil and its biological activity, *Agronomy* **12**, 1570.
[21] K. Lunz and I. Stappen (2021). Back to the roots—an overview of the chemical composition and bioactivity of selected root-essential oils, *Molecules* **26**, 3155.
[22] P. J. Linstrom and W. G. Mallard (2001). The NIST Chemistry WebBook: A chemical data resource on the internet, *J. Chem. Eng. Data* **46**, 1059-1063.
[23] E. Ioannou, A. Koutsaviti, O. Tzakou and V. Roussis (2014). The genus *Pinus*: a comparative study on the needle essential oil composition of 46 pine species, *Phytochem. Rev.* **13**, 741-768.
[24] L. Ornano, A. Venditti, C. Sanna, M. Ballero, F. Maggi, G. Lupidi, M. Bramucci, L. Quassinti and A. Bianco (2015). Chemical composition and biological activity of the essential oil from *Helichrysum microphyllum* Cambess. ssp. *tyrrhenicum* Bacch., Brullo e Giusso growing in La Maddalena Archipelago, Sardinia, *J. Oleo Sci.* **64**, 19-26.
[25] R. E. Q. Rita de Cássia, M. T. S. L. Neta, R. D. D. Sandes, N. Narain, M. de Sousa Galvão, M. S. Madruga and R. G. Costa (2019). An insight in key volatile compounds in goat milk based on their odor active values, *J. Food Sci. Nutr. Res.* **2**, 49-60.

[26] C. X. You, K. Yang, Y. Wu, W. J. Zhang, Y. Wang, Z. F. Geng, H. P. Chen, H. Y. Jiang, S. S. Du and Z. W. Deng (2014). Chemical composition and insecticidal activities of the essential oil of *Perilla frutescens* (L.) Britt. aerial parts against two stored product insects, *Eur. Food Res. Technol.* **239**, 481-490.

[27] E. Assadpour, A. Can Karaça, M. Fasamanesh, S. A. Mahdavi, M. Shariat-Alavi, J. G. Feng, M. S. Kharazmi, A. Rehman and S. M. Jafari (2023). Application of essential oils as natural biopesticides; recent advances, *Crit. Rev. Food Sci. Nutr.* 1-21. doi: 10.1080/10408398.2023.2170317

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

[29] Y. X. Feng, X. X. Lu, Y. S. Du, Y. Zheng, D. Zeng and S. S. Du (2022). Sesquiterpenoid-rich essential oils from two Magnolia plants: contact and repellent activity to three stored-product insects, *J. Oleo Sci.* **71**, 435-443.

[30] S. Basile, N. Badalamenti, O. Riccobono, S. Guarino, V. Ilardi, M. Bruno and E. Peri (2022). Chemical composition and evaluation of insecticidal activity of *Calendula incana* subsp. *maritima* and *Laserpitium siler* subsp. *siculum* essential oils against stored products pests, *Molecules* **27**, 588.

[31] S. Janaki, N. Zandi-Sohani, L. Ramezani and A. Szumny (2018). Chemical composition and insecticidal efficacy of *Cyperus rotundus* essential oil against three stored product pests, *Int. Biodeterior. Biodegrad.* **133**, 93-98.

[32] R. Lahlali, H. El Hamss, J. Mediouni-Ben Jemâa and E. A. Barka (2022). The use of plant extracts and essential oils as biopesticides, *Front. Agron.* **4**, 921965.

[33] B. I. Escher, H. M. Stapleton and E. L. Schymanski (2020). Tracking complex mixtures of chemicals in our changing environment, *Science* **367**, 388-392.

[34] P. Lizana, A. Mutis, A. Quiroz and H. Venthur (2022). Insights into chemosensory proteins from non-model insects: Advances and perspectives in the context of pest management, *Front. Physiol.* **13**, 924750.

[35] Y. C. Zhang, S. S. Gao, S. Xue, K. P. Zhang, J. S. Wang and B. Li (2020). Odorant-binding proteins contribute to the defense of the red flour beetle, *Tribolium castaneum*, against essential oil of *Artemisia vulgaris, Front. Physiol.* **11**, 819.