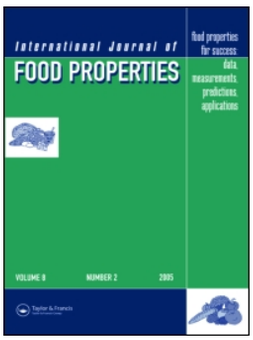

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Insecticidal and repellent efficacy of the essential oil from *Lobularia maritima* and *trans*-3-pentenenitrile against insect pests of stored grains

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ABSTRACT

The essential oil from the aerial parts of *Lobularia maritima* was investigated for its chemical composition, and its repellent and insecticidal efficacy against the grain pests *Callosobruchus maculatus, Tribolium castaneum* and *Sitophilus oryzae*. A number of 41 compounds were identified by GC-MS from which azeleonitrile (39.7%), *trans*-3-pentenenitrile (36.3%) and 4-isothiocyanato-1-butene (10.9%) were the most abundant. A fumigant bioassay-guided fractionation of the essential oil constituents led to the isolation of *trans*-3-pentenenitrile. Its structure was confirmed by EI-MS and NMR techniques. Fumigant effect of the essential oil was strong (LC_{50} = 7.48 µL/L) on *C. maculatum* and moderate on *S. oryzae* and *T. castaneum* (LC_{50} = 35.37 and 59.94 µL/L, respectively). *trans*-3-pentenenitrile showed strong fumigant effect on the three pest species (LC_{50} = 6.62–8.36 µL/L). Both the oil and *trans*-3-pentenenitrile showed strong contact effect with LD_{50} values in the range 4.84–7.81 µg/adult. The oil showed a repellency of 100% on *C. maculatus* and *S. oryzae* at concentrations higher than 0.05 and 0.1 nL/cm^2, respectively, and 93% against *T. castaneum* yet at 0.2 nL/cm^2. *trans*-3-pentenenitrile also showed 100% repellency against *C. maculatus* (≥ 0.05 nL/cm^2), *S. oryzae* (≥ 0.15 nL/cm^2) and *T. castaneum* (0.2 nL/cm^2). The results in this study indicated that the essential oil of *L. maritima* is an important source of *trans*-3-pentenenitrile which can be used in the development of insecticidal agents against the three grain pests.

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Introduction

The post-harvest grain losses caused by insect damage and other noxious organisms reach about l0-40% in the world.[1,2] Pests attacking the store products include more than 600 beetle species, 70 moth species, 40 rodent species, 150 fungal species and 355 mite species.[3] Beetles and moths are among the most destructive pests of stored grains in the world.[4] The main beetle insects include *Tribolium castaneum, Callosobruchus maculatus* and *Sitophilus oryzae*.[5] Their control has been based on synthetic pesticides.[6] However, there is a global concern derived from the intensive use of synthetic pesticides because they generate environmental pollution, pest resistance and their residues often survive in the food products.[7] These problems have led the industries to seek for drugs, repellents and other biological secondary metabolites from natural sources that can substitute synthetic chemicals.[8] One alternative is the use of essential oils obtained from aromatic plants. Some essential oils are currently used in many fields such as cosmetics, perfumery, cleaning, pharmacology, chemistry and food production.[9] In addition, essential oils would not induce resistance in grain stored pests.[10]

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Lobularia maritima (L.) is an annual plant of the Cruciferae family. It is a native halophyte found in the seashores of the Mediterranean sea. Its aerial parts are traditionally collected from the wild in Sicily (Italy) where they are consumed as a kind of vegetable in salads and other dishes.[11] *L. maritima* is popularly used in Spain as a diuretic and antiscorbutic agent and as an adstringent in gonorrhea treatment.[12] It is also cultivated as a garden plant due to its colored flowers.[13] and often intercropped with horticulture crops where its nectar and pollen attract natural enemies involved in the biological control of aphids.[14] The availability of *L. maritima* in China and other countries do it an interesting source of essential oils which might have insecticidal properties as shown for other species of the Cruciferae family.[15] However, current knowledge concerning the chemical composition of the essential oil of *L. maritima* is very scanty and its insecticidal activity has been not investigated.[16] As part of a screening program for new edible sources of insecticides, the aim of this research was to evaluate the fumigant, contact and repellent effects of the essential oil of *L. maritima* against three insect pests of stored grains and to identify the main active principle involved.

Materials and methods

Plant material

Flowering aerial parts (4.5 Kg) of *Lobularia maritima* cultivated in the Changchun Park (Jilin province) were collected early at the morning during August 2016 and stored under 4 °C. Plant samples were identified by Professor Ying Wu (Plant Science College, Jilin University, China), and a voucher specimen was stored at the Herbarium of Jilin Agriculture University (accession number YZDB1910354).

Extraction of the essential oil

The essential oil was obtained from the whole aerial parts of *L. maritima* after 3 h of steam distillation in a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C until it was analyzed and tested in bioassays.

Chemical analysis of the essential oil

The composition of the essential oil was analyzed in a gas chromatograph (GC) coupled to a mass spectrometer (MS) (70 eV). The analysis was done on a Thermo TRACE GC Agilent 5975, equipped with HP-5 MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Sample injection volume, 1 µL (diluted to 1:100 in acetone); split ratio was 30:1. The carrier gas was helium at a flow rate of 1 mL/min. The temperature program of the GC oven was initially held isothermal at 60 °C for 3 min, then ramped from 60°C to 280°C at 8°C/min and finally held at 280°C for 20 min. The temperature of the injector was kept at 280 °C; MS source temperature at 230 °C; MS quadrupole temperature at 150 °C; interface temperature at 280 °C; Mass scan, 35–450 amu; Compounds were identified by comparison of their arithmetic indexes (AI) and mass spectra with the data stored in National Institute of Standards and Technology (NIST 2008), the Wiley 275 database and those reported by Adams.[17] Relative percentages of the oil components were calculated based on peak-areas from the GC-MS total ion current (TIC) data.

Bioassay-directed fractionation

Ten ml of *L. maritima* oil were loaded on a column of silica gel 60 G (0.040–0.063 mm) which was eluted with *n*-hexane-acetone (100:1 to 0:100, v/v). The fractions recovered were grouped in nine pools (P1 to P9) based on the separation patterns observed on thin layer chromatography (TLC) plates of silica gel 60 G F_{254} (Merck) developed with acetone-*n*-hexane (1:30, v/v) as mobile phase. These pools were tested for fumigant activity. Then, the constituents of the most active pool (P5) were separated in a column of silica gel 60 G (0.040–0.063 mm) eluted with dichloromethane-methanol (6:4, v/v). The eluted fractions were grouped in three pools (P1a, P2a and P3a) based on the TLC chromatograms

developed with 95:5 chloroform/methanol as mobile phase. They were also evaluated for their fumigant effect. The most bioactive pool of the second column (P1a) is hereafterin referred to as compound 1. The ^{1}H NMR and ^{13}C NMR spectra of compound 1 were recorded in a Bruker Avance-III 400 MHz spectrometer in deuterated methanol. NMR Fourier transform, peak picking and integration were done with Bruker TopSpin software. The NMR spectra were compared with data of the NMR database of Shanghai Institute of Organic Chemistry (Chinese Academy of Science) and others available in the literature. The EI-MS of compound 1 was obtained using a JEOL JMS mass spectrometer and manually compared with spectra previously published.[18,19]

Insect cultures

C. maculatus, T. castaneum and *S. oryzae* were reared on bean grains, wheat flour mixed with yeast (10:1, w/w) and rice grain, respectively. Adult insects (7 days old) were used for bioassays. The cultures were maintained in the dark in an incubator set at 27 ± 1°C with a relative humidity of 60–70%. All experiments were carried out under the same environmental conditions.

Fumigant assays

The fumigant effect of the essential oil and the pools obtained from the bioassay guided isolation was evaluated against adults of the three insect pests of stored grains as described by Liu and Ho.[20] Six serial dilutions (100 µL to 3.1 µL) either of the essential oil or the pools were prepared in acetone. The dilutions were impregnated on discs of 20 mm diameter (20 µL per disc) of Whatman filter paper. The discs were then placed on the underside of screw caps of 24-mL glass vials. The solvent was allowed to evaporate for 15 s before the cap was placed tightly on the glass vials, each of which contained 10 adult insects inside to form a sealed chamber. Preliminary experiments demonstrated that 15 s was sufficient for the evaporation of solvents. Discs with acetone and dichlorvos (Sigma-Aldrich, China) were tested as negative control and positive controls, respectively. Five replicates were carried out for all treatments and controls, and they were incubated for 24 h. The insects were then transferred to clean vials with some culture media, returned to the incubator and observed daily for determination of end-point mortality which was reached after one week. The experiments were repeated three times. The LC_{50} values with their respective confidence limits were calculated at end-point mortality by using Probit analysis.[21] Two LC_{50} values were considered different when their 95% confidence limits were not overlapped. Probit analyses were performed in SPSS 19.0 for Windows 7 25.

Contact assays

The contact effect of the essential oil and its compound 1 was evaluated against adults of *C. maculatus, T. castaneum* and *S. oryzae* as described by Guo *et al.*[22] Range-finding studies were run to determine the appropriate testing concentrations. Aliquots of 0.5 µL of the oil from *L. maritima* diluted with acetone at five different concentrations were applied topically to the dorsal thorax of each adult insect. Ten insects were included per replicate, and five replicates were performed per dose. The negative control consisted in acetone applied instead of the oil or compound 1 whereas pyrethrum extract purchased in Sigma-Aldrich (China) was included as positive control. Both treated and control insects were then transferred to glass vials (10 insects per vial) and kept in the incubator. Insect mortality was checked after 24 h, and the LD_{50} values were calculated using Probit analysis.[21] LD_{50} values were considered different each other when their 95% confidence limits were not overlapped. Probit analyses were performed in SPSS 19.0 for Windows 7 25.

Repellent assays

The repellent effect of the essential oil and *trans*-3-pentenenitrile was evaluated against *T. castaneum, C. maculatus* and *S. oryzae* in Petri dish assays.[23] Nine cm diameter Petri dishes were used to confine beetles during the experiment. The oil and compound 1 were diluted in acetone to the concentrations of 0.05, 0.10, 0.15, and 0.20 nL/cm^2. Acetone was used as negative control. N,N-diethyl-3-methylbenzamide (DEET) provided by the National Center of Pesticide Standards (China) was tested as a positive control

at the same concentrations indicated for the oil and compound 1. Filter paper with 9 cm in diameter was cut in half and 500 µL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half served as control and was treated with 500 µL of acetone. The treated and control half discs were left to air dry for 1 min to evaporate the solvent completely. Then, both halfs were pasted with solid glue in a same Petri dish. Twenty insects were released in the center of each remade filter paper disk, and a cover was placed over each Petri dish. Five replicates were used, and the experiment was repeated three times. Counts of the insects present on each half were made after 2 h. The percent repellency (PR) for each treatment was then calculated using the formula:

$$PR(\%) = (N_C N_t)/(N_C N_t)x100$$

where Nc is the number of insects present in the negative control half and Nt is the number of insects present in the treated half. The data of percent repellency were subjected to an arcsine square-root transformation and then to one way analysis of variance (ANOVA). The differences among means were evaluated with the Tukey's HSD test. These analyses were performed in SPSS statistics 20 for Windows 2007.

Results and discussion

Chemical composition of the essential oil

The whole aerial parts of *L. maritima* yielded 1.27% (v/w) of a pale yellow oil with a pungent odor. The GC-MS analysis identified 41 compounds accounting for 97.1% of the total oil composition (Table 1; Figure 1). The oil was constituted in an 86.9% by glucosinolate-thermal degradation products which are usually released into the essential oils of Brassicaceae plants.[24] The glucosinolates are constitutive defense compounds widely distributed in the Brassicaceae family. They are degraded to release volatile derivatives such as nitriles and isothyocianates when plant tissues are wounded.[15] In the *L. maritima* oil, these derivatives were mainly azeleonitrile (39.7%) and *trans*-3-pentenenitrile (36.3%), followed by the 4-isothiocyanato-1-butene (10.9%) that likely contributed to strong smell. These results agree with previous reports showing that thermal degradation of glucosinolates is dominated by the release of nitriles over that of isothiocyanates.[25] There is only one report depicting the composition of a *L. maritima* oil extracted from aerial parts collected in Tunisia.[16] This oil showed a composition mainly dominated by oxygenated monoterpenes (74.4%) with linalool (22.4%) as the main constituent and shared only 13% of its compounds with those of the oil reported in this work. The essential oils and the volatile fraction of other extracts obtained from Brassicaceae species often contain nitriles and/or isothiocyanates derived from glucosinolate breakdown, although their contents can strongly vary according to climatic conditions, soil and changes in the extraction procedures. For example, azeleonitrile was in the volatile fraction of an aqueous root extract from *Armoracia rusticana*[26] 4-isothiocyanate belonged to the volatile fraction of an aqueous sprout extract from *Brassica oleracea* and the essential oil from flowering aerial parts of *Morettia phillaeana*.[27,28] However, the absence of glucosinolate derivatives in the Tunisian *L. maritima* suggests that they were lost during drying of the plant material or there are *L. maritima* genotypes that are poor glucosinolate producers.

Identification of compound 1

Compound 1 was obtained as an amber liquid with molecular weight of 81 and the formula C_5H_7N. It was *trans*-3-pentenenitrile with a high degree of purity. The relative intensities of the fragment ions (m/z) observed in the EI-MS were 81(45), 80(19), 66(12), 53(29), 54(100), 41(84), 39(54), 27(21) (Figure 2).[18,19] The NMR analysis confirmed this identity. ^{1} H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.83 (1 H, m, H-2), 5.38 (1 H, m, H-1), 3.09 (2 H, d, 4, H-4), 1.73 (3 H, d, 4, H-5). ^{13} C-NMR ($CDCl_3$, 400 MHz) δ (ppm): 130.87 (C-1), 118.58 (C-2), 117.96 (C-3), 20.36 (C-4), 17.60(C-5).[18]

Table 1. Constituents of the essential oil extracted from aerial parts of *L. maritima*.

No.	Retention index[a]	Compound name	Relative content
1	993	*trans*-3-Pentenenitrile	36.3
2	1009	Hexanal	0.1
3	1034	Furfural	0.1
4	1046	2E-hexenal	tr[b]
5	1050	Ethyl benzene	0.4
6	1054	5-Hexenenitrile	3.7
7	1075	Heptanal	tr
8	1086	Diethyl disulfide	tr
9	1092	α-Pinene	tr
10	1099	5-Methylhexanonitrile	tr
11	1109	2-Heptenal, (Z)-	tr
12	1112	Benzaldehyde	0.2
13	1121	Dimethyl trisulfide	tr
14	1130	6-Cyano-1-hexene	0.2
15	1139	4-Isothiocyanato-1-butene	10.9
16	1151	Octanal	0.1
17	1159	(2E,4E)-Hepta-2,4-dienal	tr
18	1166	2-Acetylthiazole	tr
19	1173	D-Limonene	tr
20	1175	Eucalyptol	0.1
21	1186	Benzene acetaldehyde	0.6
22	1205	Acetophenone	0.1
23	1209	*cis*-Linalol oxide	tr
24	1219	Isothiocyanatocyclopentane	0.4
25	1231	Undecane	0.1
26	1236	Nonanal	0.1
27	1255	2-Phenylethanol	0.3
28	1268	Benzyl cyanide	0.4
29	1310	Dodecane	0.1
30	1317	Erucinnitrile	0.3
31	1331	Dimethyl tetrasulfide	0.1
32	1408	2-Methoxy-4-vinylphenol	1.0
33	1446	4-Methyl isopulegone	0.3
34	1469	Dolichodial (6 Cl)	0.3
35	1502	Azeleonitrile	39.7
36	1515	6-Cyanoquinoline	0.1
37	1612	Spathulenol	0.2
38	1648	Cubenol	0.1
39	1659	T-Muurolol	0.4
40	1664	1,6-Diisothiocyanato hexane	0.1
41	1668	πCadinol	0.4
Total			97.1

[a]Retention index relative to n-alkanes on HP-5 MS capillary column.
[b]traces

Fumigant effect of the essential oil and trans-3-pentenenitrile

L. maritima oil and *trans*-3-pentenenitrile were tested in fumigant assays against *C. maculatus, S. oryzae* and *T. castaneum*. The LC_{50} values obtained after a week for the oil together with those of *trans*-3-pentenenitrile recovered in the bioassay guided isolation are shown in Table 2. Adults of *C. maculatus* were very sensitive (LC_{50} = 7.48 µL/L) to the essential oil of *L. maritima* which had a moderate effect on *S. oryzae* (LC_{50} = 35.37 µL/L) and *T. castaneum* (LC_{50} = 59.94 µL/L). The sensitivity of *C. maculatus* to the oil was also evident if we consider that all its adults were dead at a concentration of 20 µL/L while those of *S. oryzae* and *T. castaneum* needed 100 µL/L. The three insect pests were similarly susceptible to *trans*-3-pentenenitrile with LC_{50} values of 6.62–8.36 µL/L and 100% mortality after exposure at 20 µL/L. *trans*-3-pentenenitrile was 5.0 and 7.0 folds more active on *S. oryzae* and *T. castaneum*, respectively, than the essential oil whereas *C. maculatus* was equally sensitive to the oil and *trans*-3-pentenenitrile. This situation suggests that *trans*-3-pentenenitrile

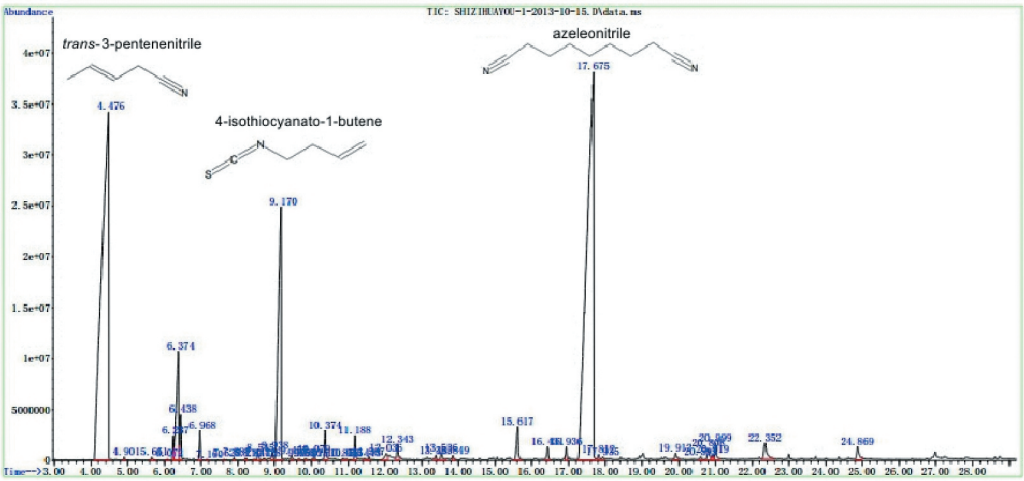

Figure 1. GC-MS chromatograms of the essential oil from aerial parts of *L. maritima*. The chemical structure of the main constituents is also presented.

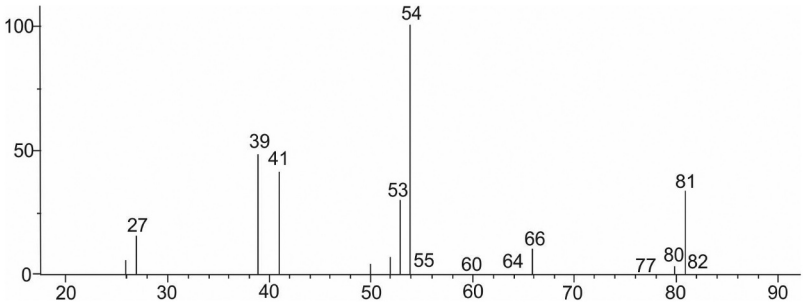

Figure 2. EI-MS spectrum of *trans*-3-pentenenitrile.

strongly contributed to the fumigant effect of the oil against *S. oryzae* and *T. castaneum*, and also that other oil constituents participated in the fumigant effect against *C. maculatum*. Both *trans*-3-pentenenitrile and the oil were several order of magnitude less active than dichlorvos on the three insect pests. However, the LC_{50} values of present work and other previously reported for essential oils and their constituents allow to conclude that the *L. maritima* oil showed a moderate fumigant effect on *S. oryzae* and *T. castaneum*, and a strong effect on *C. maculatus* whereas *trans*-3-pentenenitrile was a strong fumigant on the three insect storage species.[29]

Contact assays

The contact assays showed that neither the essential oil nor the *trans*-3-pentenenitrile exhibited a species dependent activity on the insect-stored pests (Table 3). The LD_{50} values obtained for the essential oil (LD_{50} = 5.41–7.81 μg/adult) were not significantly different from those recorded for *trans*-3-pentenenitrile (LD_{50} = 4.84–6.62 μg/adult). The contact effect was strong when compared with data reported for other essential oils and oil constituents tested against insect pests of stored grains. For example, the contact activities of *Juniperus formosana* oil and its constituent 4-terpineol against *T. castaneum* were moderate (LD_{50} = 29.14 μg/adult) and strong (LD_{50} = 7.65 μg/adult), respectively.[22] The scent of *Ostericum sieboldii* had a strong effect on *S. zeamais* (13.82 μg/adult)

Table 2. Fumigant effect of the essential oil from *L. maritima* and *trans*-3-pentenenitrile against adults of *C. maculatus, S. oryzae* and *T. castaneum*.

		LC_{50} (μL/L)[a]	LC_{95} (μL/L)	Regression equation	Standard error	Chi-square (χ^2)
Callosobruchus maculatum	Essential oil	7.48 (6.04–8.84)	16.44 (16.01–24.79)	y = −4.21 + 4.81x	0.32	14.240
	trans-3-pentenenitrile	6.62 (5.41–6.89)	15.52 (12.54–15.90)	y = −3.65 + 4.45x	0.28	11.134
	Dichlorvos	4.90×10^{-3} (2.50×10^{-3} −6.20 × 10^{-3})	8.50×10^{-3} (7.50×10^{-3} − 9.60 × 10^{-3})	Y = −2.60 + 3.70x	0.35	8.800
Sitophilus oryzae	Essential oil	35.37 (21.32–53.69)	95.1 (80.02–111.49)	y = −5.01 + 3.24x	0.37	17.020
	trans-3-pentenenitrile	7.69 (5.89–8.05)	19.69 (14.75–36.00)	y = −3.57 + 4.03x	0.28	17.380
	Dichlorvos	3.10×10^{-3} (1.80×10^{-3}-3.50 × 10^{-3})	6.10×10^{-3} (5.20×10^{-3} −8.10 × 10^{-3})	Y = −2.90 + 5.4x	0.35	10.100
Tribolium castaneum	Essential oil	59.94 (27.78–55.15)	98.47 (88.31–116.54)	y = −4.80 + 2.99x	0.37	10.020
	trans-3-pentenenitrile	8.36 (6.22–8.71)	19.89 (15.75–38.02)	y = −3.63 + 3.93x	0.28	21.207
	Dichlorvos	5.00×10^{-3} (4.20×10^{-3}-7.10 × 10^{-3})	12.10×10^{-3} (10.80×10^{-3}-15.85 × 10^{-3})	Y = −3.10 + 7.1x	0.33	9.600

[a]Upper and lower limits of the confident interval of the 95% are indicated between parenthesis.

Table 3. Contact effect of the essential oil from *L. maritima* and its constituent *trans*-3-pentenitrile against adults of *C. maculatus, S. oryzae* and *T. castaneum.*

		LD_{50} (μg/insect)[a]	Standard Error	Chi square (χ2)
Callosobruchus maculatus	Essential oil	5.41 (3.79–7.49)	0.18	8.261
	trans-3-pentenenitrile	4.84 (4.30–5.43)	0.18	5.069
	Pyrethrum extract	0.71 (0.58–0.78)	0.15	6.021
Sitophilus oryzae	Essential oil	6.23 (5.50–7.04)	0.18	1.809
	trans-3-pentenenitrile	5.47 (4.85–6.15)	0.18	4.310
	Pyrethrum extract	1.81 (1.56–1.38)	0.17	4.601
Tribolium castaneum	Essential oil	7.81 (6.94–8.78)	0.19	0.672
	trans-3-pentenenitrile	6.62 (5.86–7.46)	0.17	0.186
	Pyrethrum extract	0.45 (0.32–0.54)	0.17	0.235

[a]Upper and lower limits of the confident interval of the 95% are indicated between parenthesis.

and *T. castaneum* (8.47 μg/adult).[30] However, the contact insecticidal effect of both the *L. maritima* oil and *trans*-3-pentenenitrile was lower than that observed for the pyrethrum extract on the pest insects.[31] The equal toxicity between the oil and *trans*-3-pentenenitrile suggests that oil constituents other than *trans*-3-pentenenitrile such as 4-isothiocyanate-1-butene and azeleonitrile contribute very little to the insecticidal contact effect. Aliphatic isothiocyanates were reported with contact insecticidal effect which was attributed to the ability of these compounds to react with the amino protein groups and to cleave disulfide bonds.[32] The toxicity of nitriles and isothiocyanates has been associated to the release of cyanide acid during their metabolization in the insect midgut.[33]

Repellency assays

The repellent activity is a sublethal desirable effect that deters insects from feeding food products.[34] However, essential oil or its constituents showing a strong contact and/or fumigant effect not always have a relevant repellent activity. In this research, all the adults of *C. maculatum* and *S. oryzae* were repelled at concentrations higher than 0.05 and 0.1 nL/cm^2, respectively (Table 4). This repellent effect was similar to that observed for N,N-diethyl-m-toluamide (DEET) which was tested as reference compound. *Tribolium castaneum* was more tolerant to both the scent and DEET with 7% and 40% of the adult insects not repelled, respectively yet at the concentration of 0.20 nL/cm^2. The *trans*-3-pentenenitrile showed 100% repellency against *C. maculatus* and *S. oryzae* at concentrations equal or higher than 0.05 and 0.15 nL/cm^2, respectively, whereas *T. castaneum* required 0.20 nL/cm^2. These concentrations indicate a strong repellent effect if we compared them with those reported for other oils or oil

Table 4. Percent repellencies generated by the essential oil of *L. maritima* and the *trans*-3-pentenenitrile after an exposure of 2 h on *C. maculatus, S. oryzae* and *T. castaneum.*

		Repellency percentage[1,2]		
	Concentration (nL/L)	Essential oil	*trans*-3-pentenenitrile	DEET
Callosobruchus maculatus	0.05	91 ± 2a	100d	100d
	0.10	100d	100d	100d
	0.15	100d	100d	100d
	0.20	100d	100d	100d
Sitophilus oryzae	0.05	78 ± 3a	87 ± 6a	60 ± 4b
	0.10	87 ± 4a	98 ± 5b	94 ± 2b
	0.15	100d	100d	100d
	0.20	100d	100d	100d
Tribolium castaneum	0.05	38 ± 2a	54 ± 4b	10 ± 2 c
	0.10	52 ± 4a	71 ± 5b	21 ± 3 c
	0.15	80 ± 2a	90 ± 4a	44 ± 4b
	0.20	93 ± 3a	100d	60 ± 2b

[1]Different letters in a same row indicate significant differences ($p < 0.05$, Tukey's HSD test).
[2]Percentages are presented as mean ± standard deviation

constituents applied against insect-stored pests. For example, the oil of *Clausena anisum-olens* and its constituents myristicin (a phenylpropanoid) and *p*-cymene-8-ol (an oxygenated monoterpene) showed more than 90% of repellency when applied on adults of the *Liposcelis bostrychophila* adults at concentrations of 39.32, 6.32 and 1.22 nL/cm^2, respectively.[35] The repellent activity of essential oils have been often associated to constituents belonging to the phenylpropanoids and oxygenated terpenes with sesquiterpenoid, diterpenoid and monoterpenoid structures.[36] To the best of our knowledge, this is the first report on the repellent activity of the oil of *L. maritima* and *trans*-3-pentenenitrile.

Conclusion

This paper reported for the first time the contact, fumigant and repellent effects of the essential oil from aerial parts of *L. maritima* against three insect pests of stored grains. The fumigant effect of the oil was strong on *C. maculatum* and moderate on *S. oryzae* and *T. castaneum* while *trans*-3-pentenenitrile showed strong fumigant effect on the three pest species. Both the oil and *trans*-3-pentenenitrile showed strong contact effect. The oil showed 100% of repellency on *C. maculatus* and *S. oryzae* at concentrations higher than 0.05 and 0.1 nL/cm^2, respectively, and 93% against *T. castaneum* yet at 0.2 nL/cm^2. *trans*-3-pentenenitrile also showed 100% repellency against *C. maculatus* (≥ 0.05 nL/cm^2), *S. oryzae* (≥ 0.15 nL/cm^2) and *T. castaneum* (0.2 nL/cm^2). The findings reported in this work indicate that the oil of *L. maritima* is a source of *trans*-3-pentenenitrile which has potential to be used in the control of *C. maculatum, S. oryzae* and *T. castaneum* in stored food products.

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References

[1] Raja, N.; Albert, S.; Ignacimuthu, S.; Dorn, S. Effect of Plant Volatile Oils in Protecting Stored Cowpea *Vigna Unguiculata* (L.) Walpers against *Callosobruchus Maculatus* (F.) (Coleoptera: Bruchdae) Infestation. *J. Stored Prod. Res.* 2001, *37*, 127–132. DOI: 10.1016/S0022-474X(00)00014-X.

[2] Papachristos, D. P.; Stamopoulos, D. C. Repellent, Toxic and Reproduction Inhibitory Effects of Essential Oil Vapours on *Acanthoscelides Obtectus* (Say) (Coleoptera: Bruchidae). *J. Stored Prod. Res.* 2002, *(2002)*(38), 117–128. DOI: 10.1016/S0022-474X(01)00007-8.

[3] Rajendran, S. Postharvest Pest Losses. In *Encyclopedia of Pest Management*; Pimentel, D., Ed.; Marcel Dekker, Inc: New York, 2002; pp 654–656.

[4] Robertson, G. L. *Food Packaging: Principles and Practice*. Taylor and Francis Group. CRC Press: Boca Raton, FL, USA, 2006.

[5] Shaaya, E.; Kostjukovski, M.; Eilberg, J.; Sukprakarn, C. Plant Oils as Fumigants and Contact Insecticides for the Control of Stored-product Insects. *J. Stored Prod. Res.* 1997, *33*, 7–15. DOI: 10.1016/S0022-474X(96)00032-X.

[6] Rozman, V.; Kalinovic, I.; Korunic, Z. Toxicity of Naturally Occurring Compounds of Lamiaceae and Lauraceae to Three Stored-product Insects. *J. Stored Prod. Res.* 2007, *43*, 349–355. DOI: 10.1016/j.jspr.2006.09.001.

[7] Prakash, B.; Singh, P.; Yadav, S.; Singh, S. C.; Dubey, N. K. Safety Profile Assessment and Efficacy of Chemically Characterized *Cinnamomum Glaucescens* Essential Oil against Storage Fungi, Insect, Aflatoxin Secretion and as Antioxidant. *Food Chem. Toxicol.* 2013, *53*, 160–167. DOI: 10.1016/j.fct.2012.11.044.

[8] Kordali, S.; Kesdek, M.; Cakir, A. Toxicity of Monoterpenes against Larvae and Adults of Colorado Potato Beetle, *Leptinotarsa Decemlineata* Say (Coleoptera: Chrysomelidae). *Ind. Crop Prod.* 2007, *26*, 278–297. DOI: 10.1016/j.indcrop.2007.03.009.

[9] Regnault-Roger, C.; Vincent, C.; Arnason, J. T. Essential Oils in Insect Control: Low-risk Products in a High-stakes World. *Annu. Rev. Entomol.* 2012, *57*, 405–424. DOI: 10.1146/annurev-ento-120710-100554.

[10] Erler, F.; Ulug, I.; Yalcinkaya, B. Repellent Activity of Five Essential Oils against *Culex Pipiens*. *Fitoterapia*. 2006, *77*, 491–494. DOI: 10.1016/j.fitote.2006.05.028.

[11] Geraci, A.; Amato, F.; Di Noto, G.; Bazan, G.; Schicchi, R. The Wild Taxa Utilized as Vegetables in Sicily (Italy): A Traditional Component of the Mediterranean Diet. *J. Ethnobiol. Ethnomed.*. 2018, *14*, 14. DOI: 10.1186/s13002-018-0215-x.
[12] Chopra, R. N.; Nayar, S. L.; Chopra, L. C. *Glossary of Indian Medicinal Plants*; Council Scientific Industrial Research: New Delhi, 1986; pp 330.
[13] Tatsuzawa, F.; Usuki, R.; Toki, K.; Saito, N.; Shinoda, K.; Shigihara, A.; Honda, T. Acylated Pelargonidin 3-sambubioside-5-glucosides from the Red-purple Flowers of *Lobularia Maritima*. *J. Japan. Soc. Hort. Sci.* 2010, *79*, 84–90. DOI: 10.2503/jjshs1.79.84.
[14] Brennan, E. B. Agronomic Aspects of Strip Intercropping Lettuce with Alyssum for Biological Control of Aphids. *Biol. Cont.* 2013, *65*, 302–311. DOI: 10.1016/j.biocontrol.2013.03.017.
[15] Tsao, R.; Reuber, M.; Johnson, L.; Coats, J. R. Insecticidal Toxicities of Glucosinolate Containing Extracts from Crambe Seeds. *J. Agric. Entomol.* 1996, *13*, 109–120.
[16] Hsouna, A. B.; Dhibi, S.; Dhifi, W.; Saad, R. B.; Brini, F.; Hfaidh, N.; Mnif, W. Essential Oil from Halophyte *Lobularia Maritima*: Protective Effects against CCl_4-induced Hepatic Oxidative Damage in Rats and Inhibition of the Production of Inflammatory Gene Expression by Lipópolysaccharide-stimulated RAW 264.7 Macrophages. *RSC Adv.* 2019, *9*, 36758–36770. DOI: 10.1039/C9RA05885K.
[17] Adams, R. P. *Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press*; New York: USA, 1989.
[18] Kukula, P.; Koprivova, K. Structure-selectivity Relationship in the Chemoselective Hydrogenation of Unsaturated Nitriles. *J. Catal.* 2005, *234*, 161–171. DOI: 10.1016/j.jcat.2005.06.011.
[19] Linstrom, P. J.; Mallard, W. NIST Chemistry WebBook, NIST Standard Reference Database Number 69. https://webbook.nist.gov/chemistry/(accessed May 28, 2020).
[20] Liu, Z. L.; Ho, S. H. Bioactivity of the Essential Oil Extracted from *Evodia Rutaecarpa* Hook F. Et Thomas against the Grain Storage Insects, *Sitophilus Zeamais* Motsch. And *Tribolium Castaneum* (Herbst). *J. Stored Prod. Res.* 1999, *35*, 317–328. DOI: 10.1016/S0022-474X(99)00015-6.
[21] Sakuma, M. Probit Analysis of Preference Data. *Appl. Entomol. Zool.* 1998, *33*, 339–347. DOI: 10.1303/aez.33.339.
[22] Guo, S.; Zhang, W.; Liang, J.; You, C.; Geng, Z.; Wang, C.; Du, S. Contact and Repellent Activities of the Essential Oil from *Juniperus Formosana* against Two Stored Product Insects. *Molecules*. 2016, *21*, 504. DOI: 10.3390/molecules21040504.
[23] Zhang, J. S.; Zhao, N. N.; Liu, Q. Z.; Liu, Z. L.; Du, S. S.; Zhou, L.; Deng, Z. W. Repellent Constituents of Essential Oil of *Cymbopogon Distans* Aerial Parts against Two Stored-product Insects. *J. Agr. Food Chem.* 2011, *59*, 9910–9915. DOI: 10.1021/jf202266n.
[24] Yu, J. C.; Jiang, Z. T.; Li, R.; Chan, S. M. Chemical Composition of the Essential Oils of *Brassica Juncea* (L.) Coss. Grown in Different Regions, Hebei, Shaanxi and Shandong, of China. *J. Food Drug Anal.* 2003, *11*, 22–26.
[25] Hanschen, F. S.; Rohn, S.; Mewis, I.; Schreiner, M.; Kroh, L. W. Influence of the Chemical Structure on the Thermal Degradation of the Glucosinolates in Broccoli Sprouts. *Food Chem.* 2012, *130*, 1–8. DOI: 10.1016/j.foodchem.2011.05.109.
[26] Hertz, C.; Tran, H.; Márton, M.; Maul, R.; Baldermann, S.; Schreiner, M.; Lamy, E. Evaluation of an Aqueous Extract from Horseradish Root (*Armoracia Rusticana* Radix) against Lipopolysaccharide-induced Cellular Inflammation Reaction. *Evidence-Based Complementary Altern. Med.* 2017, *1950692*, 1–10. DOI: 10.1155/2017/1950692.
[27] Leng, C.; Zhang, Y.; Wang, M.; Wang, P.; Gu, Z.; Yang, R. Dynamic Variation of Glucosinolates and Isothiocyanates in Broccoli Sprouts during Hydrolysis. *Sci. Hort.* 2019, *255*, 128–133. DOI: 10.1016/j.scienta.2019.05.026.
[28] El-Kamali, H.; El-Egami, A. A.; Burham, B. O. Essential Oil Composition of the Flowering Aerial Parts of Sudanese *Morettia Phillaeana* (Del.) DC. *Curr. Res. J. Biol. Sci*. 2011, *3*, 100–103.
[29] Ajayi, O. E.; Appel, A. G.; Fadamiro, H. Y. Fumigation Toxicity of Essential Oil Monoterpenes to *Callosobruchus Maculatus* (Coleoptera: Chrysomelidae: Bruchinae). *J. Insects*. 2014, *917212*, 1–7. DOI: 10.1155/2014/917212.
[30] Liu, Z. L.; Chu, S. S.; Guo, H. J. Insecticidal Activity and Composition of Essential Oil of *Ostericum Sieboldii* (Apiaceae) against *Sitophilus Zeamais* and *Tribolium Castaneum*. *Rec. Nat. Prod.* 2010, *52*, 74–81.
[31] Liu, Z. L.; Chu, S. S.; Jiang, C. H.; Hou, J.; Liu, Q. Z.; Jiang, G. H. Composition and Insecticidal Activity of the Essential Oil of *Lindera Aggregata* Root Tubers against *Sitophilus Zeamais* and *Tribolium Castaneum*. *J. Essent. Oil Bear. Pl.* 2016, *19*, 727–733. DOI: 10.1080/0972060X.2014.960275.
[32] Wittstock, U.; Kliebenstein, D. J.; Lambrix, V.; Reichelt, M.; Gershenzon, J. Glucosinolate Hydrolysis and Its Impact on Generalist and Specialist Insect Herbivores. *Recent Adv. Phytochem*. 2016, *37*, 101–125.
[33] Ohkawa, H.; Ohkawa, R.; Yamamoto, I.; Casida, J. E. Enzymatic Mechanisms and Toxicological Significance of Hydrogen Cyanide Liberation from Various Organothiocyanates and Organonitriles in Mice and Houseflies. *Pestic. Biochem. Physiol.* 1972, *2*, 95–112. DOI: 10.1016/0048-3575(72)90011-9.
[34] Bouklikha, A.; Gaouar Benyelles, N.; Sampietro, D. A. Effects of Tomato Leaves Allelochemicals on Tomato Borer (*Tuta Absoluta* Meyrick) in Tlemcen Region, Algeria. *Allelopathy J.*. 2018, *43*, 131–138. DOI: 10.26651/allelo.j/2018-43-1-1136.

[35] You, C. X.; Jiang, H. Y.; Zhang, W. J.; Guo, S. S.; Yang, K.; Lei, N.; Ping, M.; Feng Geng, Z.; Du, S. S. Contact Toxicity and Repellency of the Main Components from the Essential Oil of *Clausena Anisum-olens* against Two Stored Product Insects. *J. Insect Sci.* 2015, *15*, 87. DOI: 10.1093/jisesa/iev071.

[36] Zaio, Y. P.; Gatti, G.; Ponce, A. A.; Saavedra Larralde, N.; Martinez, M. J.; Zunino, M. P.; Zygadlo, J. A. Cinnamaldehyde and Related Phenylpropanoids, Natural Repellents, and Insecticides against *Sitophilus Zeamais* (Motsch.). A Chemical Structure-bioactivity Relationship. *J. Sci. Food Agric.* 2018, 15, 5822–5831. doi: 10.1002/jsfa.9132.