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RESEARCH ARTICLE

Insecticidal and repellent efficacy against stored-product insects of oxygenated monoterpenes and 2-dodecanone of the essential oil from *Zanthoxylum planispinum* var. *dintanensis*

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Abstract

Essential oils (EOs) extracted from leaves (EL) and fruit pericarp (EFP) of *Zanthoxylum planispinum* var. *dintanensis* were analyzed for their chemical composition by GC-MS technique and evaluated for their fumigant, contact toxicity and repellency against three stored-product insects, namely *Tribolium castaneum*, *Lasioderma serricorne*, and *Liposcelis bostrychophila* adults. Results of GC-MS analysis manifested that EL and EFP of *Z. planispinum* var. *dintanensis* were mainly composed of oxygenated monoterpenes. Major components included linalool, sylvestrene and terpinen-4-ol. The obvious variation observed between two oil samples was that EL contained 2-dodecanone (11.52%) in addition to the above mentioned components, while this constituent was not detected in EFP. Bioassays of insecticidal and repellent activities were performed for EL, EFP as well as some of their individual compounds (linalool, terpinen-4-ol and 2-dodecanone). Testing results indicated that EL, EFP, linalool, terpinen-4-ol and 2-dodecanone exhibited potent insecticidal and repellent activities against the three target insects selected. Among the three individual compounds, 2-dodecanone was significantly toxic to *T. castaneum* (LD_{50} = 5.21 μg/adult), *L. serricorne* (LD_{50} = 2.54 μg/adult) and *L. bostrychophila* (LD_{50} = 23.41 μg/cm^2) in contact assays and had beneficial repellent effects on *L. serricorne* at 2 and 4 h post-exposure. The anti-insect efficacy of *Z. planispinum* var. *dintanensis* EO suggests it has potential to be used as botanical insecticide or repellent to control pest damage in warehouses and grain stores.

Keywords *Zanthoxylum planispinum* var. *dintanensis* · *Tribolium castaneum* · *Lasioderma serricorne* · *Liposcelis bostrychophila* · Insecticidal activity · Repellency

Introduction

Zanthoxylum L. is a large genus of the family Rutaceae (Appelhans et al. 2018). Plants in the genus are small trees or shrubs widespread in Asia and native to subtropical as well as warm temperate areas (Epifano et al. 2011; Perichet et al. 2018). Their dried pericarps are usually used as spices and flavoring agents in the preparation of daily dishes (Singh and Shikha 2017). As early as in ancient China, people stored animal medicinal materials with dried pericarps or whole fruits of this genus and thus to prevent some traditional Chinese medicine of animal origins suffering from pest damage and mildew erosion (Chen 2009). At present, many research articles about insecticidal and repellent activities of *Zanthoxylum* are available. Plants of *Zanthoxylum* were confirmed to have remarkable bioactivities against stored-product insects. Essential oils (EOs) and organic solvent extracts from *Z. armatum*, *Z. dimorphophyllum*, *Z. dimorphophyllum* var. *spinifolium*, *Z. dissitum*, *Z. piasezkii*, *Z. stenophyllum*, and *Z. xanthoxyloides* were demonstrated to have repellent activities against a variety of stored-product insects, including *Tribolium castaneum*, *Lasioderma serricorne* (Zhang et al.

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2017), and *Acanthoscelides obtectus* (Fogang et al. 2012). EOs of *Z. armatum* and *Z. dissitum* possessed fumigant or contact toxicity against *T. castaneum* and *L. serricorne* (Wang et al. 2015a, b). *Z. xanthoxyloides* was discovered to have insecticidal activity and could inhibit progeny production of *A. obtectus* (Fogang et al. 2012). The fraction of *Z. zanthoxyloides* and *Z. bungefoundanum* were effective as an oviposition suppressant of *Callosobruchus maculatus* (Ogunwolu and Odunlami 1996) and *Sitotroga cerealella* (Ge and Weston 1995). The fruit pericarp of *Z. schinifolium* possessed feeding deterrent activity against *T. castaneum* and *Sitophilus zeamais* (Liu et al. 2009), and notably, benzophenanthridines isolated from its stem barks exerted significant effects on *T. castaneum* (Wang et al. 2015c). Some lignans containing methylenedioxy group in the stem bark of *Z. armatum* also showed strong antifeedant activity (Zhang et al. 2018).

Stored-product insects can be easily and frequently found in warehouses and storage facilities, causing insect infestations on processed products and commodities. Pest damage directly triggered qualitative and quantitative losses of stored grains, dried fruits, medicinal herbs, and so on (Wijayaratne et al. 2018; Rumbos and Athanassiou 2017). The popular method for controlling stored-product insects is largely dependent on chemical synthetic insecticides, whose extensive use has increased the burden on the environment and brought negative impact on human health (Boulogne et al. 2012; Hubert et al. 2018). The development of insecticide resistance also aroused major concern in many countries (Boyer et al. 2012; Barres et al. 2016). Naturally occurring insecticides attracted great interest among researchers worldwide due to their low acute toxicity against non-target organisms, easy degradability in nature, and high effectiveness against various pests. EO is a special class of natural products that are synthesized through secondary metabolic pathways of plants and responsible for the specific flavor and scent of aromatic plants. They are currently being considered for developing plant protection products on account of their expected advantages (Pavela and Benelli 2016).

Zanthaoxylum planispinum var. *dintanensis* is an endemic and unique species in Guizhou Province, China, mainly distributed in the typical Karst valley. Since its quality is excellent and it plays a significant role in Karst rocky desertification harnessing, this *Zanthoxylum* variety has become an important tree species with economic and ecological benefits in that area (Chen and He 2009). It has been cultivated for hundreds of years for its excellent characteristics. However, the local industry of *Z. planispinum* var. *dintanensis* began to decline in recent years due to the influence of natural, social, and economic factors. How to reasonably develop and utilize the featured plant resources has become the key to solving the issue of industrial recession (Wei and Zuo 2016). *Z. planispinum* var. *dintanensis* is rarely reported up to now as a member of the *Zanthoxylum* genus for EO composition and related bioactivities. Research on its insecticidal and repellent activities is almost entirely blank. The evaluation of anti-insect properties can offer a new idea for comprehensive utilization and development of its plant resources, which may be helpful to address the problem emerging in the local industry. It also contributes to enriching the research on bioactivities of the *Zanthoxylum* against storage pests.

Tribolium castaneum (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) are common stored-product insects in botanical warehouses with a worldwide distribution (Abdelghany et al. 2010; Green 2014). They are accustomed to infesting and damaging raw grains, cereals, and the like for a living, which could finally lead to the deterioration of stored products. Pest damage poses a constant threat to the management of storage facilities and warehouses, and meanwhile brings irretrievable huge waste of resources and economic losses (Boyer et al. 2012). In this work, the three kinds of insects aforementioned were selected as target insects. EOs from leaves and fruit pericarp of *Z. planispinum* var. *dintanensis* were evaluated for their fumigant, contact toxicity, and repellency against these insects for the first time. Fruit pericarp is generally the traditional medicinal organ of *Zanthoxylum* plants, while the non-medicinal parts including leaves tend to be discarded. Here, the EO of *Z. planispinum* var. *dintanensis* leaves was initially investigated. Compared with similar and already published works, this work was involved in two plant organs including vegetative and reproductive organs and three modes of actions for bioassays were adopted, so as to more roundly assess the potential of *Z. planispinum* var. *dintanensis* against storage pests. It was expected to provide some basis for the development of botanical insecticides or repellents and comprehensive utilization of plant resources especially abandoned portions.

Materials and methods

Plant material

The leaves and fruit pericarp of *Z. planispinum* var. *dintanensis* were collected in August 2017 at Zhenfeng, Guizhou Province, China. Identification of the collections was verified by Dr. Q. R., Liu (College of Life Sciences, Beijing Normal University, Beijing, China), and the voucher specimens (BNU-CMH-Dushushan-2017-09-01-001; BNU-CMH-Dushushan-2017-09-01-002) were deposited in the Herbarium (BNU) of College of Resources Science and Technology, Faculty of Geographical Science, Beijing Normal University.

Essential oil extraction

The fresh leaves (1.3 kg) and fruit pericarp (0.2 kg) of *Z. planispinum* var. *dintanensis* were air-dried at room temperature. The two organs were separately pulverized with 6202 Mini-type Super High-speed Pulverizer (Xinzhen Enterprise Co., Ltd., Taiwan, China) and subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. The distilled oils were dehydrated with anhydrous sodium sulfate (Na_2SO_4) after extraction to remove residual water drops. Their volumes were recorded by observing the graduated part of Clevenger-type apparatus for yield calculation. The two oil samples were stored in sealed containers and protected from light in a refrigerator at 4 °C prior to GC-MS analysis and bioassays.

Insect rearing conditions

T. castaneum, *L. serricorne*, and *L. bostrychophila* were obtained from laboratory cultures. They were maintained in dark incubators at 28–30 °C and 70–80% relative humidity. *T. castaneum* and *L. serricorne* were reared on wheat flour mixed with active yeast (10:1, *w*/*w*). *L. bostrychophila* were bred at a mixture of wheat flour, milk powder, and active yeast (10:1:1, *w*/*w*/*w*). Moisture content of the wheat flour was about 12–13%. The unsexed adults about 7 ± 2 days old were adopted for the following bioassays.

GC-FID and GC-MS analysis

The two oil samples were analyzed by GC–flame ionization detector (FID) and GC-MS using a Thermo Finnigan Trace DSQ instrument (Thermo Finnigan, Lutz, FL, USA) equipped with a capillary column of HP-5MS (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a constant flow of 1 mL/min. The temperature program was similar for both GC-FID and GC-MS. The injector temperature was 250 °C, and the volume injected was 1 μL of 1% solution (*v*/*v*, diluted in acetone). The column temperature was programmed from 50 °C held for 2 min, then at 2 °C/min to 150 °C, and held for 2 min, and finally at 10 °C/min to 250 °C, and held for 5 min. Spectra were scanned from 50 to 550 m/z. A homologous series of *n*-alkanes (C_8–C_{40}) were used for the determination of retention indices (RI). The identification of constitutes was made by comparing their mass spectra with those stored in NIST 05 and Wiley 275 libraries (Adams 2001) and retention indices. Quantification analysis was performed on a Thermo Finnigan Trace DSQ equipped with a flame ionization detector (FID), essentially identical to that used in GC-MS analyses. Relative percentages of the individual components were obtained from GC-FID analysis reports by peak area integration without application of correction factors.

Fumigant toxicity assay

The fumigant toxicity against *T. castaneum* and *L. serricorne* was tested as described by Liu and Ho (1999). Preliminary experiments (3 concentrations set 50, 10, and 2%) were conducted to determine appropriate ranges of testing concentrations in the formal experiment. A serial of testing solutions with five concentrations was prepared in *n*-hexane for EOs/compounds. Ten adults were firstly introduced into a screw-top glass vial (diameter 2.5 cm, height 5.5 cm, volume 27.5 mL). Then, 10 μL of the testing solution was pipetted using a micropipette onto a filter paper (diameter 2 cm). The solvent was allowed to evaporate for 20 s since *T. castaneum* was sensitive to *n*-hexane. Each filter paper was placed on the underside of the screw cap of the glass vial, and the cap was immediately screwed tightly. In order to prevent insect contact with the treated filter paper, fluon was properly coated inside each glass vial. As for *L. bostrychophila*, the experimental method of Zhou et al. (2012) was adopted. Pre-experiments (3 concentrations set 5, 1, and 0.2%) were done to define the appropriate ranges of testing concentrations. A filter paper strip (3.5 × 1.5 cm) was treated with 10 μL of appropriate concentrations of EOs/compounds. The impregnated filter paper was placed in the bottom of a glass jar (250 mL). Ten insect adults in a glass bottle (8 mL) were placed into the larger glass jar and exposed for 24 h. Finally, the screw-top glass vial and the glass jar were sealed with parafilm. *n*-Hexane was used as the negative control. Five replicates were performed for each treatment and control. The number of dead insects was checked after 24 h.

Contact toxicity assay

The method from Liu and Ho (1999) was employed for the evaluation of contact toxicity against *T. castaneum* and *L. serricorne*. Pre-experiments were carried out to define the appropriate testing concentrations. A serial of testing solutions with five concentrations was prepared in *n*-hexane for EOs/compounds. Aliquots (0.5 μL) of the solutions were applied topically to the dorsal thorax of each insect. Ten insects were adopted for each concentration. Ten insects treated were then transferred to a glass vial and kept in incubators. Bioassays of contact toxicity against *L. bostrychophila* referred to the method by Zhou et al. (2012). A filter paper (diameter 5.5 cm) was treated with 300 μL of each testing solution. After the solvent was evaporated, the filter paper was fixed on the bottom of a Petri dish (diameter 5.5 cm). Ten adults were put in the Petri dish. Then, the dishes were covered and kept in incubators. *n*-Hexane was used as the negative control. All treatments and controls were replicated five times. The number of dead insects was counted after 24 h.

Repellent activity assay

The repellent activity of EOs/compounds was investigated according to the reference (Zhang et al. 2011). Petri dishes were used to confine insects here. For *T. castaneum* and *L. serricorne*, the testing solutions of EOs/compounds with three concentrations (78.63, 15.73, and 3.15 nL/cm^2) were prepared in *n*-hexane. *n*-Hexane was used as the negative control, and DEET (*N,N*-diethyl-3-methylbenzamide) was the positive control. The filter paper (9 cm in diameter) was cut into two halves. Each concentration with 500 μL was applied separately to one half-filter paper with a micropipette, as uniformly as possible. The other half was treated with an equal volume of *n*-hexane. Treated and control halves were air-dried to evaporate the solvent completely. The two halves were attached to their opposites and stuck in Petri dishes. As for *L. bostrychophila*, the Petri dishes and filter papers were 5.5 cm in diameter and the concentrations of EOs/compounds were set at 63.17, 12.63, and 2.53 nL/cm^2. The two halves of a filter paper were treated with 150 μL of the solution and *n*-hexane separately. In all bioassays, 20 insects were released in the center of each dish and a cover was placed over the dish. Five replicates were carried out for each concentration. The number of insects present on the treated and control portions of each dish was recorded at 2 h and 4 h post-exposure.

Data analysis

All data were analyzed using SPSS V20.0 (IBM, New York, NY, USA). In the fumigant and contact assays, LC_{50} and LD_{50} values were calculated with probit analysis (Sakuma 1998); 95% CL, related parameters were estimated. Some data were cited from previous literatures in the same experimental conditions by our research team. In the repellent assays, the percent repellency (PR) was determined by the following equation (Zhang et al. 2011):

$$\mathrm{PR}\ (\%) = [(Nc - Nt)/(Nc + Nt)] \times 100$$

where Nc = the number of insects on the control half and Nt = the number of insects on the treated half. Percentage repellency values were conducted with arcsine square root transformations for data standardization before one-way ANOVA (Tukey's HSD test). Differences at $P < 0.05$ were considered significant.

Results

Qualitative and quantitative analysis of essential oils

The yields of EL and EFP were 0.2% and 12.4% (*v*/*w*, mL/g). The results of GC-MS analysis were shown in Table 1. EL and EFP of *Z. planispinum* var. *dintanensis* were mainly composed of monoterpenoids, especially oxygenated monoterpenes as illustrated in Fig. 1. Major components included linalool (EL 71.33%; EFP 73.74%), sylvestrene (EL 6.35%; EFP 9.46%), and terpinen-4-ol (EL 6.69%; EFP 6.92%). Among these components, the relative content of linalool was up to 70% in EL and EFP. The obvious variation observed between the two oil samples was that EL included 2-dodecanone (11.52%) in addition to the abovementioned components. However, this constituent was not detected in EFP.

Fumigant toxicity

The results of fumigant toxicity under laboratory conditions were summarized in Table 2. EL and EFP showed certain fumigant toxicity against *T. castaneum* and *L. serricorne*, and EFP was more toxic than EL. Meanwhile, *L. serricorne* was more susceptible to EL and EFP than *T. castaneum* was. However, the two oil samples at the highest testing concentration (5%) failed to cause mortality in *L. bostrychophila*. It was believed that EL and EFP had no fumigant toxicity against *L. bostrychophila* in this experiment. 2-Dodecanone was toxic to *T. castaneum* (LC_{50} = 30.39 mg/L air), *L. serricorne* (LC_{50} = 7.48 mg/L air), and *L. bostrychophila* (LC_{50} = 0.82 mg/L air) by fumigation. 4-Terpinenol was the most toxic component against all these insects. For *L. bostrychophila*, the LC_{50} value of linalool was the same as that of 2-dodecanone.

Contact toxicity

The results of contact toxicity under laboratory conditions were summarized in Table 3. Data showed that EL and EFP had contact toxicity against target insects selected in this work, along with some of their major compounds. EL and EFP exhibited potent contact toxicity against *T. castaneum*, *L. serricorne*, and *L. bostrychophila.* All these insects were more sensitive to 4-terpinenol than linalool based on LD_{50} values. The toxicity of EOs and some of its monoterpenes might vary with insect species. The effectiveness of EL and EFP against Coleoptera insects *T. castaneum* and *L. serricorne* was stronger than that of linalool. However, *L. bostrychophila* showed the reverse. Furthermore, 2-dodecanone was significantly toxic to *T. castaneum* (LD_{50} = 5.21 μg/adult), *L. serricorne* (LD_{50} = 2.54 μg/adult), and *L. bostrychophila* (LD_{50} = 23.41 μg/cm^2) in contact assays. And the LD_{50} value of 2-dodecanone against *L. bostrychophila* was close to that of pyrethrins (LD_{50} = 18.72 μg/cm^2).

Repellent activity

Here, the repellent activities of EOs from *Z. planispinum* var. *dintanensis* and 2-dodecanone against stored-product insects were evaluated for the first time. Results were presented in Fig. 2. EL, EFP, and three individual compounds showed certain

Table 1 Chemical constituents identified from the essential oil of *Z. planispinum* var. *dintanensis*

Peak no.	RI exp.a	RI lit.b	Compounds	Relative content (%)		Identified method[c]
				Leaf	Fruit pericarp	
1	979	980	*β*-Pinene*	0.76	1.87	MS; RI
2	1010	1011	3-Carene*	0.73	1.73	MS; RI
3	1025	1027	Sylvestrene*	6.35	9.46	MS; RI
4	1027	1030	*β*-Phellandrene*	0.93	3.24	MS; RI
5	1088	1093	Terpinolene*	–	1.25	MS; RI
6	1105	1100	Linalool**	71.33	73.74	MS; RI
7	1140	1142	*cis-p*-Menth-2-en-1-ol**	–	0.43	MS; RI
8	1175	1177	Terpinen-4-ol**	6.69	6.92	MS; RI
9	1191	1189	*α*-Terpineol**	1.33	0.80	MS; RI
10	1392	1397	2-Dodecanone	11.52	–	MS; RI
			Total	99.62	99.44	

[a] RI exp., retention index as determined on a HP-5MS column using the homologous series of *n*-alkanes

[b] RI lit., retention index taken from the NIST 05 library

[c] MS, MS mass spectrum matching with NIST 05 and Wiley 275 libraries

*Labeled monoterpenes; **labeled oxygenated monoterpenes

repellent activities against *T. castaneum*, *L. serricorne*, and *L. bostrychophila*. From the bar diagrams given in, the percentage repellency (PR) of EL against three kinds of insects was higher than that of EFP at most of testing concentrations. At the last two concentrations, EFP demonstrated attractive effects on *L. serricorne* (15.73 and 3.15 nL/cm^2) and *L. bostrychophila* (12.63 and 2.53 nL/cm^2). When adults of *T. castaneum* were exposed for 4 h, repellency of EL, EFP, linalool, 4-terpinenol, and 2-dodecanone could be comparable with that of DEET at 78.63 nL/cm^2. At all testing concentrations, 4-terpinenol and 2-dodecanone kept good repellency against *T. castaneum* at 4 h post-exposure, as compared with the positive control. 2-Dodecanone had beneficial repellent effects on *L. serricorne* at 2 and 4 h post-exposure. EL was able to reach the same level of

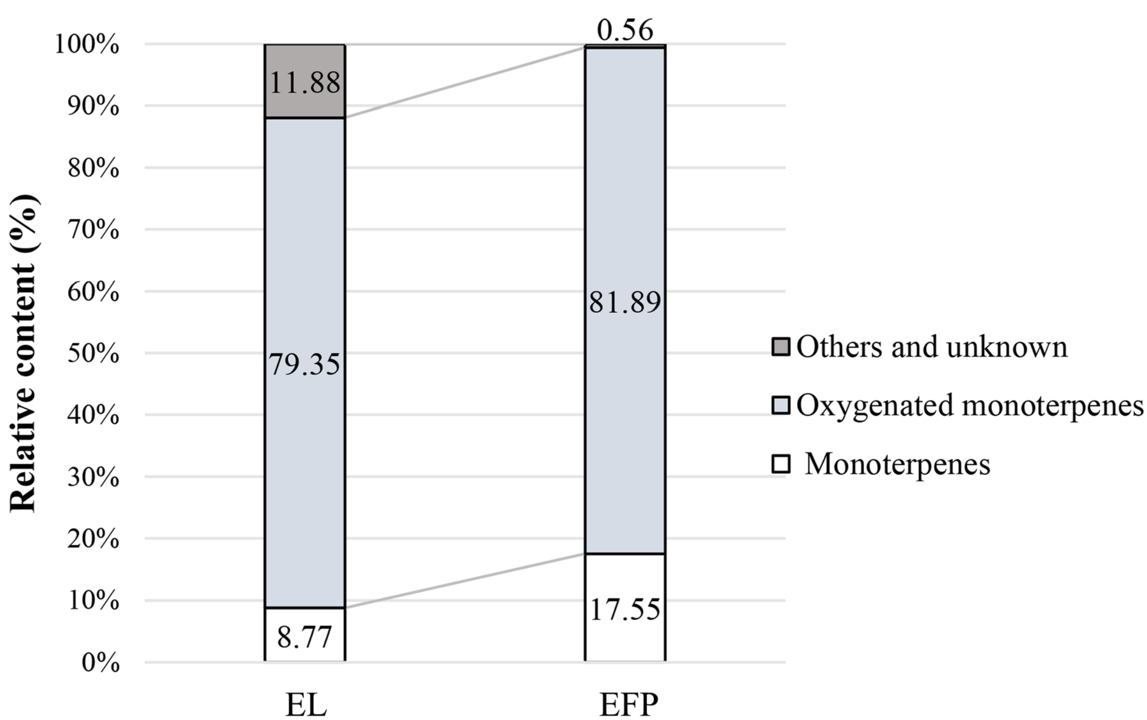

Fig. 1 Relative content of main chemical classes of essential oil components from *Z. planispinum* var. *dintanensis*. (EL and EFP: the essential oils of leaves and fruit pericarp respectively)

Table 2 Fumigant toxicity of the essential oil from *Z. planispinum* var. *dintanensis* against *T. castaneum*, *L. serricorne*, and *L. bostrychophila* after 24 h

Insectsa	Treatments	LC_{50}(mg/L air) (95% LCL–UCLb)	Slope ± SE	Chi-square χ^2 (*df*)	*P* value	
TC	EL	43.16 (38.26–48.64)	5.07 ± 0.69	8.00 (23)	0.844	
	EFP	28.28 (25.71–31.14)	5.81 ± 0.68	7.16 (23)	0.989	
	Linalool	11.60 (10.26–13.21)	3.30 ± 0.40	5.80 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	3.7 (3.3–4.3)	2.49 ± 0.31	27.56 (23)	0.233	Data from Wang et al. (2015)
	2-Dodecanone	30.39 (27.07–34.10)	5.45 ± 0.72	5.08 (23)	0.973	
	MeBr	1.75	–	–	–	Data from Liu and Ho (1999)
LS	EL	32.32 (28.61–36.43)	5.02 ± 0.67	7.21 (23)	0.891	
	EFP	13.01 (11.49–14.72)	3.87 ± 0.48	4.58 (23)	0.999	
	Linalool	46.93 (42.21–52.02)	4.20 ± 0.45	9.32 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	1.3 (0.8–1.7)	1.50 ± 0.31	12.44 (23)	0.963	Data from Wang et al. (2015)
	2-Dodecanone	7.48 (6.42–8.58)	4.06 ± 0.59	9.14 (23)	0.762	
	Phosphine	9.23 (7.13–11.37) × 10^{-3}	2.12 ± 0.27	11.96 (23)	0.971	Data from Yang et al. (2014)
LB	EL	–	–	–	–	
	EFP	–	–	–	–	
	Linalool	0.82 (0.74–0.90)	4.78 ± 0.49	13.07 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	0.08 (0.07–0.08)	4.68 ± 0.81	15.76 (23)	0.865	Data from Zhang et al. (2019)
	2-Dodecanone	0.82 (0.73–0.92)	5.72 ± 0.77	8.14 (23)	0.835	
	Dichlorvos	1.35 (1.25–1.47) × 10^{-3}	6.87 ± 0.77	–	–	Data from Liu et al. (2013)

[a] TC, *T. castaneum*; LS, *L. serricorne*; LB, *L. bostrychophila*

[b] LCL–UCL, lower confidence limit–upper confidence limit

Table 3 Contact toxicity of the essential oil from *Z. planispinum* var. *dintanensis* against *T. castaneum*, *L. serricorne*, and *L. bostrychophila* after 24 h

Insectsa	Treatments	LD_{50} (μg/adult)/(μg/cm^2) (95% LCL–UCLa)	Slope ± SE	Chi-square χ^2 (*df*)	*P* value	
TC	EL	18.68 (16.58–21.06)	5.11 ± 0.68	6.64 (23)	0.920	
	EFP	24.23 (19.28–31.20)	2.45 ± 0.35	8.62 (23)	0.801	
	Linalool	45.96 (39.91–51.79)	3.52 ± 0.44	10.58 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	19.67 (17.13–20.69)	2.13 ± 0.29	19.28 (23)	0.685	Data from Zhang et al. (2015)
	2-Dodecanone	5.21 (4.37–6.17)	3.14 ± 0.50	12.98 (23)	0.449	
	Pyrethrins	0.26 (0.22–0.30)	3.34 ± 0.32	13.11 (23)	0.950	Data from You et al. (2014)
LS	EL	10.38 (9.15–11.68)	5.10 ± 0.70	7.01 (23)	0.901	
	EFP	14.97 (12.87–17.32)	3.77 ± 0.55	4.59 (23)	0.983	
	Linalool	27.41 (24.80–30.27)	4.47 ± 0.47	10.92 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	5.42 (3.99–6.57)	1.70 ± 0.30	11.63 (23)	0.976	Data from Zhang et al. (2015)
	2-Dodecanone	2.54 (1.83–3.16)	2.28 ± 0.47	9.76 (23)	0.714	
	Pyrethrins	0.24 (0.16–0.35)	1.31 ± 0.20	17.36 (23)	0.791	Data from You et al. (2014)
LB	EL	213.69 (191.86–237.32)	6.33 ± 0.86	4.22 (23)	0.989	
	EFP	217.53 (199.56–238.30)	6.90 ± 0.96	13.17 (23)	0.435	
	Linalool	68.35 (65.27–71.97)	9.00 ± 1.00	13.28 (23)	–	Data from Cao et al. (2018)
	Terpinen-4-ol	33.10 (30.59–35.74)	6.61 ± 0.71	8.01 (23)	0.928	Data from Zhang et al. (2019)
	2-Dodecanone	23.41 (21.85–25.37)	8.59 ± 1.22	4.10 (23)	0.990	
	Pyrethrins	18.72 (17.60–19.92)	2.98 ± 0.40	10.56 (23)	0.987	Data from Yang et al. (2014)

[a] TC, *T. castaneum*; LS, *L. serricorne*; LB, *L. bostrychophila*

[b] LCL–UCL, lower confidence limit–upper confidence limit

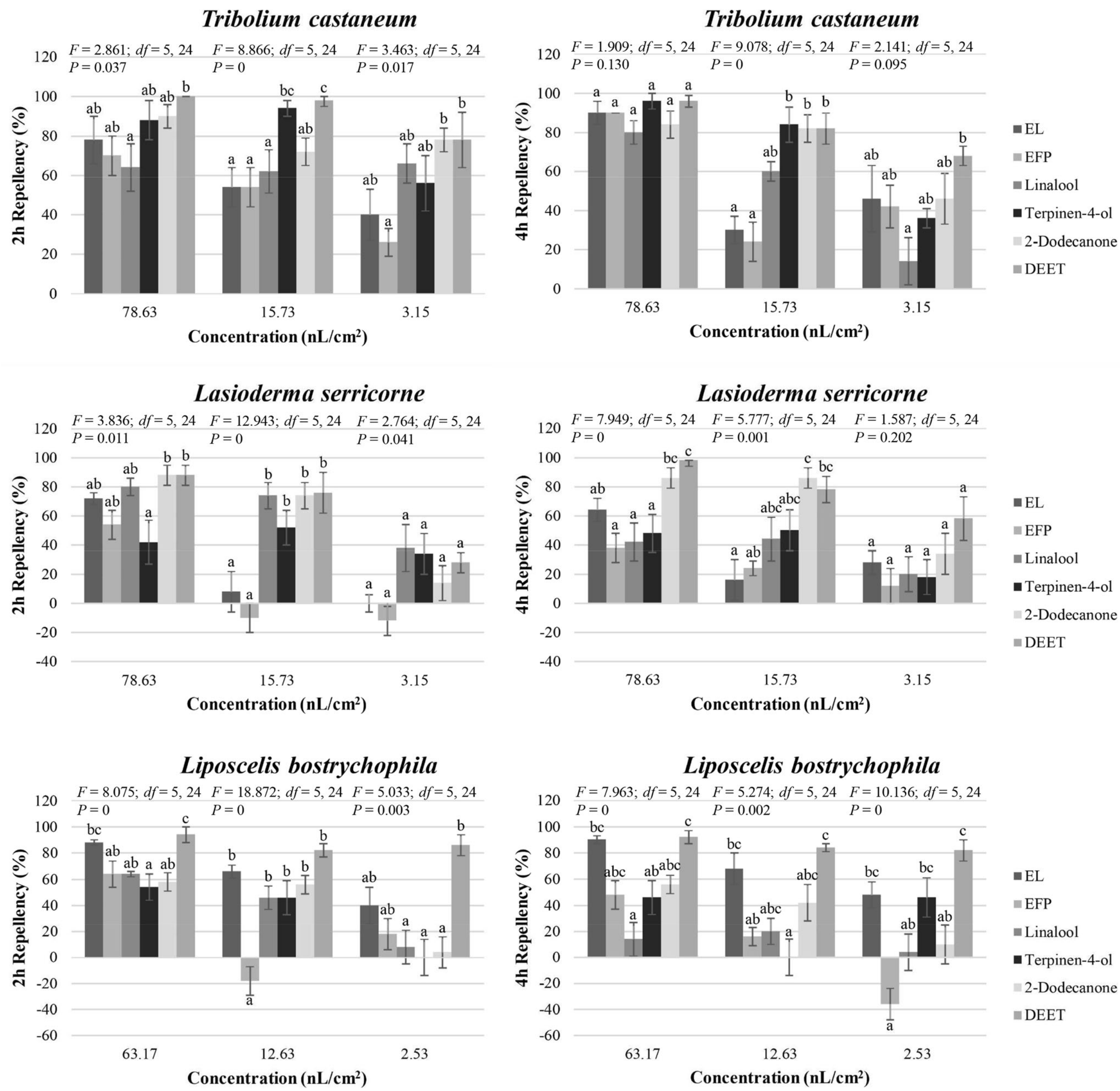

Fig. 2 Percentage repellency (PR) of the essential oils from *Z. planispinum* var. *dintanensis* against *T. castaneum*, *L. serricorne*, and *L. bostrychophila* at 2 and 4 h post-exposure. PR values were subjected to an arcsine square-root transformation before ANOVA analysis (Tukey's HSD test). Means under the same concentration condition followed by the same letters could be believed to have no statistically significant differences ($P > 0.05$). (EL and EFP: the essential oils of leaves and fruit pericarp respectively)

repellent activity as DEET did against *L. bostrychophila*, the PR value of which reached 90% at 63.17 nL/cm^2 at 4 h post-exposure.

Discussion

The significant difference between the chemical composition of two oil samples was that EL included 2-dodecanone (11.52%) in addition to linalool, sylvestrene, and terpinen-4-ol, while this constituent was not detected in EFP. Chen et al. (2008) performed GC-MS analysis of EOs in different organs of *Z. planispinum* var. *dintanensis*. They found linalool was the most abundant constituent in fruit, tender fruit, and flower, while main components in leaf were 2-dodecanone and 2-tridecanone, which was quite distinct between leaf and other organs. It was notable that the content of linalool was able to reach over 80% in the EO of fruits (Chen and He 2009). This particular species of *Zanthoxylum* could be promising to extract linalool for further development and utilization in pharmaceutical, fragrance, and cosmetics industries. In order to ensure linalool with high and stable yield in the EO, harvesting time (Dos Santos et al. 2016), storage duration (Weaver et al. 2017), extraction technique (Wang et al. 2018), and many other factors that might affect the content and proportion of chemical constituents need to be standardized. How to select favorable conditions for production remains future work and exploration.

In this work, EOs from *Z. planispinum* var. *dintanensis* and 2-dodecanone were evaluated for their insecticidal and repellent activities against stored-product insects, which have never been reported before. Major components tested all showed certain or different levels of toxicity and repellency. According to LC_{50} and LD_{50} values, terpinen-4-ol was the most active compound by fumigation and 2-dodecanone was observed to have the strongest contact toxicity against *T. castaneum* (LD_{50} = 5.21 μg/adult), *L. serricorne* (LD_{50} = 2.54 μg/adult), and *L. bostrychophila* (LD_{50} = 23.41 μg/cm^2), which might explain why EL was more toxic than EFP. 2-Dodecanone was more likely to enhance the overall contact toxicity of EL. Wang et al. (2015a) reported that 2-tridecanone was highly toxic to *T. castaneum* (LD_{50} = 5.35 μg/adult) and *L. serricorne* (LD_{50} = 5.74 μg/adult) in contact assays. 2-Dodecanone and 2-tridecanone are similar in structures and proved to have significant contact toxicity against the two beetle species mentioned above. This type of methyl ketone was probably synergistic in contact assays when mixed with some common monoterpenes. The mutual interactions between them need further investigation.

Linalool and terpinen-4-ol are common monoterpenoids in EOs of natural plants. They have been confirmed to possess insecticidal or repellent activities against a wide range of stored-product insects (Kamanula et al. 2017; Eljazi et al. 2017; Liao et al. 2016; Mbata and Payton 2013; Davoudi et al. 2011; Rozman et al. 2007). The two monoterpenoids in vapor form were highly toxic to the eggs, larvae, and adults of *T. confusum*, and displayed insect growth regulator (IGR)–like properties when applied to 3-day-old pupae, inducing the emergence of adultoids and deformed adults (Stamopoulos et al. 2007). In addition, linalool showed oviposition deterrence to exposed mated females of *Callosobruchus maculatus* (Mbata and Payton 2013). A published work (Davoudi et al. 2011) indicated that the synergist diethyl maleate (DEM) could effectively reduce doses of linalool in the management of *C. maculatus*. Decreased linalool resistance suggested that the synergist DEM combined with linalool could enhance enzymatic activity and decrease mechanism of resistance.

Z. planispinum var. *dintanensis* EO was rich in monoterpenoids. These monoterpenoids would be important ingredients in natural anti-insect properties of this plant. Some research commented that monoterpenoids were efficient inhibitors of acetylcholinesterase (AChE) from stored-product insect strains (López and Pascual-Villalobos 2015). The inhibition of AChE activity is a possible mode of action for monoterpenoids which cause a considerable mortality rate of pests. Some monoterpenoids with high concentrations in plants such as linalool can function as strong inhibitors of AChE. However, the potent inhibition of AChE does not necessarily mean great insecticidal activity. Geraniol and *R*-carvone are that case. Moreover, the inhibition is also related to the chemical structure of monoterpenoids. Some monoterpenoid ketones have been proved more inhibitory, and the conjugated double bond was speculated as the key group for stronger inhibition (López and Pascual-Villalobos 2010; Abdelgaleil et al. 2009). In our experiments, 2-dodecanone was an alkane ketone that exhibited better contact toxicity than linalool and terpinen-4-ol did. Such volatile alkane with a ketone group was possibly more toxic in contact assays against *T. castaneum*, *L. serricorne*, and *L. bostrychophila*. The presence of ketone group in alkanes could play an important role in enhancing AChE inhibitory activity thus affecting their insecticidal efficacy. Monoterpenoids could take part in different metabolic pathways. Besides the most investigated inhibition of AChE, the positive allosteric modulation of GABA and competition with octopamine in binding to its receptor are other proposed mechanisms of action on insects. Determination of molecular targets strongly motivates future work on EO-based botanical pesticides (Jankowska et al. 2017). Monoterpenoids are able to easily penetrate inside insects for their lipophilic property and interfere with physiological functions of insects (Lee et al. 2003). Therefore, it is hard to completely clarify their mode of action and even more complicated synergy or antagonism in the mixture.

Z. planispinum var. *dintanensis* was used to make superior pepper oil through processing in the food industry. The EO of it as botanical products is easily biodegradable, and its major components were effective in the control of stored-product insects. Its long-used experience and characteristic features increase the feasibility of developing eco-friendly pesticides. In recent years, green nanotechnology rapidly developed and the advancement in this field has potential to bring revolutionary changes of application to pest management approaches (Mishra et al. 2017). Emergence of nanoemulsions is likely to improve the qualities of natural EOs in insecticidal applications, which could allow to enhance stability and efficacy of EOs against pests (Balasubramani et al. 2017; Hashem et al. 2018). Even so, safety issues cannot be ignored. Natural products draw in people's interest induced by the assumption that "natural is safe" to some degree, which is actually invalid and alluring (Isman and Grieneisen 2014). Although no agencies claim *Z. planispinum* var. *dintanensis* EO would be toxic, whether natural products in plants or novel formulation technologies such as nanoemulsion are really safe for human and environment still requires a lot of experimental verification in the following work.

EL and EFP of *Z. planispinum* var. *dintanensis* contained large amounts of oxygenated monoterpenes. Major components included linalool, sylvestrene, and terpinen-4-ol. Here, 2-dodecanone (11.52%) was identified in the EL, while not detected in EFP, which was the obvious difference noticed in the two oil samples. EL, EFP, and their three individual compounds (linalool, terpinen-4-ol, and 2-dodecanone) were toxic and repellant to adults of *T. castaneum*, *L. serricorne*, and *L. bostrychophila.* Among these compounds, 2-dodecanone had significant contact toxicity against the three stored-product insects. Further investigation is required to determine if the EOs can be applied to pest management and provide long-term protection of stored-products as a botanical insecticide or repellent, not only under laboratory conditions but also in warehousing environments. In fact, the evaluation of biological efficacy under laboratory conditions is an initial step. The experiments in the real world are vital. A lot of plant EOs have been confirmed to have potential active ingredients, and research on botanical insecticides has annually surged, but the number of commercial products based on EOs is limited and remains low. The use of EO-based bio-pesticides for practical application exists weaknesses and challenges. These bio-pesticides tend to show a quick loss of efficiency against target insects in real-world applications. Booming modern preparation technology is promising to help improve this defect. Meanwhile, it is crucial to stabilize homogeneous EO composition for keeping high effectiveness and long duration, inevitability following optimization of plant growing conditions and specification of extraction processes. When it comes to the legislation and market, how to simplify the authorization requirements of legitimizing new botanical pesticides also becomes a great concern (Pavela and Benelli 2016). All these problems deserve consideration and need to be addressed in the future.

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