The Effects of Three Essential Oils on Adult Repellency, Larval Fumigant Toxicity, and Egg Hatch of Tribolium castaneum (Coleoptera: Tenebrionidae)

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The effects of three essential oils on adult repellency, larval fumigant toxicity, and egg hatch of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract

The essential oils of *Cedrus atlantica* (Endl.) (Pinales: Pinaceae), *Mentha x piperita* L. (Lamiales: Lamiaceae), and *Santalum paniculatum* L. (Santalales: Santalaceae), were evaluated for their bioactivity on red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The most pharmacologically effective oil for this beetle species was that of *M. piperita* followed by *C. atlantica* and *S. paniculatum. Mentha piperita* provided about 74% repellency (at 1 d) to adults, whereas larval fumigant mortality was nearly 50% at 8 h of exposure. The greatest apparent reduction of egg hatch occurred with *S. paniculatum* (76%) when compared with *M. piperita* (25%) after 15 d. However, we believe that all 3 essential oils could be helpful for controlling red flour beetles to some degree. After recording bioactivity for all essential oils tested, each was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to identify its chemical components. Further studies should be conducted to test the bioactivity of the individual chemical components of these oils for *T. castaneum*.

Key Words: GC-MS; bioactivity; red flour beetle; stored grain pests

Resumen

Los aceites esenciales de *Cedrus atlantica* (Endl.) (Pinales: Pinaceae), *Mentha x piperita* L. (Lamiales: Lamiaceae) y *Santalum paniculatum* L. (Santalales: Santalaceae) fueron evaluados por su bioactividad sobre el escarabajo rojo de la harina, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). El aceite más farmacológicamente eficaz para esta especie de escarabajo fue el de *M. piperita* seguido de *C. atlantica* y *S. paniculatum*. *Mentha piperita* proporcionó aproximadamente 74% de repelencia (a 1 dia) a los adultos, mientras que la mortalidad de larvales expuestas a los fumigantes fue de casi el 50% a las 8 horas de exposición. La mayor reducción de huevos sucedio con *S. paniculatum* (76%) en comparación con *M. piperita* (25%) después de 15 días. Sin embargo, creemos que los 3 aceites esenciales podrían ser útiles para controlar los escarabajos rojos de la harina hasta cierto punto. Después de registrar la bioactividad de todos los aceites esenciales analizados, cada uno de ellos fue sometido a un análisis de cromatografía de gases y espectrometría de masas (GC-MS) para identificar sus componentes químicos. Se deben realizar más estudios para probar la bioactividad de los componentes químicos individuales de estos aceites para *T. castaneum*.

Palabras Clave: GC-MS; bioactividad; escarabajo rojo de la harina; plagas de granos almacenados

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an economically important pest of stored products (Campbell & Runnion 2003). Adult and larval stages often are present in storehouses year round, and can directly affect the quantity and quality of those commodities. Considerable damage of stored grains can occur because both stages will feed on seed embryos, resulting in decreased germination (Baier & Webster 1992; Moino et al. 1998).

Synthetic fumigant insecticides commonly are used to control these pests in cereal grain storehouses. But the reliance, and often misuse, of these materials has created several problems, including environmental pollution, insect resistance, and unwanted chemical residuals in the commodity. Moreover, some products have been reported to have a direct, negative impact on the health of consumers or non-target organisms (Perez et al. 2010; Zapata & Smagghe 2010; Grünwald et al. 2014). A number of essential oils from different plant species have been reported to possess acceptable bioactivity for a variety of insect and non-insect pests (Adler et al. 2000; Isman 2000, 2006; Nerio et al. 2010). Identifying natural products with repellent and toxic fumigant properties that are environmentally benign, and efficacious, could provide viable alternatives to synthetic pesticides currently used in granary storehouses. We report here on the evaluation of the essential oils of *Cedrus atlantica* (Endl.) (Pinales: Pinaceae), *Mentha piperita* (L.) (Lamiales: Lamiaceae), and *Santalum paniculatum* (L.) (Santalales: Santalaceae) for their effectiveness to repel *T. castaneum* adults and produce larval mortality through fumigant activity, while negatively affecting egg hatch of this pest.

Materials and Methods

INSECTS

Tribolium castaneum used in this study was maintained in 250 mL glass jars containing wheat flour and yeast in the Hubei Insect

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Resources Utilization and Sustainable Pest Management Key Laboratory of Huazhong Agricultural University, Wuhan, China. Insects were reared in the laboratory at 25 ± 2 °C and 50 ± 5% RH with a 14:10 h (L:D) photoperiod.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Commercially available essential oils of sandalwood (*S. paniculatum*), peppermint (*M. piperita*), and cedarwood oil (*C. atlantica*) were purchased from Jiang Xi Xue Song Natural Medicinal Oil LTD, China. A 0.1 mL aliquot of each oil was dissolved in 10 mL of 95% ethanol to achieve a 1:10 working solution used in all studies.

Chemical constituents of each essential oil were separated by gas chromatography-mass spectrometry (GC-MS) according to the methodology of Wagan et al. (2017) using a Varian 450-GC/320-MS (Varian Medical Systems, Inc., Palo Alto, California, USA). This equipment was fitted with a HP-5MS capillary column (film thickness: 30 m × 0.25 mm i.d. × 0.25 mm) and flame ionization detector. The injector oven temperature of the GC was maintained initially at 60 °C for 3 min, ramped at 10 °C per min to 180 °C then maintained for 1 min and ramped again at 20 °C per min to 280 °C for an additional 15 min. One microliter of each essential oil was diluted with 1% hexane and injected with a split ratio of 1:10. Column pressure was maintained at 100 KPa. Helium gas, passed at a rate of 1.0 mL per min, was used as the sample carrier. Temperatures of the MS quadrupole, ion source, and transmission line were 150 °C, 230 °C, and 250 °C, respectively. Constituents were identified from the gas chromatogram database using the online libraries of MANLIB, REPLIB, PMWTox3N (NIST 2011).

ADULT REPELLENCY TEST

Adult repellency of the essentials oils to *T. castaneum* was evaluated in H-type glass tubes measuring 2.5 cm D × 20 cm H with a 15 cm connecting arm (Fig. 1). A 0.1 mL aliquot of a test solution was applied to 0.02 g of cotton wick and placed into 1 arm at the end of the H-tube. At the end of the other arm, 0.1 mL of 95% ethanol (control) was applied to another cotton wick and similarly placed. For food, 15 g of a susceptible rice variety (Taichung Native-1) were added into each arm of the H-tube with and without essential oil. To prevent the oil from contaminating rice grains, a 2.5 cm diam filter paper disc was placed between treated wicks and rice. To prevent evaporation of treatments, as well as to maintain the oil fragrance within the tube, a cotton wick was placed into the open ends of each arm. On the day of evaluation, 20 adult 10-d-old beetles were released into the center of the H-tube then placed in a covered box to maintain darkness in the same room used for rearing (25 ± 2 °C, 50 ± 5% RH, and 14:10 h L:D photoperiod). The number of insects in each arm was recorded at 1, 3, 5, and 7 d following insect release. A total of 8 repetitions were conducted for each essential oil.

EGG HATCH TEST

Following the adult repellency test, rice and beetles were removed from the H-tube and placed into 100 mL glass jars. Because the eggs cannot be accurately counted, we assumed that approximately equal numbers of eggs were deposited in each jar. Jars remained in the same room that was used for the repellency test. Rice grains were examined under a stereoscope microscope and the numbers of young larvae were recorded at 5, 10, and 20 d after placement in jars.

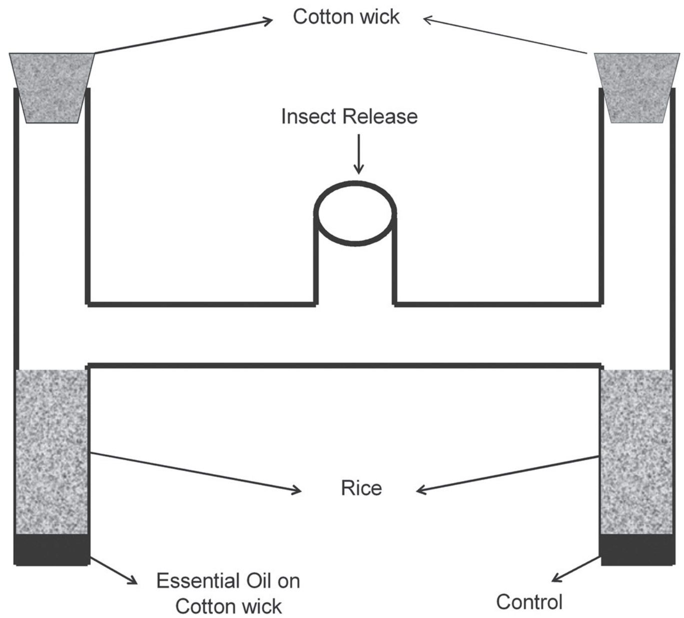

Fig. 1. Diagram of adult repellency test apparatus showing cotton wick (source of essential oils) placed at the bottom of both legs, which were half filled with rice grains.

LARVAL FUMIGANT TEST

Fumigant toxicity was conducted in airtight glass jars (100 mL). To evaluate the fumigant toxicity of essential oils, 0.1 mL of the initial 1:10 solutions was uniformly applied to separate 6 cm diam filter paper discs (Whatman N-grade 1: 11 µm) using a micropipette (Eppendorf, Inc., Hamburg, Germany). The same volume of 95% ethanol applied to a separate group of filter paper discs was used as the control. Thirty min after application, each filter paper was attached to the lid of the airtight 100 mL glass jars. Then, 20 fourth instar *T. castaneum* (2.5–3.0 mg) were placed in jars and the lids tightened. A strip of Parafilm®®M (Bemis Company, Oshkosh, Wisconsin, USA) was affixed to the outer rim of the jar lid and jar to prevent possible aeration. Insects remained on the bottom of the jars throughout the experiment and had no contact with the filter paper disc. Larval mortality was observed through the glass container; erect and immobile larvae were recorded as dead. Larval mortality was recorded at 1, 2, 4, and 8 h of exposure. There were 3 replications per oil and 8 repetitions of the fumigant test.

STATISTICAL ANALYSIS

The equation used by Liu et al. (2013) was used to calculate percent repellency: (PR %) = [(C − T) / (C + T)] × 100, where T is the number of insects in treatment and C is the number in control. Adjusted mortality rate (AMR %) was calculated as: (T − C) / (1 − C) × 100%. Mean percentage data were arcsine-square root transformed (√x + 1), or log transformed [log 10 (x + 1)], prior to analysis. A Chi-square test was used to compare mean adult beetle repellency between treatments and controls. A 1-way ANOVA and Tukey's post hoc tests were used to separately compare mean percent larval mortality in fumigant evaluations and mean egg hatch between essential oils. All analyses were performed using SPSS version 20 (IBM Corp., Armonk, New York, USA) with a significance level of $P < 0.05$. Untransformed means are presented in results.

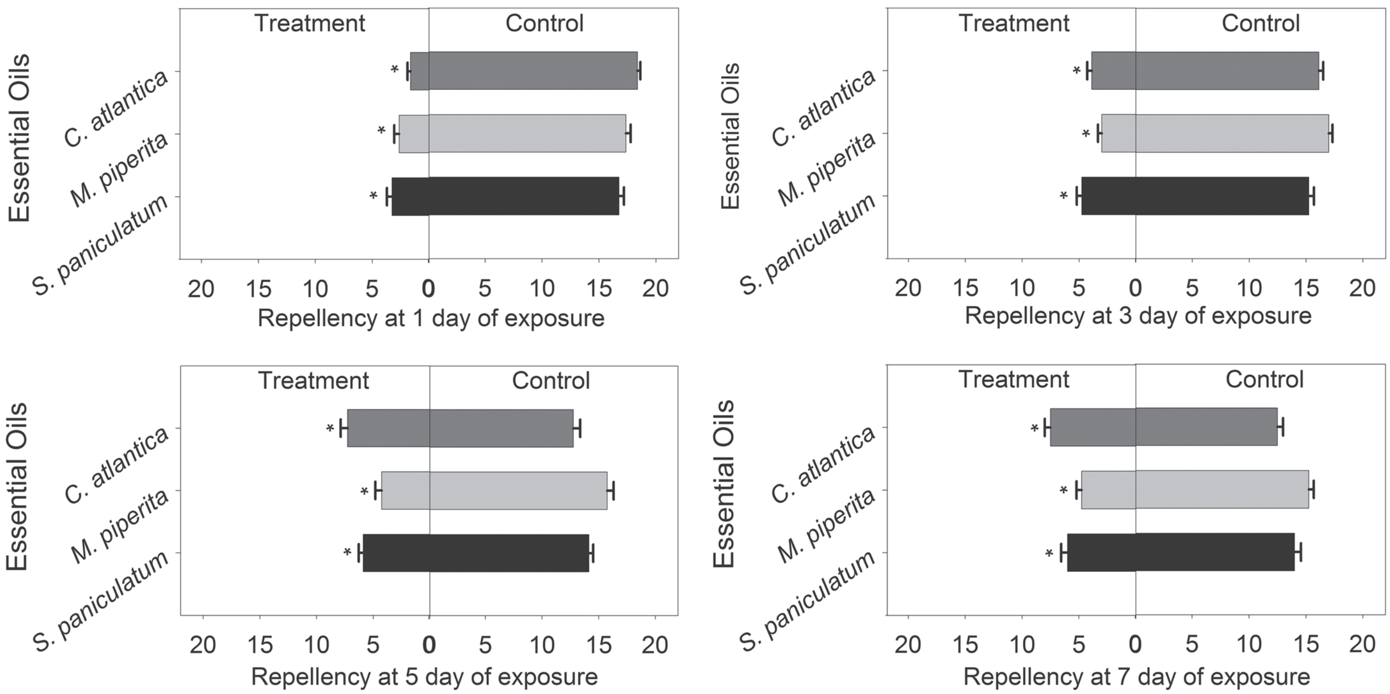

Fig. 2. Mean (± SE) repellency of adult red flour beetles at varying intervals of exposure, tested separately to 1 of 3 essential oils. Means with an asterisk for each time interval are significantly different (Chi-square test, $P < 0.05$).

Results

ADULT REPELLENCY TEST

Generally, *T. castaneum* adults were significantly repelled by all essential oils when compared with controls at 1 d ($F = 4.47$; df = 2; $P < 0.05$), 3 d ($F = 4.88$; df = 2; $P < 0.05$), 5 d ($F = 7.91$; df = 2; $P < 0.05$), and 7 d ($F = 7.32$; df = 2; $P < 0.05$) of exposure (Fig. 2). *Cedrus atlantica* provided the greatest mean repellency to beetles when compared with controls at the first d of exposure ($\chi^2 = 68.04$; df = 1; $P < 0.001$) (Fig. 2). Repellency decreased over time, but was still significantly greater for this essential oil than controls at 3 d ($\chi^2 = 33.12$; df = 1; $P < 0.001$), 5 d ($\chi^2 = 6.17$; df = 1; $P = 0.01$), and 7 d ($\chi^2 = 5.08$; df = 1; $P = 0.02$). *Mentha piperita* provided an intermediate level of beetle repellency between *C. atlantica* and *S. paniculatum* (Fig. 2), and maintained a significantly greater level than controls at 1 d ($\chi^2 = 50.36$; df = 1; $P < 0.001$), 3 d ($\chi^2 = 44.67$; df = 1; $P < 0.001$), 5 d ($\chi^2 = 28.83$; df = 1; $P < 0.001$), and 7 d ($\chi^2 = 23.68$; df = 1; $P < 0.001$). The essential oil of *S. paniculatum* provided the least repellency to adult *T. castaneum* but was still significantly greater than controls at 1 d ($\chi^2 = 41.14$; df = 1; $P = 0.00$), 3 d ($\chi^2 = 19.98$; df = 1; $P < 0.001$), 5 d ($\chi^2 = 14.22$; df = 1; $P = 0.00$), and 7 d ($\chi^2 = 13.33$; df = 1; $P < 0.001$) (Fig. 2).

Among all the essential oils, *M. piperita* averaged greater repellency to *T. castaneum* adults, with mean percentages of 73.75, 70.00, 57.50, and 52.50% at 1, 2, 5, and 7 d, respectively (Fig. 3). Whereas *C. atlantica* appeared to be a strong repellent at first observation, its effectiveness was reduced with time, and its repellency was at level 3 by the end of the experiment. The repellency percentages were observed to be 83.75, 61.25, 27.50, and 25.00% at 1, 3, 5, and 7 d, respectively (Fig. 3). *Santalum paniculatum* displayed the lowest repellency in the first 2 observation times; when the other oil repellency decreased, *S. paniculatum* became second in the repellency index. The mean percentages were 67.50, 52.50, 41.25, and 40.00%, as recorded at 1, 3, 5, and 7 d, respectively (Fig. 3).

EGG HATCH TEST

For egg hatch, all essential oils showed significant difference with control at 5 d ($F = 14.99$; df = 3; $P < 0.01$), and 10 d ($F = 49.47$; df = 3; $P < 0.01$), but not at 15 d ($F = 65.24$; df = 3; $P > 0.01$) of exposure. Essential oil from *M. piperita* apparently decreased egg hatch when compared with *S. paniculatum* and *C. atlantica*. It showed a significant difference with the other 2 oils at the first 5 d of exposure, whereas no significant differences were observed at 10 and 15 d of exposure (Fig. 4).

LARVAL FUMIGANT TEST

Generally, significant differences in larval *T. castaneum* toxicity existed among essential oils and control when evaluated as fumigants, and generally increased with increased exposure time at 1 h ($F = 3.78$; df = 3; $P < 0.02$), 2 h ($F = 3.04$; df = 3; $P < 0.04$), 4 h ($F = 19.28$; df = 3; $P < 0.01$), and 8 h ($F = 39.75$; df = 3; $P < 0.01$) (Fig. 5). *Mentha piperita* displayed a significantly higher percentage of toxicity throughout the study when compared with the essential oils and control, whereas *S. paniculatum* was the second most toxic to larvae. *Cedrus atlantica* averaged least effective, though not significantly less effective than *S. paniculatum* (Fig. 5).

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Gas chromatography and mass spectrometry analyses identifying chemical constituents, retention times, and yield percentages of each extract for the 3 essential oils used in our study revealed 18 chemical constituents from *M. piperita*, 13 from *S. paniculatum*, and 13 from *C. atlantica* (Table 1).

Discussion

Among the 3 essential oils evaluated in this study, *M. piperita* was found to have the strongest repellency to adult *T. castaneum* during

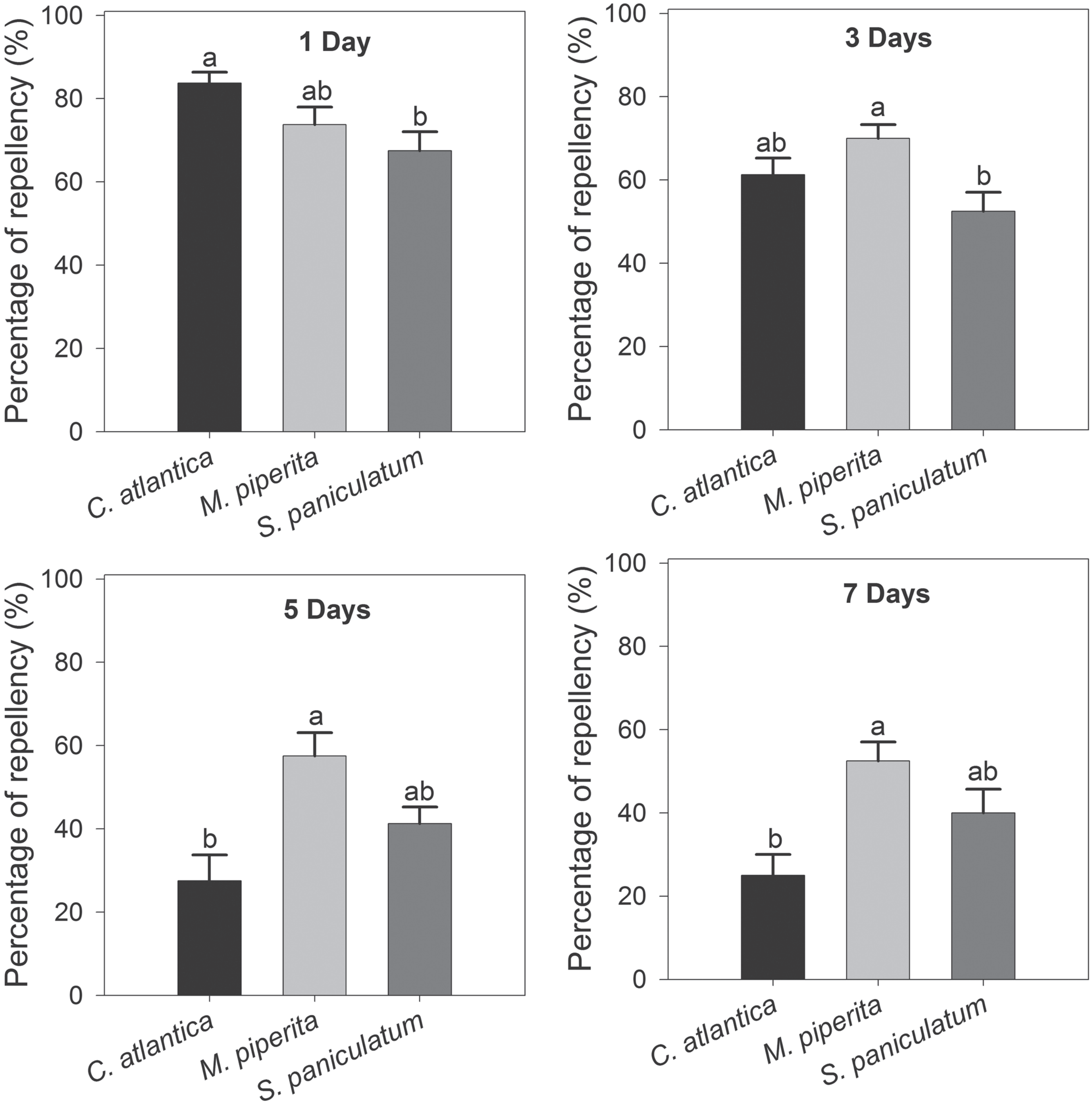

Fig. 3. Mean percent (± SE) repellency of adult red flour beetles at varying intervals of exposure, tested separately to 1 of 3 essential oils. Means with a different letter for each time interval are significantly different (Tukey's HSD post hoc test, $P < 0.05$).

the majority of the 7 d exposure period. Recently, several studies on the repellency of *Mentha* have confirmed this effect with many insect species (Khater et al. 2009). At concentrations of 500 ppm and 1,000 ppm, the essential oil from *M. piperita* has been found to effectively repel 50% of cabbage aphids, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), after 24 h of exposure with continued repellent activity that lasted up to 48 h (Wubie et al. 2014). *Mentha piperita* (peppermint) essential oil also has been reported to exhibit repellent activity to the mosquito *Culex pipiens* L. (Diptera: Culicidae) (Erler et al. 2006).

The repellency of *S. paniculatum* essential oil to adult *T. castaneum* was noticeably less when compared with *M. piperita,* and was intermediate between the latter oil and *C. atlantica*. Ritchie et al. (2006) reported that burning sandalwood sticks (containing 0.05% *S. paniculatum* essential oil) prevented about 73% of adult *Verrallina funerea* (Theobald) and *Ve. lineata* (Taylor) (Diptera: Culicidae) mosquitoes from landing and probing on humans for 3 h when exposed under field conditions. Moreover, sandalwood is traditionally burned to repel insects in some Pacific Islands (Ananthapadmanabha & Venkatesha-Gowda 2011).

We found that exposure of *T. castaneum* eggs to all 3 essential oils resulted in a reduction in egg hatch. It has been reported that volatile essential oils can have a direct toxic effect on insect eggs that may re-

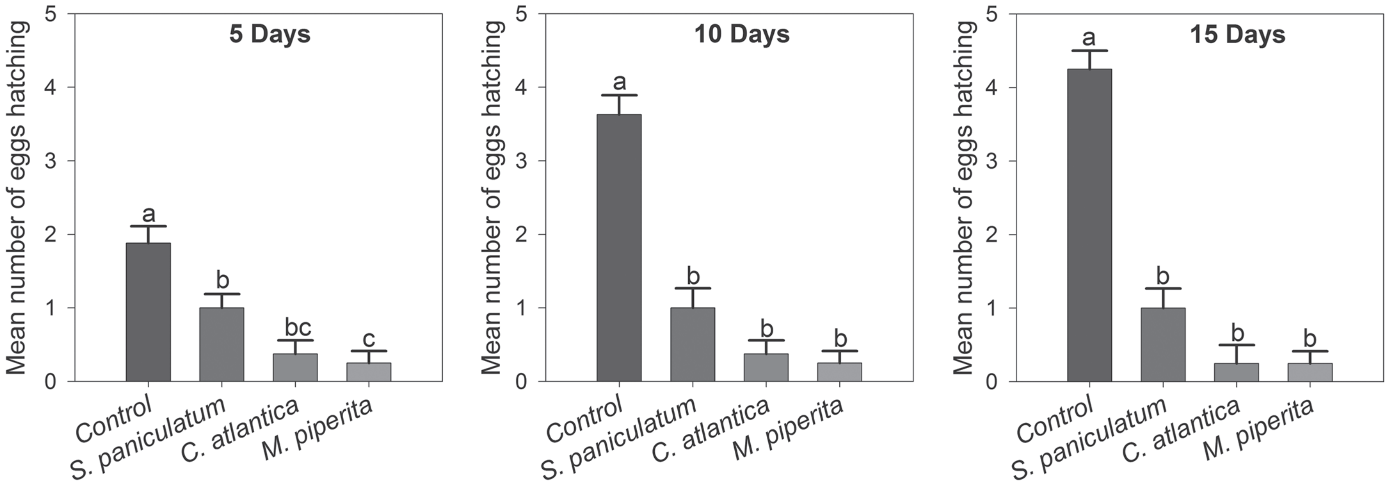

Fig. 4. Mean red flour beetle egg hatch (± SE) during exposure to rice grains treated with 1 of 3 essential oils at varying exposure times. Means with a different letter for each time interval are significantly different (Tukey's HSD post hoc test, $P < 0.05$).

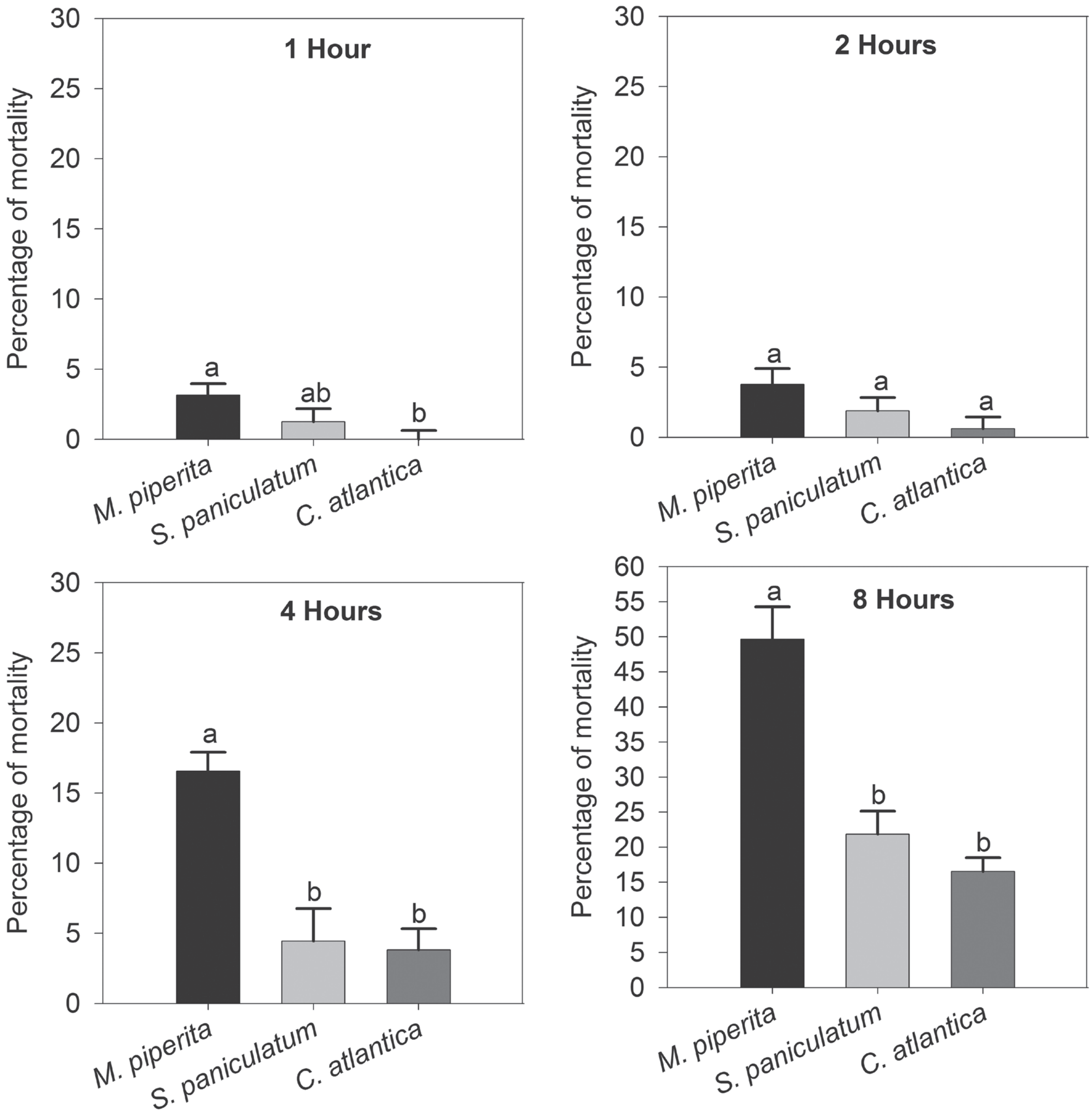

Fig. 5. Mean percent (± SE) mortality of red flour beetle larvae during separate exposure, as a fumigant, to 1 of 3 essential oils, at varying exposure times. Means

Table 1. Gas chromatography-mass spectrometry extraction results of 3 essential oils.

Components	Retention Time (min)	Percentage of Total (%)
Mentha piperita		
alpha.-Pinene	6.03	2.79
Sabinene	6.47	0.92
beta.-Pinene	6.55	2.42
3-Octanol	6.69	0.49
Benzene, 1,2,3,4-tetramethyl	7.01	2.77
D-Limonene	7.07	6.13
Eucalyptol (1,8-Cineole)	7.11	0.88
Isopulegol	8.20	1.23
l-Menthone	8.27	20.86
L-Menthal one	8.34	9.82
(+)-Neomenthol	8.37	4.84
Menthol	8.47	32.86
neo-Menthol	8.54	1.13
Pulegone	8.92	2.01
3-Cyclohexen-1-ON, 2-isopropyl-5-methyl	9.04	1.12
Neomenthylacetate	9.23	4.32
beta.-Bourbonene	9.98	0.47
trans-Caryophyllene	10.25	0.77
Santalum paniculatum		
Cyclohexene, 4-methyl-1-(1-methylethyl)-	6.58	0.29
Isopulegol	8.17	1.03
Acetic acid, phenylmethyl ester	8.24	1.94
3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl	8.55	2.94
2(3H)-Furanone, 5-hexyldihydro	9.77	2.29
Isolongifolene	10.11	9.38
Diethyl Phthalate	11.25	18.52
alpha.-Cadinol	11.71	2.85
alpha.-Bisabolol	11.81	4.84
Tricyclo[20.8.0.0e7,16]Triacontan, 1(22),7(16)-Diepoxy	12.79	3.40
Cembrene	12.49	1.09
Naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)	13.20	1.72
Ethylene brassylate	13.65	13.39
Cedrus atlantica		
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	8.45	0.26
Carvacrol methyl ether	8.85	1.38
1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl	10.32	11.66
Widdrene	10.48	13.75
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	10.59	1.93
beta.-Cedrene	10.65	1.01
Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene	10.74	2.05
Thujopsene-(I2)	10.89	9.95
beta.-Himachalene	11.02	1.77
Cedrol	11.59	8.79
alpha.-Cedrol	11.68	2.36
alpha.-Bisabolol	11.85	2.39
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)	14.06	1.42

sult in reduced egg hatchability (Schmidt et al. 1991). Indeed, Gurusubramanian & Krishna (1996) observed this with the volatile oil of *Allium sativum* L. (Asparagales: Amaryllidaceae) on the eggs of *Earias vittella* Fab. (Lepidoptera: Noctuidae), *Spodoptera litura* Fab. (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and *Dysdercus koenigii* Fab. (Hemiptera: Pyrrhocoridae)

We found that the essential oil of *M. piperita* produced the most toxicity as a fumigant to larval *T. castaneum* compared to the other essential oils evaluated. The lowest fumigant toxicity to *T. castneum* larvae was achieved with *C. atlantica* essential oil with a mortality rate of 6.7% at 8 h continuous exposure.

In summary, all essential oils in our study provided some level of adult repellency, fumigant action on larvae, or negative influence on egg hatch of *T. casteneum*. The essential oil of *M. piperita* was found to have the strongest repellent activity (at 3, 5, and 7 d), reduction of egg hatch, and larval toxicity at all time intervals. The essential oil of *C. atlantica* was found to have the second strongest adult repellency and egg hatch reduction but was ranked third in larval toxicity. The essential oil of *S. paniculatum* was found to have the second strongest larval toxicity but it was the least effective of the 3 oils for adult repellency and reduction of egg hatch. Our study proved that essential oils of *M. piperita*, *C. atlantica*, or *S.*

paniculatum could be helpful for controlling red flour beetle populations in grain storage facilities. We recommend that future studies be conducted on these essential oils to determine their efficacy to other stored product pests. In addition, further investigation of the bioactivity of individual chemical constituents of each essential oil obtained from the gas chromatograph-mass spectrophotometer extractions is warranted.

Acknowledgments

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