Study on the insecticidal activity compounds of the essential oil from *Syzygium aromaticum* against stored grain insect pests

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Abstract

Insect pests are a major cause of damage in stored grain around the world. To control the stored grain insects, synthetic insecticides have been used extensively for many years, resulting in insect populations that are resistant to insecticides. Consequently there is an interest to find alternatives to chemical pesticides. The essential oil from *Syzygium aromaticum* (clove oil) has a number of bioactive compounds. The chemical constituents of the clove oil were analyzed by GC-MS, and 9 of 18 compounds were identified. The main compound (83%) was 2-methoxy-4-(2-propenyl)-phenol the second most common compound (12%) was trans-caryophyllene. These two pure compounds and clove oil were tested for toxicity and repellency against *Rhyzopertha dominica, Sitophilus oryzae and Tribolium castaneum*. The pure compounds were tested at the dosages found in clove oil. The mortality from 2-methoxy-4-(2-propenyl)-phenol was not significantly different from clove oil, suggesting that the activity of clove oil was solely due to this major compound. The repellency results were more complex. 2-methoxy-4-(2-propenyl)-phenol was more repellant than clove oil. Trans-caryophyllene was less toxic and less repellant than both clove oil and 2-methoxy-4-(2-propenyl)-phenol. The potential for these compounds to be used to control stored product insects is discussed.

Keywords: Essential oils, *Syzygium aromaticum*, Clove oil, Insecticidal activity compounds, Stored grain insects

1. Introduction

Rhyzopertha dominica (F.) (lesser grain borer), Sitophilus oryzae (L.) (rice weevil), *Tribolium castaneum* (Herbst) (red flour beetle) are cosmopolitan pests of grain. In China, these insects damage paddy, wheat, maize, potato and their processed products (Li, 2004). These pests not only cause damage in the warehouse, but also in rice processing factories. In southern China, they are active throughout the year.

At present, the major method to reduce damage caused by stored grain pests is chemical control (Liang, 1994; Yang, 2004; Collins, 2006). Chemical control has several advantages; fast acting, the low cost, controls most insect pests, but the “3R” (Resistance, Resurgence and Residue) problems have become more and more serious (Li, 1994; Hu, 2001).

Based on the current problems cause by the chemical pesticides, the environmentally friendly pesticides against stored products pests are being developed to replace chemical pesticides. Developing plant resources and extracting essential oils from plants as grain protectants is an area of considerable recent research (Cao, 2002; Hu, 2001; Hou,2001; Yan, 2007). Plant essential oils have several advantages; low mammalian toxicity, low resides on grain, and novel chemical structures. Given that essential oils are very different in chemical structure than the currently used stored-product insecticides, we do not expect the insects that have resistance to the commonly used insecticides to also be resistant to the essential oils. Therefore, essential oils fulfill the requirements of pesticides in the 21st century and they may be widely used to stored product pest control (Zhang, 2004).

The essential oil from *Syzygium aromaticum* (clove oil) possesses many compounds with biological activity, and it is used to control insects, fungus, mildews in stored grains (Kong, 2004; Han, 2006; Lou, 2006; Shang, 2007). There are several compounds in clove oil, but the specific activities of these various compounds against stored-product pests has never been examined. The objective of this study was to examine the insecticidal activity of compounds from the clove oil against the major stored grain insect pests.

2. Materials and methods

2.1. Culturing insects

Insects were cultured at 25° ± 1°C and r.h. 70%-80%. *Rhyzopertha dominica* and *S. oryzae* were cultured on wheat, and *T. castaneum* was reared on a mixture of crushed wheat, oatmeal, yeast in the ratio of 3:3:1. After 7 d, the adults were removed. All of the experimental insects were the newly emerged adults 1 to 14 d old. The wheat grain was from China Grain Reserves Corporation, oatmeal and yeast were purchased from the market. In order to kill all insects, the wheat was heated to 60°C for 2-3 h before use and moisturized after heating until the content of water was about 14%. The paddy used for experiments was the Five-star Mew Rice with water content of about 13%. It was produced in Xinhui city and Lianshan County, Guangdong Province.

2.2. Essential oils

The clove oil was provided by Guangzhou Gaoshangmei Fine Chemical Co., Ltd. The compounds from clove essential oil used for tests were shown in Table 1.

Table1 The compounds from essential oils used for test.

No.	Compounds	Purity (%)	Formula	CAS number	Relative content in essential oil (%)
D5	2-methoxy-4-(2-propenyl)- phenol	99.0	C10H12O2	97-53-0	83.13
D6	trans-caryophyllene	98.5	C15H24	87-44-5	12.42

* The compounds were provided by Happy & Excited Guangzhou Biotech Ltd. Co.

2.3. GC-MS analysis

The compounds from essential oils were analyzed by Finnigan TRACE Gas Chromatography - Mass Spectrometry. The oils sample was diluted 10 fold with ethanol, and then 0.3 μL of that was taken to inject in GC.

Conditions: DB-1 column: 30m × 0.25mm; Ionization mode EI: 70 eV; Mass range: 35-395 amu; Operating temperatures: maintain 60°C for 1 min, then heat to 90°C at 100C/min, to 150°C at 5°C/min, to 300°C at 10°C/min. and at the end, maintain 3000C for 5 min.

2.4. Repellent activity

The repellent activity was examined by insecticide-impregnated filter paper, following the methods described by Jilani (1990). A filter paper of 9 cm in diameter was cut in half. One half was dipped in the acetone diluted solution containing essential oils, the other half as a control was dipped in acetone solution. After the acetone evaporated form both two halves, they were fixed together again by a transparent plastic at the bottom. A stainless steel ring of 9 cm in diameter was placed on the filter paper. The wall of the ring was wiped by a layer of Teflon to prevent escape. Thirty adults of the pests were put into one ring. Five replications were used for each concentration. The distribution of insects on the two halves of the filter paper was examined at 12, 24, 36, 48, 60 and 72 h after treatment. Percentage repellency was calculated as follows.

$$PR(\%)=(Nc - Nt)/ Nc\times100\% \qquad (1.1)$$

PR=Percentage repellency, %;

Nc=Average number of insects on the untreated area after the 6 exposure intervals;

Nt=Average number of insects on the treated area after the 6 exposure intervals;

2.5. Toxicity

The essential oil was dissolved with acetone in order to get different concentrations of solutions. Respectively, the solutions and a certain amount of paddy were well-mixed. After the volatilization of acetone, 50 g paddy and 30 adult insects were put into a jar of 250 mL. Each treatment had five replications and the control was treated with acetone. The mortality of insects was examined 3, 7, 10 and 14 d after treatment. The morality and corrected mortality was calculated as follows.

$$M (\%)=Nd / Nt\times 100\% \qquad (1.2)$$

$$CM (\%)=((Mt-Mc)/(1-Mc)) \times 100\% \qquad (1.3)$$

M=Mortality, %;
CM=Corrected mortality, %;
Nd=Number of dead pests;
Nt=Number of pests used for test;
Mt=Mortality of treatment, %;
Mc=Mortality of control, %.

3. Results

3.1. Component analysis of the essential oil from S. aromaticum

GC-MS technology was used to analyze the components of the essential oil from S. aromaticum (Fig. 1). Eighteen components were detected (Fig. 1, Table 2). Nine main components (SI> 800) were identified, of which the peak area was 98.68% of the total ion peak area. The most important component was 2-methoxy-4-(2-propenyl)-phenol (83.13%), followed by trans-caryophyllene (12.42%). The proportions of the other 7 components were all above 1%. The content of a-caryophyllene (SI> 800) was about 1.69%.

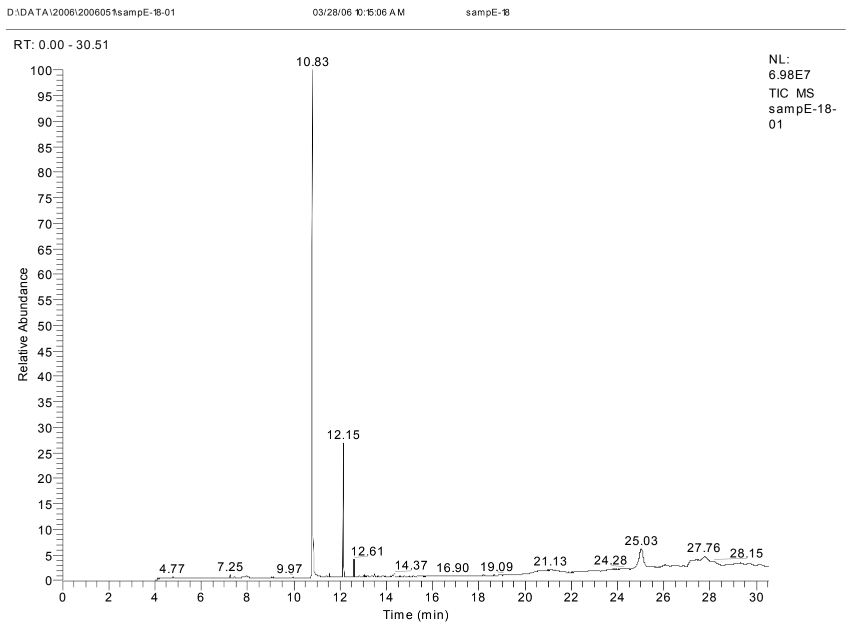

Figure 1 Total ion current chromatogram of the essential oil from *S. aromaticum*

Table 2 Components of the essential oil from *S. aromaticum* detected by GC-MS.

Peak no.	Retention time (min)	Compound	Formula	Similarity	Relative content in essential oil (%)
1	7.25	(2S-trans)-5-methyl-2-(1-methylethyl)-cyclohexanone	C10H18O	908	0.29
2	7.44	(2S-trans)-5-methyl-2-(1-methylethyl)-cyclohexanone	C10H18O	807	0.07
3	7.55	menthofuran	C10H14O	711	0.03
4	7.74	L-(-)-menthol	C10H20O	830	0.11
5	7.83	L-(-)-menthol	C10H20O	799	0.18
6	7.96	2-hydroxy-benzoic acid, methyl ester	C8H8O3	741	0.25
7	9.02	chavicol	C9H10O	793	0.07
8	9.11	2h-1-benzopyran	C9H8O	702	0.06
9	10.83	2-methoxy-4-(2-propenyl)- phenol	C10H12O2	920	83.13
10	11.13	2-methoxy-4-(2-propenyl)- phenol	C10H12O2	709	0.23
11	11.43	1,2-dimethoxy-4-(2-propenyl)- benzene	C11H14O2	699	0.18
12	11.54	cedrene	C15H24	814	0.34
13	12.15	trans-caryophyllene	C15H24	943	12.42
14	12.61	a-caryophyllene	C15H24	902	1.69
15	13.04	2-methoxy-4-(2-propenyl)-phenol	C10H12O2	702	0.16
16	13.48	tau-cadinol	C15H26O	803	0.31
17	14.27	25-desacetoxy-bccurbitacin b	C30H44O6	727	0.16
18	14.37	caryophyllene oxide	C15H24O	841	0.32

3.2. Repellency

We tested the repellency and toxicity of the essential oil of S. aromaticum and its two main chemical components: 2-methoxy-4-(2-propenyl)-phenol (Code: D5) and trans-caryophyllene (Code: D6). The essential oil, D5 and D6 were repellent *R. dominica*, *S. oryzae* and *T. castaneum* (Table 3). D5 had highest repellency grade on all 3 tested pest species. The mean repellency rates of D5 on R. dominica, S. oryzae and *T. castaneum* were 93, 71 and 97% respectively, which was significantly higher than that of D6 on all 3 pest species, and significantly higher than that of the clove oil on S. oryzae and *T. castaneum*. The percentage repellency of D6 on *R. dominica* was significantly lower than that of the clove oil, but significantly higher than the clove oil with *S. oryzae*. This suggests that D5 was the main ingredient with repellent activity.

Table 3 Repellency of the essential oil of *S. aromaticum* and its two main compounds against stored grain insect pests.

Insect	Essential oil and compound	Repellency + SE (%) Duration (h) 12	24	36	48	60	72	Mean repellency (%)
R. dominica	*S. aromaticum*	79.1 ± 0.5 a	66.0 ± 18.4 a	70.5 ± 11.6 a	60.9 ± 14.7 ab	68.5 ± 9.2 ab	64.5 ± 19.9 a	68.2 ± 11.6 a
	D5	96.5 ± 0.0 a	95.2 ± 3.3 a	91.5 ± 1.3 a	92.9 ± 0.0 a	91.3 ± 2.6 a	93.7 ± 4.7 a	93.5 ± 0.7 a
	D6	11.1 ± 11.1 b	0.0 ± 0.0 b	16.7 ± 16.7 b	19.1 ± 19.1 b	38.5 ± 16.7 b	11.1 ± 11.1 b	16.1 ± 9.6 b
S. oryzae	*S. aromaticum*	4.2 ± 4.2 c	0.0 ± 0.0 b	4.2 ± 4.2 b	21.5 ± 6.2 b	33.8 ± 19.7 a	42.9 ± 0.8 b	17.8 ± 2.2 c
	D5	94.0 ± 1.3 a	17.4 ± 1.2 ab	92.3 ± 4.6 a	81.2 ± 5.9 a	75.3 ± 2.7 a	68.8 ± 3.8a	71.5 ± 2.1 a
	D6	59.1 ± 12.6 b	35.9 ± 11.4 a	71.9 ± 12.4 a	63.9 ± 16.6 a	44.4 ± 11.2 a	42.5 ± 11.3 b	52.9 ± 3.4 b
T. castaneum	*S. aromaticum*	13.0 ± 13.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	2.2 ± 2.2 b
	D5	92.8 ± 2.2 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.9 ± 1.2 a	95.3 ± 1.2 a	97.7 ± 1.2 a	97.4 ± 0.5 a
	D6	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b

* Tested dosage: oil was 600 µg/cm2, D5 was 500 µg/cm2, D6 was 240 µg/cm2; Means followed with same letters in the same insect species within the same column are not significantly different at 0.05 level by Duncan's multiple range test.

3.3. Toxicity

All compounds were toxic to the three species tested (Table 4). The lowest mortality was with D5 and D6 against *T. castaneum* after 3 d. As expected, mortality increased with the time. There was no significant difference in mortality against all 3 pest species between D5 and the clove oil, except for D5 against *T. castaneum*. But the mortalities of D6 on all pests were very low, and were lower than the clove oil against *R. dominica* and *S. oryzae*. After 14 d, D5 and the clove oil had similar mortality, whereas mortalities of D6 were lower than that of D5 and the clove oil treatments. As with the repellency tests, D5 was the compound responsible for the mortality of the clove oil.

Table 4 Toxicity of the essential oil of *S. aromaticum* and its two main compounds against stored grain insect pests.

Insect	Essential oil and compound	Mortality + SE (%) Duration (d) 3	7	10	14
R. dominica	*S. aromaticum*	33 ± 6 a	41 ± 7 a	60 ± 11 a	80 ± 6 a
	D5	27 ± 5 a	31 ± 6 ab	49 ± 10 a	67 ± 5 a
	D6	6 ± 2 b	13 ± 2 b	16 ± 1 b	19 ± 1 b
S. oryzae	*S. aromaticum*	1 ± 1 a	35 ± 2 a	44 ± 2 a	51 ± 2 a
	D5	4 ± 3 a	37 ± 8 a	43 ± 6 a	51 ± 5 a
	D6	1 ± 1 a	9 ± 5 b	14 ± 8 b	21 ± 7 b
T. castaneum	*S. aromaticum*	1 ± 1 a	3 ± 1 a	11 ± 2 a	22 ± 4 a
	D5	0 ± 0 a	1 ± 1 a	4 ± 1 b	21 ± 5 a
	D6	0 ± 0 a	1 ± 1 a	2 ± 1 b	10 ± 2 a

* Tested dosage: oil was 2000 mg/kg (0.2%W/W), D5 was 1660 mg/kg, D6 was 250 mg/kg; Means followed with same letters in the same insect species within the same column are not significantly different at 0.05 level by Duncan's multiple range test.

4. Conclusions

Two-methoxy-4-(2-propenyl)-phenol (D5) is the major compound of clove oil, with a proportion of 83%, followed by trans-caryophyllene (D6), which is 12%. The clove oil and both its two components had repellent and toxicity activity on the 3 important stored grain insect pest species, *R. dominica, S. oryzae* and *T. castaneum*. For mortality, there was no significant difference between clove oil and 2-methoxy-4-(2-propenyl)-phenol and very little mortality with trans-caryophyllene. Therefore it can be concluded that 2-methoxy-4-(2-propenyl)-phenol (D5) is responsible for the mortality of clove oil and the compounds did not act synergistically together. The repellency is more complicated to interpret, with different species reacting differently; D5 was more repellant than the clove oil for S. oryzae and *T. castaneum*, suggesting that some of the other compounds in clove oil may mask the repellency of D5. D6 also had some activity alone suggesting that not all the repellency in the clove oil was due to D5.

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