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Fumigant Activities of 2 Essential Oils Extracted from Dried Fruits of *Zanthoxylum bungeanum*a gainst the Adults of *Sitophilus zeamais*

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Abstract: Prickly ash tree, *Zanthoxylum bungeanum* (Maxim), which is a deciduous shrub, is widely grown in southwest China, especially in Sichuan Province and Chongqing municipality. The dried fruits of *Zanthoxylum bungeanum* are important condiment and traditional Chinese medicine. Chinese farmers have a long history to use the dried fruits and other parts of *Zanthoxylum bungeanum* to control stored grain insect pests. The essential oils extracted from the dried fruits of *Zanthoxylum bungeanum* are the promising resources of insecticide and fungicide. In this paper, the fumigation activities of green prickly ash essential oil and red prickly ash essential oil against the adults of *Sitophilus zeamais* Motschulsky were researched at 20, 23, 26, 29, 32, and 35℃. The results showed that essential oil, temperature and essential oil concentration influenced the mortality significantly. At 29, 32, and 35℃, 12μL/L and 15μL/L doses of green prickly ash essential oil led to 100% mortality after 24 h fumigation. Meanwhile, red prickly ash essential oil caused 100% mortality at 16 and 19μL/L essential oil dosage under the same temperatures. In addition, LC_{50} values at 30℃ after 24, 36, 48, and60 h fumigation were also studied and the results indicated that LC_{50} value of green prickly ash essential oil was smaller than that of red prickly ash essential oil after the same fumigation time. The objective of this research was to provide reference data for using essential oils of *Zanthoxylum bungeanum* to control stored grain insect pests.

Key words: essential oil of *Zanthoxylum bungeanum*, *Sitophilus zeamais*, fumigant activities

With the increasing concerns to ecological environment, food safety and human heath, the stored product insect pests control approaches must be safe, high efficient and environmentally friendly. Because botanic insecticides possess the advantages of low mammalian toxicity, easy degradation and less possibility to develop resistance for pests, the researchers in the world are trying their best to develop insecticides from the botanic resources (Shaaya et al., 1997; Zhang et al., 2004). Prickly ash tree, *Zanthoxylum bungeanum*, which belongs to *Zanthoxylum* Lin., Rutaceae, is widely grown in southwest China, especially in Sichuan Province and Chongqing municipality. The dried fruits of *Zanthoxylum bungeanum* are important condiment and traditional Chinese medicine (Sun and Duan, 1996). Volatility of *Zanthoxylum bungeanum* enssential oil and its monomers is strong and its scent is easily emitted. After the grains are treated, the scent residue can be gradually emitted. Hence, it is a promising resource of insecticide and fungicide (Jiang et al., 1992; Liu et al., 1994; Lu et al., 1995). In recent years, some researchers have been conducting experiments to use *Zanthoxylum bungeanum* to develop new botanic insecticide.

Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a cosmopolitan pest of stored products and it was reported as the most important pest on stored maize in many countries including China (Yang et al., 1993; Zhang et al., 1998). The control of *S. zeamais* mainly depends on chemical approaches. The main chemicals used at present are phosphine and methyl bromide. However, *S. zeamais* has developed high resistance to phosphine and methyl bromide will be phased out in China in 2015 due to its ozone depleting (Wang et al., 1998). In order to solve the problems of chemicals, people are more and more interested in developing botanical insecticides, which have become research focuses in many countries (Zhang et al. 2004). The fumigation activities of green prickly ash essential oil and red prickly ash essential oil against the adults of *Sitophilus zeamais* were reported in this paper and the objective of this research was to provide reference data for using essential oils of *Zanthoxylum bungeanum* to control stored grain insect pests.

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Material and Methods

Insects. *Sitophilus zeamais* was maintained at Chongqing Key Laboratory of Entomology & Insect Control Engineering, Southwest University, Chongqing, China. The insects were reared on wheat in 1 - liter glass jars at 30℃ ± 0.5℃, 70% - 80% RH, and a photoperiod of 0:24 (L:D) in the laboratory.

Essential Oils

Green prickly ash essential oil (85% AI) and red prickly ash essential oil (85% AI) were provided by Mountain Simian Prickly Ash Company of Jiangjing, Chongqing.

Effect of Temperature and Essential Oil Concentration on Toxicities

The 1L glass jars were used in the fumigation. At 20, 23, 26, 29, 32 and 35℃, adults were fumigated 24 hours under the green prickly ash essential oil concentrations of 6, 9, 12, and 15μL/L and red prickly ash essential oil concentrations of 10, 13, 16, 19μL/L. 30 adults of mixed age were used for each treatment. Adults were placed in glass jar. Filter paper slip (1cm × 15cm) was glued to jar lid at one side. The filter paper of the other side was applied with quantitative essential oils with micropipette and was quickly placed in the glass jar and the plastic film was used to wrap the glass jars to make them sealed. Put jars in the incubator which was set at certain temperature and 24 h dark. Each treatment has 3 replications. Contrasts were set up without essential oil. Mortality was checked after 24 h fumigation.

LC_{50} Values of 2 Prickly Ash Essential Oils on S. zeamais under Different Fumigation Time

The same fumigation method as above was adopted. Fumigation time were 24, 36, 48, 60, 72 h, respectively, and fumigation temperature was 30℃. At 24h fumigation time, the concentrations of green prickly ash essential oil were 6.5, 7.5, 8.5, 9.5, 10.5, 11.5 μL/L and the concentrations of red prickly ash essential oil were 12.5, 13.5, 14.5, 15.5, 16.5, 17.5 μL/L, respectively. At 36 h fumigation time, the concentrations of green prickly ash essential oil were 6, 7, 8, 9, 10, 11 μL/L and the concentrations of red prickly ash essential oil were 9, 10, 11, 12, 13, and 14 μL/L, respectively. At 48 h fumigation time, the concentrations of green prickly ash essential oil were5, 6, 7, 8, 9, and 10 μL/L and the concentrations of red prickly ash essential oil were 9, 10, 11, 12, 13, 14μL/L, respectively. At 60 h fumigation time, the concentrations of green prickly ash essential oil were5, 6, 7, 8, 9, 10 μL/L and the concentrations of red prickly ash essential oil were 8, 9, 10, 11, 12, 13 μL/L, respectively. At 72 h fumigation time, the concentrations of green prickly ash essential oil were 2, 3, 4, 5, 6, 7 μL/L and the concentrations of red prickly ash essential oil were 4, 5, 6, 7, 8, and 9 μL/L, respectively. These concentrations were chosen to make the mortality from 16% to 84%. Each treatment had 30 adults, 3 replications.

Data Analysis

Insect mortality were corrected by using Abbott's formula (Abbott, 1925). Mortality data were transformed using arcsine ($x^{0.5}$) and ANOVA was carried out using SPSS software. Duncan's multiple range tests was used to test the difference significance and IRM software (developed by Southwest University) was used to obtain LC_{50} values and regression equations.

Results

Effect of Temperature and Prickly Ash Essential Oil Concentration on the Activities

Under the condition of 24 h fumigation time and different temperature, the activities of green prickly ash essential oil and red prickly ash essential oil against adults of *S. zeamais* were listed in Table 1 and Table 2. Table 1 and Table 2 showed the corrected mortality increased significantly as the temperature and essential oil concentration increased. At 29, 32, and 35℃, with the green ash essential oil dose of 12 and 15μL/L, the corrected mortalities reached up to 100% after 24 h fumigation. Meanwhile, for red prickly ash essential oil, when the essential oil concentrations were 16 and 19μL/L and the temperature was higher than 26℃, the corrected mortalities were also 100%. ANOVA showed essential oil, temperature and essential oil concentration affected the corrected mortality significantly (for green prickly ash essential oil, its temperature factor: $F = 34.287$; df = 5, 48; $P = 0.000$; concentration factor: $F = 129.004$; df = 3, 48; $P = 0.000$; temperature × concentration interaction: $F = 5.600$; df = 15, 48; $P = 0.000$; for red prickly ash essential oil, its temperature factor: $F = 65.260$; df = 5, 48; $P = 0.000$; concentration factor: $F = 162.712$; df = 3, 48; $P = 0.000$; temperature × concentration interaction: $F = 4.345$; df = 15, 48; $P = 0.000$).

Table 1. The activities of green prickly ash essential oil against adults of *S. zeamais* Under different temperatures (24 h)

Temperature(℃)	Corrected mortality (%) (mean ± SE)			
	6μL/L	9μL/L	12μL/L	15μL/L
20	13.20 ±5.00 a	48.33 ±10.68 a	91.67 ±3.53 a	99.00 ±1.00 ab
23	25.67 ±4.67 ab	77.67 ±12.45 b	90.00 ±0.00 a	94.67 ±2.33 a
26	31.67 ±1.33 b	73.20 ±7.91 ab	91.27 ±2.98 a	95.67 ±4.33 ab
29	46.67 ±4.98 bc	93.23 ±2.11 bc	100.00 ±0.00 b	100.00 ±0.00 b
32	68.33 ±5.21 c	96.67 ±1.33 c	100.00 ±0.00 b	100.00 ±0.00 b
35	91.33 ±8.67 d	100.00 ±0.00 c	100.00 ±0.00 b	100.00 ±0.00 b
F	20.484	8.649	18.733	2.271
Df	5,12	5,12	5,12	5,12
P	0	0.001	0	0.114

Note: The data shows the average of three duplicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

Table 2. The activities of red prickly ash essential oil against adults of *S. zeamais u*nder different temperatures (24 h)

Temperature(℃)	Corrected mortality (%) (mean ± *SE*)			
	10μL/L	13μL/L	16μL/L	19μL/L
20	23.33 ±6.89 a	51.67 ±5.55 a	66.33 ±6.67 a	82.33 ±5.33 a
23	38.33 ±2.73 ab	75.00 ±6.56 b	95.67 ±1.33 b	99.00 ±1.00 b
26	47.33 ±7.84 c	78.33 ±6.77 bc	100.00 ±0.00 c	100.00 ±0.00 b
29	62.33 ±5.24 cd	91.00 ±1.00 c	100.00 ±0.00 c	99.00 ±1.00 b
32	69.33 ±8.41 d	88.67 ±1.33 c	100.00 ±0.00 c	100.00 ±0.00 b
35	93.00 ±2.00 e	100.00 ±0.00 d	100.00 ±0.00 c	100.00 ±0.00 b
F	15.365	21.425	49.989	12.871
df	5,12	5,12	5,12	5,12
P	0	0	0	0

Note: The data shows the average of three duplicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

LC_{50} values of 2 prickly ash essential oils on *S. zeamais* under different fumigation times

The LC_{50} values of essential oils from both green prickly ash and red green prickly ash decreased as the fumigation time increased (Table 3 and 4). For green prickly ash essential oil, LC_{50} values after 24 and 72 h fumigation were9.88 μL/L and 2.24 μL/L, respectively. The LC_{50} value was 4 times shorter at 72 fumigation time than that at 24 h fumigation time, which demonstrated green prickly ash essential oil possessed a longer residue period. Similarly, for red prickly ash essential oil, the longer the fumigation time, the better the fumigation efficacy. Hence, the essential oil amount could be relatively reduced as the fumigation time was increased. In addition, based on the Table 3 and Table 4, we found that LC_{50} of green prickly essential oil was smaller than that of red prickly ash essential oil after the same fumigation time, which proved that the efficacy of green prickly ash essential oil was better than that of red prickly ash essential oil.

Table 3. The fumigation activities of green prickly ash essential oil against adults of *S. zeamais u*nder different treatment time (30℃)

Treatment Time (h)	Regression Equation (Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
24	−3.25 +8.29X	0.994	1.258 *	9.88 ±0.18	15.61 ±0.82
36	0.17 +5.52X	0.993	1.373 *	7.50 ±0.20	14.91 ±0.90

Treatment Time (h)	Regression Equation(Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
48	-0.71 + 6.59X	0.986	3.258 *	7.34 ± 0.15	13.05 ± 0.74
60	-1.76 + 8.55X	0.996	1.029 *	6.17 ± 0.12	9.61 ± 0.32
72	3.88 + 3.19X	0.989	1.695 *	2.24 ± 0.16	7.35 ± 0.66

Table 4. The fumigation activities of red prickly ash essential oil against adults of *S. zeamais* under different treatment time(30℃)

Treatment time (h)	Regression Equation(Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
24	-9.20 + 12.41X	0.982	3.652 *	13.94 ± 0.17	18.92 ± 0.52
36	-3.92 + 8.16X	0.979	4.630 *	12.41 ± 0.19	18.73 ± 0.97
48	-3.29 + 7.91X	0.966	5.526 *	11.15 ± 0.19	18.00 ± 0.99
60	-2.41 + 7.46X	0.971	4.960 *	9.86 ± 0.18	16.38 ± 0.89
72	2.71 + 3.79X	0.987	1.451 *	4.02 ± 0.26	10.93 ± 1.00

Discussion

China is the largest country to grow *Zanthoxylum L.* in the world. Except for northeast and inner Mongolia, it is widely cultivated in many provinces such as Henan, Shangxi, Gansu, Hunan, Hubei, Yunan, Guizhou, Sichuan, Chogqing etc. (Zheng, 2000; Bi et al., 2002, 2003). Among the prickly ash growing provinces, Chongqing and Sichuan are the most famous places for prickly ash production, because people in these two places like hot and spicy food, in which the dry fruits of prickly ash are the most important condiments. *Zanthoxylum* L. as a traditional Chinese medicine has a long history. People used the dry fruits and other part of prickly ash tree to cure different diseases (Shi et al., 2003). As to insecticidal and fungicidal activities, Chinese people like to place the dry fruits and leaves of prickly ash in their stored grains to control insect and mold pests. Therefore, the research on insecticidal activity of prickly ash is focused on stored insect pests. Ge et al. (1995) proved that prickly ash essential oil caused *S. cerealella* to refuse to feed and inhibited oviposition. Dube et al. (1990) found that essential oil of prickly ash possessed fungicidal and repellent activities. He et al. (1980) reported that the bags dipped in water containing prickly ash dry fruits could make the paddy rice over summer without any insect and mold problems. Lu et al. (1995) separated and identify the components of volatile oil and found β - phellandrene and linalool could kill adults of *Tribolium confusum*. Jiang et al. (1992) demonstrated that essential oil of prickly ash could be used as a fumigant to control *Sitophilus zeamais*. Our research indicated that at 29, 32, and 35℃, 12μL/L and 15μL/L doses of green prickly ash essential oil led to 100% corrected mortality after 24 h fumigation. Meanwhile, red prickly ash essential oil caused 100% corrected mortality at 16 and 19μL/L essential oil dosage under the same temperatures. Our result is in agreement with that of Jiang et al. (1992). We couldn't make sure the components to be acted as insecticide in prickly ash essential oils. Whether β - phellandrene or linalool function as insecticidal components in prickly ash essential oils need to be further studied.

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