0212

Toxicity of Ethyl Formate on Adults of *Liposcelis entomophila*

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Abstract: The fumigation activities of ethyl formate (EtF) against adults of *Liposcelis entomophila* (Enderlein) were researched by the sealed-jar fumigation method under the conditions of different EtF dosages, temperatures and exposure times in laboratory. The results indicated EtF dosage, temperature and exposure time affected the fumigation efficacy significantly. EtF showed a high fumigation activity within short exposure time and EtF performed better at relatively low temperature than at relatively high temperature. At 20℃, 25℃ and 30℃ after 24 h fumigation, the LC_{50} values were 7.769、8.964 and 9.900 μL/L, respectively.

Key words: ethyl formate, *Liposcelis entomophila*, fumigation activities

Liposcelis spp, also named as booklice, paperlice and psocids, is a kind of small insect which belongs to Liposcelididae, Psopotera. *Liposcelis* spp has been becoming one of the most important stored product insect pests in the tropical and subtropical regions all over the world (Rejendran, 1994; Chen et al., 2003). The five important species among *Liposcelis* in the world are *Liposcelis bostrychophila* Badonnel, *L. entomophila*, *L. paeta* Pearman, *L. decolor* (Psocoptera), and *L. pearmani* Lienhard. Economic importance of *Liposcelis bostrychophila* and *L. entomophila* was reported in many Asian countries, such as China, India, Thailand, Philippine, Singapore, Malaysia, and Indonesia (Leong et al., 1995; Wang et al., 1999). There are five kinds of booklice damage (Chen et al., 2003). The first is booklice's direct feeding, which cause weight and quality losses of stored grain. The germination rate of wheat seed decreased significantly as the result of booklice damage. 4% – 5% weight loss of stored paddy rice was suffered because of psocid occurrence. Secondly, dead psocid, excretion, ecdysis, and psocid fragment contaminated the stored grain and other stored products. Thirdly, psocid was the vector of some diseases and it was a potential anaphylactogen, which led to skin allergic action for the allergic people. Fourthly, high population density of psocid cause grain moisture increase, temperature increase and grain molding. Fifthly, high psocid population density caused the psychological pressure for most people and also made the people feel uncomfortable. In Europe, existence of psocids in food production facility was the reason of consumer's complaint (Tuner, 1987). In Australia, psocid was reported as small bugs vs. big problems (Reuss et al., 1994). In China, *Liposcelis bostrychophila* and *L. entomophila* had become the dominant species in "Two Low Storage of oxygen and phosphine" and "Three Low Storage of oxygen, phosphine, and temperature" national warehouses (Wang et al., 1999).

The main chemicals to control psocids are phosphine and methyl bromide. But Resistance of psocids to chemicals was very serious because of irrational chemical use. Due to small psocid size and strong resistance to chemicals, it was easy to neglect its existence. Hence, it is becoming more and more difficult to control them. Furthermore, Methyl bromide has been phased out in 2005 in developed countries and will be phased out in 2015 in developing countries including China, so it is urgent to find new fumigants to be used as alternatives to methyl bromide and phosphine. Ethyl formate (EtF) is a promising and environmental friendly fumigant (Muthu et al., 1984; Ren et al. 2000; Damcevski et al., 2000), which was registered as dry fruit fumigants in 2002 in Australia (Ren et al. 2003, 2006; Damcevski et al., 2006). The purpose of this research was to evaluate the fumigation activity of EtF on the adults of *L. entomophila* under the conditions of different temperature, fumigation time and EtF concentration and expected to provide data information for developing EtF as an alternative of Methyl bromide and phosphine to control *L. entomophila*.

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Material and Methods

Insect. *L. entomphila* Badonnel was collected from simulative warehouse at Chongqing Key Laboratory of Entomology & Insect Control Engineering, Southwest University, Chongqing, China. The insects were reared on the mixture of whole wheat flour, brewer's yeast and milk powder(10∶1∶1) in 1 – liter glass jars at 27℃ ±0.5℃、75% – 80% RH、and a photoperiod of 0∶24(L∶D) in the laboratory.

Ethyl Formate

Ethyl formate (AI > 98.00%) was produced by Shanghai Chemical Reagent Group of China.

Effect of Temperature and EtF Concentration on Toxicity

1L glass jars were used in the fumigation. At 16, 19, 22, 25, 28, 31 and 34℃, booklice adults were fumigated 24 hours with EtF concentrations of 7, 9, 11 and13 μL/L. 30 adults of 24h old age were placed in a plastic box(d = 2 cm, h = 1 cm) for each treatment and then the plastic box wrapped with nylon gauze was placed at the bottom of the 1 000mL jars. The filter paper with quantitative EtF was placed in the glass jar and the Plastic film was used to seal the glass jars. At last the jars were put intoan incubator which was set at certain temperature and all dark. Each treatment has 3 replications. Controls were set up without EtF fumigation. Mortality was checked 24h after fumigation.

Effect of Fumigation Time and EtF Concentration on the EtF Efficacy

Same fumigation method as abovewas adopted. At 30℃, the EtF concentrations were 7, 9, 11, 13μL/L and fumigation times were 12, 24, 36, 48 and 60 h, respectively. Each treatment has 30 adults, 3 replications. Mortality was checked after fumigation.

LC_{50} Values of EtF on Liposcelis entomophila

The same fumigation method as the above in 1.2.1 was adopted. Fumigation time was 24 h and fumigation temperatures were 20, 25, and 30℃, respectively. At each temperature, 5 to 7 concentration levels were set up as follows. At 20℃, the concentration levels were 7, 7.5, 8, 8.5, 9, 9.5, 10μL/L, respectively. At 25℃, the concentrations were 8, 9, 10, 11, 12, 13μL/L, respectively. At 30℃, the concentrations were 7, 8, 9, 10, 11, 12 μL/L, respectively. These concentrations related temperatures were chosen to make the mortality from 16% to 84%. Each treatment had 30 adults, 3 replications.

Data Analysis

All the data concerning 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 EtF Concentration on the Activities of EtF

The fumigation activities of EtF against L. entomphila under different temperatures and EtF concentrations after 24 h fumigation were listed in table1. Table 1 showed that EtF demonstrated relatively good activities on the adults of L. entomphila. At 19℃, the best effectiveness of EtF against the adults of L. entomphila. was obtained. Betweent16 and 22℃, the corrected mortalities reached up to 90% at 11 μL/L and 100% mortality was obtained when the EtF concentration was 13 μL/L. The corrected mortality decreased as temperature increased when the temperature ranged from 19 to 34℃, which indicated that the EtF efficacy was better at relatively low temperature than at relatively high temperature. Two-way ANOVA showed the effects of temperature, EtF concentration, and temperature EtF concentration interaction on the corrected mortalities were significant (for temperature: $F = 55.338$, $df = 6, 56$, $P = 0.000$; for concentration: $F = 265.059$, $df = 3, 56$, $P = 0.000$; for temperature EtF concentration interaction: $F = 3.726$, $df = 18, 56$, $P = 0.000$).

Table 1. The fumigation activities of EtF against *L. entomphila* under different temperatures(24 h)

Temperatures(℃)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
16	34.33 ±8.69 c	77.00 ±1.53 de	98.33 ±1.67 e	100.00 c
19	31.00 ±5.86 bc	86.77 ±1.94 e	99.00 ±1.00 e	100.00 c
22	27.67 ±2.67 bc	80.00 ±5.00 e	93.33 ±3.33 de	100.00 c

Temperatures(℃)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
25	22.57 ±2.57 abc	62.33 ±6.23 cd	84.33 ±1.33 cd	99.00 ±1.00 c
28	16.67 ±1.93 ab	54.43 ±2.94 bc	70.00 ±5.13 bc	84.33 ±4.67 b
31	13.33 ±1.93 a	40.67 ±1.86 b	66.67 ±5.24 b	76.67 ±10.53 b
34	23.00 ±4.16 abc	25.33 ±7.67a	45.00 ±2.89 a	50.00 ±5.77 a
F	3.055	18.817	24.435	22.120
Df	6,14	6,14	6,14	6,14
P	0.04	0.000	0.000	0.000

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

Effect of Fumigation Time and EtF Concentration on the EtF Efficacy

Two-way ANOVA showed fumigation time and EtF concentration affected the corrected mortalities significantly (for fumigation time: $F = 12.975$; $df = 4, 40$; $P = 0.000$; for concentration: $F = 71.687$; $df = 3, 40$; $P = 0.000$), but the effect of the fumigation time × EtF concentration interaction on the corrected mortality was not significant ($F = 0.651$; $df = 12, 40$; $P = 0.785$) at 30℃. When the EtF concentrations were the same, the corrected mortality increased as the fumigation time increased (Table 2). Under the condition of 60 h fumigation time and 13 μL/L dose, the corrected mortality was 100%. Meanwhile, when the fumigation time was the same, the efficacy improved as the EtF concentration increased.

Table 2. The fumigation activities of EtF against *L. entomphila* under different time and concentration (30℃)

Treatment time(h)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
12	10.00 ±2.89 a	36.67 ±10.91 a	40.17 ±10.33 a	67.67 ±4.63 a
24	18.00 ±6.01a	40.67 ±2.03 ab	60.00 ±10.15 ab	85.00 ±7.57 b
36	13.33 ±3.33 a	41.67 ±1.67 ab	72.67 ±1.45 bc	89.33 ±2.33 b
48	15.67 ±2.96 a	67.67 ±9.02 ab	76.33 ±2.03 bc	95.67 ±2.96 bc
60	28.33 ±6.01a	70.67 ±10.35 ab	86.67 ±3.76 c	100 c
F	0.797	2.755	6.562	9.897
df	4,10	4,10	4,10	4,10
P	0.554	0.088	0.007	0.002

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

LC_{50} Values of EtF against Adults of *L. entomphila*

LC_{50} values of EtF against adults of L. entomphila at 20, 25, 30℃ were listed in table 3. Table 3 showed LC_{50} value was smaller at 20℃ than that at 25 and 30℃, which demonstrated the efficacy of EtF at 20℃ against this booklice was better than that at 30℃. Based on the regression equations, the relatively big rates of slope proved the susceptibility of the adults of L. entomphila was consistent and the improvement of fumigation efficacy could be obtained by increasing EtF concentration.

Table 3. The LC_{50} Values of Ethyl Formate against Adults of *L. entomphila* at Different Temperatures

Temperatures (℃)	Regression equation (Y =)	R	X^2	LC_{50}/μL · L^{-1}	LC_{95}/μL · L^{-1}
30	−1.801 +6.8304x	0.962	6.636 *	9.900 ±0.20	17.237 ±1.18
25	−6.656 +12.139x	0.977	5.297 *	8.964 ±0.13	12.246 ±0.28

Discussion

Ethyl formate, as an old fumigant, has been used as a fumigant for dried fruits for many years (Ren, 2006). For the past few years, the phase out of methyl bromide and resistance of stored product insect pests to phosphine drove reevaluation of EtF efficacy. Muthu (1984), Hilton and Banks (1997) reported that EtF could control stored product insect pests effectively. The researchers in Australia used EtF to fumigate stored wheat and sorghum in unsealed conditions and their results show EtF killed insect pests within short time period. Damcevski and Annis (2000, 2006) studied the fumigation efficacies of EtF on *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium confusum* in the laboratories and the results also demonstrated that EtF had satisfactory fumigation activities in a short fumigation time. The toxicities varied with the stored product insect pest species. In China, Tang et al. (2006) researched the fumigation activities of EtF against *Sitophilus oryzae* and *Tribolium castaneum* in the laboratory and he proved that EtF killed insects in a short time and the toxicities of EtF were better at relative low temperature than at relative high temperature. Our research also showed that EtF killed most psocids in 24 hour fumigation time at 11μL/L and 13μL/L dosage, especially at 13μL/L dosage. Our results about EtF quick killing insect pests were in consistent with that of Tang and other researchers from Australia. Temperature is an important factor to affect the efficacy of EtF. We found that the efficacy of EtF was much better at 16, 19, and 22℃ than that at 31 and 34℃, which demonstrated that EtF functioned much more effectively at relatively low temperatures. This finding was also in agreement with that of Tang et al., who tested the fumigation activities of EtF on *Sitophilus oryzae* and *Triboium castaneum*. We speculated that EtF decomposed quicker at higher temperature than at lower temperature. Contrary to phosphine fumigation, in which its toxicity increased with temperature, we thought that EtF was more suitable for the fumigation at relatively low temperature. Our data about EtF fumigation activities was obtained under the condition of empty jar and could be used as reference data for empty warehouse fumigation to control *L. entomphila*. In the real warehouse filled with stored grains, many factors such as the grain species, bulk grain height, insect species and its developmental stage, and other environmental factors affects the fumigant efficacy. These factors that affect the ethyl formate efficacy need to be further researched in the future.

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