Fumigation activities of ethyl formate on different strains of *Liposcelis bostrychophila*

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DOI: 10.5073/jka.2010.425.301

Abstract

The psocid, *Liposcelis bostrychophila* is a prevalent insect pest in large grain depots in P.R. China. Our previous research proved that ethyl formate killed psocid adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature. In this paper, fumigation activities of ethyl formate on DDVP and PH_3 resistant strains of *L. bostrychophila* were studied by the sealed jar fumigation method under different ethyl formate concentrations, fumigation times, and temperatures. Results showed that treatment time and concentration significantly affected fumigation effectiveness of ethyl formate against *L . bostrychophila* adults at 30°C. The 50% lethal concentrations (LC_{50}) of ethyl formate against two resistant strains increased as the temperature increased from 20° to 30°C. At 27±0.5°C and 24 h fumigation, the LC_{50} were significantly lower for DDVP and PH_3 resistant strains than that of the susceptible strains and the LC_{50} of DDVP resistant strains were significantly lower than that of PH_3 resistant strains.

Keywords: Fumigation activity, Ethyl formate, *Liposcelis bostrychophila*, Resistant strain

1. Introduction

The psocid, *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae)has a worldwide distribution and is commonly found in various processed and unprocessed dry foods in households, granaries, and warehouses. Apart from causing measurable damage to stored grain (Rees and Walker, 1990), infestations of *L. bostrychophila* can also cause health problems among storage and warehouse workers (Sidik et al., 1986). In P. R. China, *L. bostrychophila* has posed an alarming threat to stored grains, especially in the storage facilities where CA and insecticide combined treatments are commonly practiced (Wang, 1997; Wang et al.,1999). The main chemicals to control *L. bostrychophila* are phosphine and methyl bromide, but resistance of *L. bostrychophila* to chemicals was very serious because of chemicals abuse (Chen et al., 2003). Due to its small size and strong resistance to chemicals, it was easy to miss its presence. Hence, it is becoming more and more difficult to control. Furthermore, methyl bromide will be phased out in the year 2015 in China, so it is urgent to find new fumigants to be as the alternatives of methyl bromide and phosphine. Ethyl formate (EtF) is a promising and environmental friendly fumigant, which was registered as dry fruit fumigants in 2002 in Australia (Ren and Mahon, 2006). The previous research proved that EtF killed *L. bostrychophila* adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature (Li et al., 2006). The purpose of this research was to evaluate the fumigation activity of EtF on the adults of the DDVP and PH_3 resistant strains of *L. bostrychophila* at different temperatures, fumigation times and EtF concentrations to provide data for developing EtF as an alternative of phosphine to control resistant strains of *L. bostrychophila.*

2. Materials and methods

2.1. Insects

Stock colonies of susceptible strain of *L. bostrychophila* were collected from a simulative warehouse at Chongqing Key Laboratory of Entomology & Pest Control Engineering, Southwest University, Chongqing, China. The insects were reared on an artificial diet consisting of whole wheat flour, brewer's yeast and milk powder (10:1:1) in a temperature controlled room at 27 ± 0.5°C, in complete darkness. Cultures were set up in glass bottles (250 mL) with a nylon screen cover and kept in desiccators (5000 mL), in which the relative humidity (r.h.) was controlled with saturated NaCl solution to maintain

75-80% r.h.. The resistant strains of *L. bostrychophila* to dichlorvos (DDVP-R) and phosphine (PH_3-R) were created as follows: At monthly interval, the booklice were treated with DDVP acetone solution of appropriate concentrations and about 75% mortality was maintained in each treatment. After 85 treatments, the strain that exhibited 22.36-fold resistance to DDVP was obtained and used as the DDVP-R. As to phosphine resistant strain, at monthly intervals, the booklice was treated with phosphine and about 75% mortality was maintained each time. After 85 treatments, the strain with 4.51-fold resistance was regarded as booklice resistance strain to phosphine (PH_3-R).

2.2. Fumigants

Ethyl formate (>98.00%) was produced by Shanghai Chemical Reagent Group of China. Dichlorvos 80% (DDVP) was from Chongqing Pesticides Co. and phosphine (Aluminium phosphide >56%) from Shangdong Jining Pesticide factory.

2.3. Effect of fumigation time and EtF concentration on the efficacy of EtF against L. bostrychophila

The 1-L glass jars were used in the fumigation. At 30°C, the EtF concentrations of 10, 12, 14 and 16 µL/L, and fumigation times of 12, 24, 36, 48 and 60 h were adopted. The detailed methods were as follows. Thirty 5-day-old adults were collected for each treatment. Adults were placed in a plastic box (diameter 2 cm, Length 1 cm), wrapped in nylon gauze and then the wrapped plastic box was placed at the bottom of a 1-L jar. The filter paper containing a known quantity of EtF was placed in the glass jar and a plastic film was used to seal the jar. The jars were placed in the incubator set at controlled temperature in the dark. Each treatment was replicated three times. Controls groups were kept without EtF fumigation. Mortality was checked after fumigation.

2.4. LC_{50} of L. bostrychophila at different fumigation time and temperatures

The same fumigation method as described in section 1.2.1. was adopted. The fumigation times were designed as 24 and 48 h. The temperatures were 20°, 25° and 30°C, respectively. Five to seven EtF concentrations were used for each resistant strain. For DDVP resistant strain, for 24 h exposure time at 20°C, EtF concentrations were 6, 7, 8, 9, 10, 11 and 12 µL/L; at 25°C, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L; at 30°C, EtF concentrations were 8, 9, 10, 11, 12 and 13 µL/L. For 48 h exposure time, at 20°C, EtF concentrations were 6.5, 7, 7.5, 8, 8.5, 9 and 9.5 µL/L; at 25°C, EtF concentrations were 7, 8, 9, 10, 11 and 12 µL/L; at 30°C, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L. For PH_3 resistant strain, under 24 h fumigation, at 20°C, EtF concentrations were 7, 7.5, 8, 8.5, 9, 9.5 and 10 µL/L; at 25°C, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L; at 30°C, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L. Under 48 h fumigation, at 20°C, EtF concentrations were 6, 6.5, 7, 7.5, 8, 8.5 and 9 µL/L; at 25°C, EtF concentrations were 9, 9.5, 10, 10.5, 11, 11.5 and 12 µL/L; at 30°C, EtF concentrations were 11, 12, 13, 14, 15, 16 and 17 µL/L. EtF concentration designs were based on the corrected mortalities of 16% ~ 84%. Each treatment had three replications.

2.5. LC_{50} comparison of L. bostrychophila strains to ethyl formate

The same fumigation method as the above in 1.2.1. was adopted. Under the conditions of 27±0.5°C and 24 h fumigation, LC_{50} were measured for DDVP and PH_3 resistant and susceptible strains. For DDVP-R, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L. For PH_3-R, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L. For susceptible strain, EtF concentrations were 11, 12, 13, 14, 15, 16 and 17 µL/L. Each treatment had three replications.

2.6. 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.

3. Results

3.1. Effect of fumigation time and EtF concentration on the EtF efficacy

Under the conditions of different fumigation time and EtF concentration, the toxicities of EtF against *L. bostrychophila* DDVP resistant strain were listed in Table 1. The corrected mortality increased gradually as the fumigation time increased at fix EtF concentration. The concentration of 14 µL/L EtF led to 90%

corrected mortality within 36 h of fumigation. When the fumigation time was the same, high EtF concentration increased EtF toxicity against *L. bostrychophila*. The concentration of 16 µL/L of EtF caused more than 90% corrected mortality within 24 h fumigation and 100% corrected mortality within 48 h fumigation. Two-way ANOVA showed fumigation time affected the corrected mortality of *L. bostrychophila* DDVP resistant strain significantly (F = 165.175; df = 4, 40; P = 0.0000), so did the EtF concentration (F = 256.835; df = 3, 40; P = 0.000). However, the interaction between EtF concentration and fumigation time influenced the corrected mortality insignificantly (F = 1.297; df = 12, 40; P = 0.258).

Table 1 Effectiveness of ethyl formate against *L. bostrychophila* of DDVP-R strain at different exposure times and concentrations at 30°C.

	Corrected mortality (%)			
Treatment time (h)	**10µL/L**	**12µL/L**	**14µL/L**	**16µL/L**
12	14.47±2.23 a	33.00±5.77 a	46.67±3.33 a	60.33±3.33 a
24	41.33±4.33 b	68.00±1.00 b	82.00±1.00 b	95.67±2.96 b
36	45.33±3.93 b	76.67±2.03 bc	90.00±1.73 c	97.67±2.33 b
48	56.67±2.03 c	80.00±1.73 c	94.33±1.33 c	100±0.00 b
60	64.67±2.33 c	89.00±1.00 d	100±0.00 d	100±0.00 b
F	40.487	46.222	136.351	26.367
df	4,10	4,10	4,10	4,10
P	0.000	0.000	0.000	0.000

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

The toxicity of EtF against PH_3 resistant strain was listed in Table 2. The efficacy of EtF against *L. bostrychophila* increased as the fumigation time and EtF concentration increased. Fumigation time (F = 35.741; df = 4, 40; P = 0.0000) and EtF concentration (F = 76.757; df = 3, 40; P = 0.000) affected the corrected mortality significantly, but the interaction between them insignificantly (F = 0.841; df = 12, 40; P = 0.635).

Table 2 Effectiveness of ethyl formate against *L. bostrychophila* of PH_3-R strain at different exposure times and concentrations at 30°C.

	Corrected mortality (%)			
Treatment time (h)	**10µL/L**	**12µL/L**	**14µL/L**	**16µL/L**
12	17.67±2.91a	29.00±6.11 a	38.67±5.67 a	62.33±2.33 a
24	19.00±4.16 a	45.33±6.22 ab	62.33±12.33 ab	82.00±6.66 b
36	21.00±2.00 a	51.33±2.96 b	72.00±11.00 bc	83.00±0.00 b
48	32.00±9.00 a	55.67±4.33 b	79.00±6.11 bc	89.00±1.00 b
60	64.33±1.33 b	78.00±6.66 c	91.00±4.16 c	99.00±1.00 c
F	14.324	9.538	5.547	19.152
df	4,10	4,10	4,10	4,10
P	0.000	0.002	0.013	0.000

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

3.2. LC_{50}s of L. bostrychophila at different fumigation time and temperatures

The fumigation toxicity of EtF against DDVP resistant strain was listed in Table 3. The table showed that the LC_{50} increased as the temperature increased. Moreover, the LC_{50} was affected by temperature. Within 24 h exposure time, the LC_{50} value was 7.874 µL/L at 20°C, and the LC_{50} value was 10.18 µL/L at 30°C, which meant the EtF efficacy at lower temperature was more effective than that at high temperature.

Table 3 The LC_{50} of ethyl formate against *L. bostrychophila* of DDVP-R strain at different exposure times and temperatures.

Treatment Time (h)	Temperatures (°C)	Regression equation (Y=)	r	LC_{50}(μL/L)	X^2
24	20	-1.599+7.3650x	0.998	7.874±0.15	3.533*
	25	-5.290+10.327x	0.983	9.920±0.13	6.728*
	30	-4.580+9.5050x	0.994	10.18±0.15	1.150*
48	20	-2.691+8.7970x	0.979	7.486±0.11	3.382*
	25	-4.285+9.5130x	0.991	9.463±0.14	2.574*
	30	-3.194+8.2680x	0.988	9.796±0.15	3.582*

For PH_3 resistant strain, the LC_{50} change tendency was the same as that of DDVP resistant strain (Table 4). Based on the regression equations, we found relatively large slope rates, which meant that the susceptibility of both *L. bostrychophila* resistant strain to EtF was consistent and increasing the EtF concentration could improve fumigation efficacy.

Table 4 The LC_{50} of ethyl formate against *L. bostrychophila* of PH_3-R strain at different exposure times and temperatures.

Treatment Time （h）	Temperatures (°C)	Regression equation (Y=)	r	LC_{50}(μL/L)	X^2
24	20	-8.678+9.976x	0.993	8.678±0.11	1.321*
	25	-7.161+0.145x	0.975	11.91±0.15	8.529*
	30	-0.543+0.398x	0.972	14.13±0.40	2.270*
48	20	-1.905+7.792x	0.967	7.695±0.12	5.043*
	25	-2.993+7.778x	0.991	10.65±0.16	2.589*
	30	-6.207+9.926x	0.981	13.46±0.17	4.624*

3.3. LC_{50} comparison of the L. bostrychophila strains to ethyl formate

Table 5 showed that LC_{50} of DDVP and PH_3 resistant strains decreased significantly compared with the LC_{50} of susceptible strain. Meanwhile, LC_{50} for DDVP resistant strain was obviously smaller than that for PH_3 resistant strain, which explained that the two resistant strains under the DDVP and PH_3 pressure were more susceptible to EtF and EtF could be used to control *L. bostrychophila* DDVP and PH_3 resistant strains.

Table 5 Susceptibility of the L. bostrychophila strains of DDVP-R and PH_3-R and susceptible strain to ethyl formate at 27±0.5°C.

strains	LC_{50} (μL/L)	r	Regression equation(Y=)	X^2
DDVP-R	9.9801±0.14	0.987	-5.590+9.927x	6.547*
PH_3-R	12.141±0.18	0.991	-7.261+1.105x	8.427*
S	13.583±0.19	0.989	-4.645+8.586x	6.746*

4. Discussion and conclusions

The resistance of stored grain insect to fumigants has been increasingly serious since fumigants began to be used (Ding et al., 2002). At present, the widely used fumigants are only PH_3 and methyl bromide, but methyl bromide will be phased out in developing countries by 2015 and PH_3 abuse cause serious resistance problems for main stored product insect pests. Hence, scientists from developed and developing countries all over the world are trying their best to explore new alternatives to control insect pests. EtF, as an old fumigant, which has been used as a fumigant for dried fruits for many years, aroused scientist's interest. It was ever reported that EtF could control stored product insect pests effectively (Muthu et al., 1984; Hilton and Banks, 1997). The researchers at SGRL, CSIRO in Australia used EtF to fumigate stored wheat and sorghum in unsealed conditions and their results showed that EtF killed insect pests within several hours (Ren and Mahon, 2006). The fumigation efficacies of EtF on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) in the laboratories were also studied and the results also demonstrated that EtF had satisfactory fumigation activities in a short fumigation time (Damcevski and Annis, 2006). The toxicities varied with the stored product insect pest species. In China,

Tang et al. (2006 a, b) researched the fumigation activities of EtF against *S. oryzae* and *T. castaneum* in the laboratory and he proved that EtF killed insects in a short time and the toxicities of EtF were better at lower temperatures than at high temperatures. In a previous research Li et al., (2006) showed that EtF killed psocid adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature. This paper proved that EtF also killed DDVP and PH_3 resistant strains booklice in relatively short time period, and temperature and EtF concentration affected EtF efficacy significantly. EtF susceptibility for two resistant strains that LC_{50} were smaller that of susceptible strain and the LC_{50} between the DDVP and PH_3 resistant strains differed significantly. LC_{50} values for DDVP resistant strain were obviously smaller than that of PH_3 resistant strain, which showed EtF could be considered to be the fumigant to control the booklice which had developed resistance to DDVP. The toxicity data of EtF against other insect pests and its mechanism of action need to be further researched.

Acknowledgments

This work was supported in part by the Scientific Research Foundation of State Education Ministry for Returned Oversea Scholars (Grant No. (2009) 1590), and by Natural Science Foundation Project of CQ CSTC, 2009BB0010.

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