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Fumigation Efficacy of Ethyl Formate against *Tribolium Castaneum* (Herbst)

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Abstract: The lethal effects of ethyl formate (EF) on eggs, early larvae, late larvae, pupae and adults of *T. castaneum* were systematically studied in the laboratory under different dosages, treatment times and temperatures. The results indicate that treatment time and temperature significantly affect fumigation efficacy of EF on *T. castaneum*. There was a significantly higher fumigation activity of EF within 48h, and the fumigation efficacy at lower temperatures was better than that at higher temperatures. At 20, 25 and 30℃ and 24h fumigation, the LC_{50} values of adults were 24.24, 27.52 and 29.95 μL/L, respectively; whereas 48h of fumigation produced corresponding LC_{50} values of 22.18, 25.74 and 27.30 μL/L, respectively. EF was most effective against eggs and least effective against pupae. EF was most effective against *T. castaneum* in a simulated wheat storehouse and least effective in a simulated paddy rice storehouse. The corrected mortalities of *T. castaneum* in upper, middle and lower layers of simulated wheat and maize storehouses were 100% with a dose of 70 g/m^3 after 24 h treatment at 30℃, whereas in a paddy storehouse *T. castaneum* can survive at all layers. When the EF dose was 90 g/m^3, the corrected mortality in the upper layer was 100%, in the middle layer 19.3% and in the lower layer it was zero.

Introduction

Grain storage is a key method to use the grain resources properly. However, grain can be infested by insects, mites and other organisms during storage, which cause heavy losses in both quality and quantity[1-3]. *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a worldwide storage pest. It feeds on many kinds of grains and their products, oil crops, dry fruits and herbs. Adults have an odour gland which can cause a mouldy smell when populations are large, causing problems to grain store owners and significant grain loss[4].

Grain storage facilities depend mainly on PH_3 and methyl bromide to control *T. castaneum*. Resistance of stored products insect pests to PH_3 has been recorded. Methyl bromide will be phased out because it depletes the ozone-layer. Scientists around the world are looking for alternatives that overcome insect resistance and that are not ozone depleters [2-5].

Ethyl formate is an environment-friendly fumigant. It is a colorless liquid with a boiling point of 54.1℃ under normal temperatures. It was registered as fumigant for dried fruit in Australia in 2002[6-8].

EF has some inherent advantages as fumigant: firstly, it has wide natural occurrence in a range of foods such as vegetables, fruit, grain and animal products. Secondly, fumigation with EF does not adversely affect product quality or seed germination. Thirdly, it breaks down on the commodity after fumigation rather than being desorbed, and its breakdown products are also naturally occurring components of food[7-10].

This study aimed to understand the fumigant activity of EF on adult and immature stages of *T. castaneum* under different conditions. This research can provide information for using EF commercially for controlling stored product insects as an alternative to methyl bromide.

Materials and Methods

Insects and Ethyl Formate

Insects were obtained from the Applying Insects and Mites Laboratory of SWU (Southwest University, Chongqing, China), and grown on a mixture of whole wheat and brewer's yeast (whole wheat: yeast is 20∶1). One week after oviposition, the adults were removed from the food and the off-spring were grown at 32 ± 1℃, RH75% ± 5%, and 24h dark. Newly emerged and healthy adults were selected for the experi-

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ments.

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

Effect of EF Concentration and Fumigation Time on the Effectiveness

One liter jars were used in the fumigations. At 25℃ the treatment times were 12,24, 36,48 and 60h at EF concentrations of 30,35, 40,45 μL/L,respectively. Adults of *T. castaneum* were placed at the bottom of the 1 000mL jars, then measured amounts of EF were dropped onto the filter paper at the bottom of the fumigation box(d = 2cm, h = 1cm). Nylon gauze was used to wrap the box as quickly as possible so that *T. castaneum* would not make direct contact with EF. Plastic film was used to seal the jars. The jars were incubated in the dark at the designated temperature. Each treatment contained 50 adults,and there were 3 replications. Controls were identical except unfumigated. Mortality was checked after 24h fumigation.

Temperature Effect on the EF Efficacy

The same fumigation method as described above was used. The EF concentrations were 26,28,30,32 μL/L at temperatures of 16,19, 22,25,28,31 & 34℃ respectively. Fumigation time was 24h.

LD_{50} of EF Against *T. castaneum*

The same fumigation method as described above was used. *T. castaneum* adults were treated for 24h at 20℃ at concentrations of 21,22, 23,24,25,26 & 27 μL/L;at 25℃ at 24,25.5, 27,28.5,30,31.5 & 33 μL/L; at 30℃, 26, 28,30,32,34 & 36 μL/L. Fumigation for 48h at 20℃ at concentrations of 20,21,22,23,24, 25 & 26 μL/L;at 25℃ at concentrations of 22, 24,26,28 & 30 μL/L; at 30℃ at concentrations of 22,24,26,28,30 & 32 μL/L. These concentrations and related time/temperature combinations were chosen to achieve mortality from 16% to 84%. Each treatment contained 50 adults and there were 3 replications.

Fumigation Activity Comparison of EF against Immature Stages of *T. castaneum*

The methods used were similar to those used by Obeng-Ofori(2005)[11]. 3000 adults of mixed sex were reared on 8.4kg culture media with whole wheat: yeast = 20: 1. Seven days after oviposition, the parent adults were sieved out. Every 100g of the culture media with eggs were separated and put into jars. Nylon gauze was used to seal the jars which were incubated at 32 ± 1℃. After 1d, the jars were randomly placed into dryers, and the jars with the eggs were fumigated with EF at 20℃ & 30℃ for 24h in the dark in the incubators. Then after 8,16 & 23d, similar experiments were carried out to test the efficacy of EF against early larvae, late larvae and the pupae. Immature insects were taken out and reared in the chamber. The numbers of adults were checked 7 weeks later. Each treatment had three replications. The controls were set up without EF fumigation.

Fumigation Activity of EF to Adults of *T. castaneumn* Simulative Storehouse

The big glass dryers with 18L volume were used as to simulate a storehouse. Ten kg of wheat, 10kg maize and 7.5kg of paddy rice were placed in each dryer respectively. Four stages of *T. castaneum* were put into a special film box, in which both sides were covered with nylon gauze to prevent the insects escaping. The boxes each with 50 insects were assigned to the upper, middle or lower layer in the dryer, which was kept at 30 ± 1℃ for 12h. EF was added to each dryer for 24h. The mortality was checked after fumigation. Each treatment and controls had three 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 test was used to test the difference significance and IRM software(developed by Southwest University) was used to obtain LD_{50} values and regression equations.

Results

Effect of EF Concentration and Fumigation Time on the Activities of EF

EF showed satisfactory results under the EF concentration and fumigation time (Table 1). Corrected mortality increased with the fumigation time for the same concentration of EF. Under the same fumigation time, the corrected mortality also increased as the EF concentration increased. Two – way ANOVA indicated that fumigation time and concentration affected the corrected mortality significantly ($P < 0.01$). Under the conditions of fumigation time of 36h and an EF concentration of 30 μL/L, the corrected mortality was 81.88%. The corrected mortality even reached 93.96% when concentration was 35 μL/L, and the corrected mortality was 100% when the concentration was 40 μL/L. When the concentration reached 45 μL/L, the corrected mortality was 100% after 12h fumigation. This indicated that EF as fumigant killed adults quickly.

Table 1. The fumigation activities of ethyl formate against *T. castaneum* adults in different treatment time and concentrations (25℃)

Treatment time/h	Corrected mortality/%			
	30μL/L	35μL/L	40μL/L	45μL/L
12	60.00 ±7.21 a	78.00 ±5.77 a	92.67 ±1.76 a	100.00
24	70.67 ±2.91 ab	87.33 ±5.21 ab	96.67 ±1.76 b	100.00
36	81.88 ±3.49 bc	93.96 ±2.33 bc	100.00 c	100.00
48	89.19 ±2.44 c	97.97 ±1.17 c	100.00c	100.00
60	87.16 ±4.73 c	98.65 ±0.68 c	100.00 c	100.00
F	6.716	6.373	10.646	–
df	4,10	4,10	4,10	–
P	0.007	0.008	0.001	–

Temperature effect on EF Fumigation Activity

The temperature affected the fumigant activity on adults of *T. castaneum* significantly ($P < 0.01$, Table 2). Lower temperatures can provide better fumigant activity than higher temperatures. The corrected mortality of adults of *T. castaneum* decreased significantly ($P < 0.01$) when temperature increased over the range 16 to 34℃.

Table 2. The effects of treatment temperature on ethyl formate activity (24h)

Temperatures/℃	Corrected mortality/%			
	26μL/L	28μL/L	30μL/L	32μL/L
16	75.52 ±3.13 d	83.67 ±3.53 d	91.84 ±3.12 d	100.00 e
19	69.39 ±4.71 cd	81.63 ±7.07 d	88.44 ±5.93 d	95.24 ±2.97 de
22	59.73 ±3.08 c	71.81 ±3.49 cd	81.88 ±6.97 cd	86.58 ±5.24 cd
25	41.61 ±4.19 b	55.70 ±4.19 bc	67.79 ±4.19 bc	77.85 ±4.65 bc
28	36.00 ±4.62 b	50.67 ±5.21 ab	60.00 ±6.93 ab	65.33 ±2.91 ab
31	30.00 ±5.29 ab	40.00 ±6.43 ab	50.00 ±3.46 ab	58.00 ±8.08 a
34	21.48 ±2.01 a	36.24 ±4.08 a	43.62 ±3.08 a	53.02 ±5.24 a
F	25.110	12.864	10.780	18.535
df	6,14	6,14	6,14	6,14
P	0	0	0	0

LC_{50} Values of EF Against adults of *T. castaneum*

Table 3 shows the LC_{50} values of ethyl format against adults of *T. castaneum* using different fumigation times and temperatures. The LC_{50} value is smaller at lower temperatures than at higher temperatures when treatment time is the same. It confirmed that EF displayed better fumigation activity at lower temperature than higher temperature. LC_{50} value was lower at 48h fumigation time than that under 24 h fumigation time, which indicated that increased fumigation time could improve EF efficacy.

Fumigation Activity to the Immature Stages of T. *castaneum*

Table 4 shows that EF was toxic to the immature stages of *T. castaneum* and its fumigant activity varied significantly ($P < 0.01$) according to life stage. EF was most toxic to eggs and least toxic to pupae. The corrected mortality is higher at 20℃ than at 30℃. A concentration of 35 μL/L led to more than 90% mortality of all stages. Egg and younger larvae were all killed at 20℃. The controls produced more than 800 adults and 1 000 pupae, which also showed the good fumigation activity of EF against immature stages of *T. castaneum*.

Table 3. The LC_{50} values of ethyl format against adults of *T. castaneum*

Time/h	℃	Regression equation Y =	r	LC_{50} (μL/L)	LC_{95} (μL/L)
24	20	−18.24 + 16.79x	0.9785	24.24 ± 0.14	32.20 ± 0.85
	25	−13.27 + 12.69x	0.9974	27.52 ± 0.21	37.08 ± 0.80
	30	−12.77 + 12.03x	0.9862	29.95 ± 0.26	41.03 ± 1.02
48	20	−13.42 + 13.68x	0.9795	22.18 ± 0.16	32.51 ± 1.07
	25	−13.55 + 13.15x	0.9962	25.74 ± 0.22	34.33 ± 0.86
	30	−9.94 + 10.40x	0.9892	27.30 ± 0.27	39.28 ± 1.26

Table 4. Ethyl formate activity against immature stages of *T. castaneum*

Stages	Temperatures (℃)	Mortality/%		
		25 μL/L	30 μL/L	35 μL/L
Eggs	20	87.10 ± 0.63 e	98.08 ± 0.52 f	100.00 f
	30	81.90 ± 0.93 d	93.89 ± 0.62 e	100.00 f
Early larvae	20	83.08 ± 1.07 d	95.18 ± 0.66 e	100.00 f
	30	77.98 ± 1.01 c	90.01 ± 1.02 d	96.94 ± 0.27 d
Late larvae	20	74.01 ± 1.83 c	86.10 ± 1.58 c	98.23 ± 0.31 e
	30	67.35 ± 1.74 ab	81.02 ± 1.49 b	92.28 ± 1.13 b
Pupae	20	69.08 ± 1.79 b	82.41 ± 0.90 bc	95.09 ± 0.55 c
	30	63.30 ± 1.52 a	74.95 ± 2.39 a	90.27 ± 0.88 a
	F	39.936	47.992	135.316
	df	7,16	7,16	7,16
	P	0	0	0

The Fumigation Activities of EF to *T. castaneum* in Simulative Storehouses

The fumigation activities of EF when simulated in a storehouse showed the fumigant was most effective in a wheat storehouse and least effective in a paddy storehouse. The corrected mortalities of *T. castaneum* in the upper, middle and lower layers in the wheat and maize storehouses were 100% with a dose of 70 g/m^3 after 24 h fumigation at 30℃, whereas in paddy storehouse *T. castaneum* survived in all layers. In the paddy rice simulated warehouse, using an EF dose of 90 g/m^3, the corrected mortality in the upper layer was 100%, in the middle layer 19.3% and zero percent in the lower layer.

Discussion

Our results showed that EF is effective for controlling adults of *T. castaneum*. At 40 μL/L for 36h or 45μL/L for 12h, the corrected mortality of adults of *T. castaneum* reached up to 100%. EF showed its rapid activities as a fumigant. EF was more toxic at relatively low temperatures, in contrast to previous publications[citations] which were carried out under different conditions. Further work will be undertaken to confirm our results.

Our research indicated that EF showed good fumigation activity against eggs of *T. castaneum*. Fumigant activity varied according to the insect stage. The susceptibility to EF of *T. castaneum* life stages decreased from the egg, to young larvae, to old larvae and to the pupal stage. The respiration rate of insects is known to be relatively low when they are in the egg and pupal stage and hence the reduced efficacy of common fumigants against eggs and pupa[16]. In contrast to these previous studies, EF was toxic to the egg stage but less toxic to the pupal stage.

Phosphine and methyl bromide are the most commonly used chemicals used today for grain storage disinfestation. However, methyl bromide will be phased out in China because of its damage to the ozone layer and resistance of stored grain insect pests to PH_3 develops fast because of the inappropriate use[2,10]. It is very urgent to find some kinds of new fumigants for the stored product protection to all the researchers. EF is a relatively new chemical, which shows promise as an alternative to PH_3 and methyl bromide[12-15]. Based on the results above, our research indicates that EF can be used as a potential alternative fumigant in grain storage in the future.

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Table 5. The fumigation activity of ethyl formate against adults of T. *castaneum* in a simulated storehouse

	Wheat, Ethyl Formate (g/m^3)			Maize, Ethyl Formate (g/m^3)			Rice, Ethyl Formate (g/m^3)		
	50	60	70	50	60	70	70	80	90
upper	100 b	100 a	100	100 b	100 b	100	91.3 ±5.7 b	99.3 ±0.7 c	100 c
middle	98.7 ±1.3 b	100 a	100	96.0 ±3.1 b	100 b	100	7.3 ±6.4 a	13.3 ±4.8 b	19.3 ±9.3 b
lower	90.6 ±3.3 a	98.7 ± 1.3 a	100	74.0 ±2.3 a	94.7 ± 2.4 a	100	1.3 ±1.2 a	0 a	0 a
F	10.076	1	–	24.220	17.557	–	40.864	219.661	121.245
df	2,6	2,6	–	2,6	2,6	–	2,6	2,6	2,6
P	0.012	0.422	–	0.001	0.003	–	0	0	0

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[34] Table 5 The fumigation activity of ethyl formate against adults of *T. castaneum* in a simulated storehouse