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Toxicity of Ethyl Formate to Three Stored Grain Insects in Absence of Grain

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Abstract: The effects of treatment time, temperature and concentration of ethyl formate (EF) against *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus zeamais* were studied in absence of grain. Some dosages of EF achieved complete kill within 24h in a sealed system. Mortality of each of the three pests decreased with increasing temperature at a fixed dosage. Mortality of *T. castaneum* and *R. dominica* at 16℃ was significantly different to that at 24 and 32℃ ($p < 0.05$). Mortality of *S. zeamais* at 16, 24 and 32℃ were significantly different to each other ($P < 0.05$). The LC_{50} values of *T. castaneum*, *R. dominica* and *S. zeamais* were 25.97, 12.50 and 7.45μL/L respectively with EF over 24h at 24℃.

Key words: Ethyl formate, fumigation, *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus zeamais*

Introduction

Currently, worldwide, methyl bromide and phosphine are the mainly used fumigants. However, methyl bromide has been classified as an ozone-depleting substance under the Montreal Protocol and its use as a fumigant will be phased out before 2015 worldwide [1]. Furthermore, resistance of stored grain pests to phosphine is growing and spreading, the dosage of phosphine and its frequency of use are increasing in response to this resistance, with decreasing quality of the fumigated products and increasing cost of pest control[2]. Developing a new type of fumigation to replace methyl bromide and reducing the dosage of phosphine is a priority in the current plan[3]. Ethyl formate is one of the closely studied candidate replacements in the process [4].

There are many reports on EF as fumigant for dry fruit and stored grain. It was reregistered as a fumigant for dried fruit and nuts in Australia in 2002[5]. Damecevski et al[6]. researched the effect of humidity on the fumigation activity of EF in empty containers. The results showed that r. h. influenced the observed mortality. The higher the r. h., the lower dosage of EF was required to achieve 99% mortality [6]. Tang Peian et al. researched various aspects of the fumigation activity of EF to *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Liposcelis bostrychophila* in empty containers[7-10]. They showed that the sensitivity of different species and different stages of insects to EF were different. The better efficacy of EF was showed at lower temperatures. This was not consistent with previous reports that higher temperatures are better for EF fumigation [2].

T. castaneum, *R. dominica* and *S. zeamais* are the main stored grain pests in China. There are many reports about the efficacy of EF against *Sitophilus oryzae* and *T. castaneum*, but there is little information on the efficacy of EF against *S. zeamais* and *T. castaneum*. Therefore, we chose *T. castaneum*, *R. dominica* and *S. zeamais* as test insects so that our studies could be compared with the previous ones and new data on the efficacy of EF against *S. zeamais* and *T. castaneum* was obtained.

In this paper, we determined the LC_{50} values of EF to *T. castaneum*, *R. dominica* and *S. zeamais* adults at 16℃, 24℃ and 32℃.

Materials and Methods

Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27 ± 1℃ and r. h. 75% ± 5%. *Sitophilus zeamais* was reared on whole wheat, *Rhyzopertha dominicad* on broken wheat, *Tribolium castaneum* on whole wheat flour with 5% yeast. The wheat to be used was sterilised at

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Editor's note: No concentration measurements were taken for EF during the fumigation. It may be that no EF was present after 24h.

80℃ for 2h. The moisture content was then adjusted to 13% ± 1% w. b. Adults (7 – 14days old) were used in this study.

Chemicals

The EF used was analytical grade (≥97% purity), purchased from Tianjin Basifu Chemical Ltd.

Toxicity Studies

Fumigation was carried out in 1L airtight jars. Firstly, 15 test insects were placed in the jar. Secondly, measured quantities of EF were added to a filter paper (311cm) which was glued vertically inside the jar. Thirdly, the jar was closed as soon as possible, and the lid was sealed on with parafilm. Finally, the jar was placed in the incubators at constant temperature and humidity. Each bioassay was carried out in duplicate with five EF dosages and an undosed control.

Fumigations were carried out at 16, 24 and 32℃, with exposure times of time was 24, 48, 60 and 72h.

Statistical Analysis of Data

The mortality results were analysed statistically using SPSS data processing software[11].

Results Mortality of adult insects to EFTables 1, 2 and 3 show the LC_{50} values for EF on *T. castaneum*, *R. dominica* and *S. zeamais*. At the same temperature, LC_{50} values of for each of the three species of insect were not significantly different ($P > 0.05$) at specific treatment times (Tables 1, 2 and 3), showing that EF had a rapid insecticidal ability and suggesting that all the ethyl formate had been lost (destroyed) within 24h.

The LC_{50} values of EF for *T. castaneum*, *R. dominica* and *S. zeamais* were 25.97, 12.50 and 7.45μL/L respectively, for 24h at 24℃, showing that *T. castaneum* was the most tolerant of the three species to EF.

The effect of Temperature to Fumigation activity of EF

Table 4 showed the observed mortalities with selected initial concentrations of EF of the three insects at different temperatures for 24h exposure. Lower mortalities to the selected dosage were observed at higher temperatures. This trend was statistically significant for each of the species tested ($P < 0.05$).

Discussion

Rapid Toxicity of EF

EF has a rapid impact on the test insects, giving high mortality within 24h in the absence of grain. No increase in effectiveness was observed when the exposure was extended to 72 h (Tables 1, 2 and 3). Damcevski et al. [5] studied that the potential of EF for use as a rapid disinfectant giving very high mortality (99%) in adult insects and high mortality (95%) in other stages with a 24h exposure period; and Aharoni et al. [6] found that a 3 h fumigation of grapefruit infested with the California red scale, *Aonidiella aurantii* (Maskell), at a concentration of 1.5% EF resulted in 100% mortality of all stages of the insect. In comparison, fumigation with phosphine needs 7 – 10 days. EF has great potential for grain fumigation[13].

The Effect of Temperature to EF

Generally, fumigants are more effective at higher temperatures. The volatility of the chemical is greater and the respiration of insects is higher. Consequently, it is easier to kill the insects. The experimental results, given here, showed that the efficacy of EF was better at relatively lower temperature in empty containers, which is consistent with the results of Tang et al. [13]. We suggest that the physiological state of insects is poor in the relatively low temperature. Despite the lower volatility of EF, a smaller quantity of the toxicant is effective against the pests. Alternatively, the toxicant may be able to penetrate more easily into the body of the insects through the intersegmental membrane and other parts. The presence of grain may affect the fumigation efficiency of dosages of EF in other ways, such as penetration of EF through the grain bulk, the sorption of EF on the grain and so on. Temperature can directly affect these factors. The effect of temperature on EF toxicity that we observe in an empty container may thus be different from the results observed in the presence of grain by others, where effectiveness increases with temperature. Further research is required.

Table 1. LC_{50} values of EF to *T. castaneum*.

Temperature (℃)	Exposure time (hours)	LC_{50} (μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	17.94 (17.57, 18.11)	0.60	0.54
	48	17.70 (17.31, 18.11)	0.55	0.80

Temperature (℃)	Exposure time (hours)	LC_{50} (μL/L) (95% ConfidenceIntervals)	Slope	P
	60	17.70 (17.31, 18.11)	0.55	0.80
	72	17.44 (16.95, 17.93)	0.43	0.54
24	24	25.97 (24.90, 27.44)	0.19	0.91
	48	25.18 (24.28, 26.29)	0.21	0.97
	60	24.51 (23.63, 25.51)	0.22	0.96
	72	24.36 (23.48, 25.33)	0.22	0.98
32	24	26.16 (24.57, 29.13)	0.15	0.14
	48	25.59 (24.21, 27.85)	0.18	0.11
	60	25.64 (24.12, 28.27)	0.17	0.08
	72	25.53 (24.01, 28.13)	0.18	0.06

Table 2. LC_{50} values of EF to *R. dominica*.

Temperature (℃)	Exposure time (hours)	LC_{50} (μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	10.45 (9.87, 11.12)	0.37	0.64
	48	10.11 (9.56, 10.72)	0.40	0.85
	60	9.83 (9.33, 10.38)	0.45	0.93
	72	9.58 (9.02, 10.21)	0.36	0.57
24	24	12.50 (11.90, 13.22)	0.38	0.42
	48	11.81 (11.22, 12.51)	0.41	0.53
	60	11.81 (11.22, 12.51)	0.41	0.53
	72	11.70 (11.12, 12.37)	0.42	0.63
32	24	16.61 (15.29, 18.11)	0.20	0.12
	48	16.44 (15.11, 17.72)	0.20	0.11
	60	16.23 (14.66, 18.05)	0.16	0.08
	72	15.46 (14.25, 16.72)	0.15	0.51

Table 3. The LC_{50} values of EF to *S. zeamais*.

Temper - ature (℃)	Exposure time (hours)	LC_{50} (μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	6.00 (5.60, 6.41)	0.60	0.21
	48	5.78 (5.38, 6.18)	0.61	0.18
	60	5.70 (5.14, 6.26)	0.60	0.12
	72	5.66 (5.25, 6.06)	0.61	0.27
24	24	7.45 (6.84, 8.03)	0.33	0.29
	48	7.16 (6.54, 7.73)	0.34	0.28
	60	7.01 (6.36, 7.59)	0.33	0.16
	72	6.53 (5.49, 7.35)	0.34	0.06
32	24	9.52 (8.94, 10.19)	0.33	0.60
	48	8.62 (7.74, 9.62)	0.20	0.71
	60	7.65 (5.94, 9.19)	0.17	0.07
	72	6.36 (4.54, 7.56)	0.20	0.10

Table 4. Mortalities to EF of three insects at different temperatures for 24h exposure[a]

Insect species	Fumigant concentration (μL/L)	Mortality		
		16℃	24℃	32℃
Tribolium castaneum	25	100 ± 0.00a	60.00 ± 6.67b	53.33 ± 11.55b
Rhyzopertha dominica	11	55.56 ± 7.70a	17.78 ± 7.70b	6.67 ± 6.67b
Sitophilus zeamais	8	97.78 ± 3.85a	64.44 ± 10.18b	28.89 ± 13.88c

[a] Results are the means ± SD ($n = 3$). Means within rows followed by the same letter are not significantly different ($p > 0.05$, LSD Fisher's multiple range test).

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References

[1] Cao A C. Methyl bromide and its alternatives. Pesticides, 2003, 6: 1 – 4

[2] Bai X G. Storage and control of pests. Beijing: Science Press, 2002, 451 – 478

[3] Guo D L, Pu W, Yan X P, Tao C. Storage gas and progress of fumigation overseas. Grain Storage, 2004, 6: 44 – 47

[4] Liang Q. Feelings of the 6th International Gas storage products with the fumigation. Grain Storage, 2003, 5: 43 – 49

[5] Le T V, Ren Y L. Natural levels of ethyl formate in stored grains determined using an improved method of analysis. Journal of Stored Products Research 2004, 40: 77 – 85

[6] Damcevski K A, Annis P C. Influence of grain and relative humidity on the mortality of *Sitophilus oryzae* (L.) adults exposed to ethyl formate vapour. Journal of Stored Products Research 2006, 42: 61 – 74

[7] Tang P A, Deng Y X, Wang J J et al. Studies on the fumigation activities of ethyl formate against the adults and immature stages of *Tribolium castaneum* herbst. Journal of Southwest Agricultural University, 2006, 1: 61 – 65

[8] Tang P A, Deng Y X, Wang JJ et al. Fumigation activity of ethyl formate against the adults and immature stages of *Sitophilus oryzae* (L.). Journal of Plant Protection, 2006, 2: 178 – 182

[9] Li J, Deng Y X, Wang J J et al. Fumigation lethal effect of ethyl formate on the adults of *Liposcelis bostrychophila*. Journal of Southwest Agricultural University, 2006, 5: 858 – 862

[10] Tang P A, Deng Y X, Wang J J et al. Research advance of ethyl formate controlling stored – product insects. Grain Storage, 2006, 1: 13 – 17

[11] Jia C S. Calculating the LC_{50} of insecticides with software SPSS. Entomological Knowledge, 2006, 3: 414 – 417

[12] Simpson T, Bikoba V, Mitcham E J. Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries. Postharvest Biology and Technology, 2004, 34: 313 – 319

[13] Bell C H. Fumigation in the 21st century. Crop Protection, 2000, 19: 563 – 569