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Toxic Activity of Allicin on Several Stored Product Pests

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Abstract: The fumigant effect of allicin was tested on the larvae, pupae and adults of *T. castaneum*, *Oryzaephilus surinamensis* and *Cryplotestes ferrugineus*. The LC_{50} of 3 – days exposure of adults to allicin was 0.68, 0.86 and 0.99 L/L respectively. The LC_{50} of 3 – days exposure of the larvae to allicin was 0.11, 0.12 and 0.36 L/L respectively, indicating that the larvae were much more sensitive than adults to allicin. Trials in the laboratory using allicin with wheat that imitated a commercial f resulted in LC_{50} values for adults of the three species of 8.29, 7.61 and 8.26 L/L respectively. An allicin concentration of 5 L/L suppressed pupal emergence of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* by 85.56%, 94.44% and 100.00 % respectively. Low concentrations of allicin were effective in controlling these stored product pests, but micro-encapsulation may be necessary to conceal its odour and to allow future commercialisation of this promising fumigant.

Introduction

Phosphine (PH_3) is a toxic gas used to protect stored commodities against insect pest infestation. It is by far the most widely used fumigant of stored products worldwide, because it is inexpensive to apply and leaves little or no residue (Cao *et al.* 2006). With the emergence of high level phosphine resistance in insect populations in various regions of the world, there is increasing interest in determining the mode of action and the mechanisms whereby insects acquire resistance to this fumigant (Cao 2000; Zhang *et al.* 2004). The future use of phosphine as a commercial fumigant could be threatened by the further development of resistant strains.

Many alternatives have been tested to replace methyl bromide fumigation for stored product and quarantine uses. Recently, the secondary metabolites of some plants have been formulated as botanical pesticides for plant protection, since they do not leave residues toxic to the environment, they have lower toxicity to mammals and medicinal properties for humans (Catherine 1995).

Crude extracts and a steam-distilled oil fraction of garlic were found to be larvicidal against several species of mosquitoes and could be used as a potential grain protectant against *Tribolium castaneum* and *S. zeamais* (Lu *et al.* 2003). Hexane extracts of garlic were also found to be insecticidal to *Liposcelis entomophilia*, *R. dominica* and *T. castaneum* (Lv *et al.* 2006). Chemical analysis showed that the major chemical component of garlic oil is allicin (Yin 2002).

In our research, the fumigant effect of commercial allicin was tested on the stored product insects *T. castaneum*, *Oryzaephilus surinamensis* and *Cryplotestes ferrugineus*.

Materials and Methods

Insects

T. castaneum, *O. surinamensis* and *C. ferrugineus* colonies was obtained from Qihe storage of Shandong province, Jiyuan storage and Zhidian storage of Henan province respectively. These insects were reared for several generations in our laboratory. *T. castaneum* was reared on wheat flour mixed with yeast (7 : 1, w/w) while *O. surinamensis* and *C. ferrugineus* were reared on wheat flours mixed with oatmeal and yeast (7 : 3 : 1) and maintained in the dark in incubators at 30 ± 1℃ and 70% – 80% RH.

Commercial Allicin

Commercial allicin was purchased from Sudong Chemical Plant Laboratory in Nantong of Jiangsu Province. The purity (98.5%) was confirmed by gas chromatography.

Toxicity Bioassay in Laboratory

Fumigation toxicity of allicin was tested in the laboratory using a sealed jar (Zhang *et al.* 2001). Three adults were put into the 300mL jar and a filter paper (9cm Length, 1cm width) soaked with different concentrations of allicin

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was hung in the jar. Fifteen adults were used in each treatment and three replicated in this assay. The control jar was similarly treated but without allicin in the filter paper. The jars with treated and control were kept in a controlled climate room at 28 ± 2℃, 24 h darkness and 75% 5% RH. The phosphine was dissipated on time and the numbers of dead adults counted after exposure periods of 6h, 12h, 24h, 48h, 72h, 96h, 120h and 144h respectively.

The toxicity of allicin against larvae and pupae were carried out with the same methods described for the adults. The phosphine was released and the number of dead larvae counted at the times described above. The other conditions were same as those described for the adults. The number of adults that emerged was observed every day until the adult number remained constant. The emergence rate of pupae was calculated.

Imitation of a Commercial Fumigation

The methods for imitating a commercial fumigation in the laboratory were the same as those used in the toxicity bioassay, but the difference was that there was 110g of wheat in each sealed jar (300mL). The fumigation time was 24h, 48h, 72h, 96h, 120h and 144h.

Statistical Analysis

Every experiment was replicated at least five times and values were expressed as mean SE. LC$_{50}$ and LC$_{95}$ values were calculated by Probit Analysis (SAS Institute 2004). The modified mortality of larval and pupae were calculated by the following formula: The modified mortality = (treatment mortality control mortality)/(1 – control mortality) * 100%.

Results and Analysis

Toxicity of Allicin Against Adults

The toxicity of allicin to adults of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* was determined using different exposure times and concentrations. The relationship between logarithm of concentration (x) and probability of death (y) was fitted a with linear regression equation (Table 1).

The LC$_{50}$ and LC$_{95}$ of adults exposed to allicin were significantly reduced with extended fumigation times. The LC$_{50}$ and LC $_{95}$ of allicin against *T. castaneum* was 1.51 and 4.23μL/L respectively when the time was 6h. When the time was 72h, the LC$_{50}$ and LC$_{95}$ of allicin for *T. castaneum* were 0.68 and1.27μL/L, respectively. The results indicate that for the same LC the concentration of allicin could be reduced if the fumigated time was prolonged.

The LC$_{50}$ and LC$_{95}$ of allicin against *T. castaneum* were less than that of *O. surinamensis* and *C. ferrugineus* when the fumigated time was held constant e. g., LC$_{50}$ was 79.07% and 68.69% that of *O. surinamensi* and *C. ferrugineus* when the exposure time was 72h. This suggested that *T. castaneum* was the most sensitive to allicin of the three species of adults.

Table 1. Comparison of toxicity of allicin against three species of adult grain pests

Adults	time /h	Regression equation/y =	LC$_{50}$/μL. L^{-1} (95% confident range)	LC$_{95}$/μL. L^{-1} (95% confident range)	r	χ^2
T. castaneum	6	4.345 +3.671x	1.51(1.413 –1.619)	4.23(3.612 –5.205)	0.991	5.169
	12	4.892 +3.720x	1.07(0.993 –1.144)	2.96(2.648 –3.403)	0.972	18.733
	24	5.227 +4.605x	0.89(0.823 –0.958)	2.03(1.886 –2.217)	0.917	14.317
	48	5.662 +5.841x	0.77(0.700 –0.834	1.47(1.391 –1.566)	0.950	7.030
	72	6.014 +6.170x	0.68(0.604 –0.756)	1.27(1.188 –1.346)	0.949	5.155
O. surinamensis	6	3.981 +1.759x	3.80(2.930 –5.862)	32.74(16.199 –111.675)	0.985	1.897
	12	4.224 +1.670x	2.92(2.373 –4.038)	28.19(14.74 –84.679)	0.974	3.788
	24	3.671 +1.856X	2.28(1.919 –2.955)	26.64(14.014 –79.535)	0.959	11.510
	48	4.760 +1.715x	1.38(1.215 –1.581)	12.57(8.048 –25.600)	0.948	19.339
	72	5.108 +1.656x	0.86(0.704 –1.000)	8.48(5.775 –15.723)	0.930	10.058
C. ferrugineus	6	3.819 +2.447x	3.04(2.540 –4.002)	14.32(9.110 –29.170)	0.959	9.207
	12	4.124 +2.255x	2.45(2.117 –2.995)	13.11(8.679 –24.573)	0.917	18.461
	24	4.269 +2.217x	2.14(1.885 –2.530)	11.79(8.037 –20.935)	0.994	1.458
	48	4.656 +1.954x	1.50(1.344 –1.697)	10.43(7.207 –18.137)	0.963	7.532
	72	5.012 +1.883x	0.99(0.853 –1.111)	7.37(5.379 –11.771)	0.969	5.998

Imitation of a Commercial Fumigation in the Laboratory

In order to test the fumigation effect of allicin in storage, an imitation of a commercial fumigation was carried out using wheat. Perhaps allicin could be absorbed by the wheat and the toxicity could be proportional to the rate of absorption. The rate of adsorption is the ratio of LC_{50} of insect in bottles with bottles and blank bottles (Table 2).

The adsorption rate was the lowest when allicin fumigation time was extended to the fourth day for adults of *O. surinamensis* and *C. Ferrugineus*; and to the sixth day for adult is *T. castaneum*. The results indicate that the optimum fumigation time for allicin against stored product insects was 4 to 6 days. The adsorption rate decreased when the fumigation time was longer. The fumigated time could be adjusted in the future according to the known adsorption rate.

Table 2. Comparison adsorption and toxicity and mimicked toxicity effect of allicin with three species of adults

Adult	LC50 and adsorption rate	1d	2d	3d	4d	5d	6d
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	0.89	0.77	0.68	0.59	0.51	0.38
T. castaneum	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	13.49	10.17	8.29	6.98	6.02	4.92
	Adsorption rate	15.16	13.21	12.19	11.83	11.80	12.95
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	2.28	1.35	0.86	0.76	0.62	0.51
O. surinamensis	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	28.74	15.99	9.24	5.10	4.41	4.06
	Adsorption rate	12.61	11.84	10.74	6.71	7.11	7.96
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	2.14	1.50	0.99	0.61	0.57	0.51
C. ferrugineus	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	23.02	15.87	12.36	7.56	4.19	3.38
	Adsorption rate	10.76	10.58	12.48	12.39	7.35	6.63

Fumigant Toxicity of Allicin Against Larvae

The toxicity of allicin to larvae of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* was determined using different exposure times and concentrations. The relationship between logarithm of concentration (x) and probability of death (y) was fitted a with linear regression equation (Table 3). The LC_{50} and LC_{95} of larvae could be decreased as the fumigated time was prolonged. Compared with the LC_{50} and LC_{95} achieved in 6h, in 72h they declined to 85.14% and 87.99% respectively. The quantity of allicin could be reduced for the same level of mortality if the fumigation time was correspondingly prolonged.

Table 3. Toxicity of allicin against larval of *T. castaneum*, *O. surinamensis* and *C. ferrugineus*.

Adults	time /h	Regression equation/y =	$LC_{50}/\mu L.\ L^{-1}$ (95% confident range)	$LC_{95}/\mu L.\ L^{-1}$ (95% confident range)	r	χ^2
	6	5.348 + 2.650x	0.74 (0.649 − 0.884)	3.08 (2.112 − 5.799)	0.971	4.662
	12	5.985 + 3.075x	0.48 (0.428 − 0.531)	1.64 (1.310 − 2.291)	0.956	9.122
T. castaneum	24	6.558 + 2.668x	0.26 (0.202 − 0.311)	1.08 (0.900 − 1.403)	0.996	24.229
	48	7.542 + 3.293x	0.17 (0.111 − 0.220)	0.53 0.465 − 0.616)	0.985	9.177
	72	8.028 + 3.213x	0.11 (0.046 − 0.176)	0.37 (0.278 − 0.446)	0.978	3.796
	6	4.656 + 1.647x	1.62 (1.257 − 2.397)	16.11 (8.004 − 53.770)	0.972	3.799
	12	5.129 + 1.624x	0.83 (0.700 − 1.020)	8.57 (5.073 − 19.959)	0.957	6.557
O. surinamensis	24	5.440 + 1.717x	0.55 (0.461 − 0.653)	5.03 (3.361 − 9.363)	0.964	6.020
	48	5.890 + 2.018x	0.36 (0.290 − 0.430)	2.36 (1.833 − 3.402)	0.967	8.690
	72	6.891 + 3.060x	0.24 (0.180 − 0.296)	0.83 (0.730 − 0.965)	0.989	14.915

Adults	time /h	Regression equation/y =	LC_{50}/μL. L^{-1} (95% confident range)	LC_{95}/μL. L^{-1} (95% confident range)	r	χ^2
C. ferrugineus	6	3.979 + 2.728x	2.37(1.918 – 3.376)	9.49(5.752 – 23.109)	0.963	5.448
	12	4.308 + 2.625x	1.83(1.573 – 2.306)	7.76(5.131 – 15.343)	0.954	8.179
	24	6.081 + 2.802x	1.05(0.919 – 1.195)	5.44(3.851 – 9.485)	0.959	5.544
	48	5.875 + 2.976x	0.51(0.406 – 0.598)	1.83(1.573 – 2.197)	0.936	14.850
	72	6.553 + 3.541x	0.36(0.254 – 0.460)	1.06(0.935 – 1.208)	0.929	11.183

Toxicity of Allicin to Pupae

The effects on emergence of pupae exposed to different concentrations of allicin fumigant are shown in Table 4. Emergence was completely prevented in *T. castaneum* using a concentration of 5μL/L. An allicin concentration of 4μL/L reduced emergence of *T. Castaneum*, *O. surinamensis* and *C. Ferrugineus* pupae to 67.78, 80.00 and 93.33% respectively. The results suggest that, of the three species, *C. ferrugineus* pupae were the most sensitive to allicin.

Table 4. Modified mortality of pupae fumigated with different concentrations of allicin

Pupae	Modified morality/%				
	1/μL. L^{-1}	2/μL. L^{-1}	3/μL. L^{-1}	4/μL. L^{-1}	5/μL. L^{-1}
T. castaneum	10.00 ± 3.85 b	25.56 ± 4.01 c	52.22 ± 4.01 c	67.78 ± 1.11 c	85.56 ± 1.11 c
O. surinamensis	32.22 ± 2.94 a	50.00 ± 5.77 b	68.89 ± 4.01 b	80.00 ± 1.92 b	94.44 ± 1.11 b
C. ferrugineus	41.11 ± 4.84 a	66.67 ± 3.85 a	86.67 ± 1.11 a	93.33 ± 1.92 a	100.00 ± 0.00 a

Discussion

This study showed that allicin had a significant fumigant effect on different life stages of three species of insects, especially on *T. castaneum*. These species were sensitive to allicin at low fumigant concentrations, perhaps because this is their first exposure to this fumigant. Research on the how each species metabolises allicin may provide further insight on how to optimise its use as a fumigant.

Papachr and Stamopulosd (2002) showed that the fumigation concentration should be increased or the fumigated time should be extended in order to optimise the fumigant's affect when used with grains that differ in their ability to absorb allicin. Our research showed that the fumigation effect was rapid and mortality increased with concentration. When the fumigant time was prolonged, the concentration of allicin could be reduced.

The fumigated concentration of allicin in the laboratory was very low, but it needed to be much higher when allicin was used with wheat. Absorption rate in wheat decreased gradually over time. Wheat absorbed significant quantities of allicin rapidly. Toxic concentrations of allicin diffused into the grain interior showing that it was highly permeable reaching insects in the interior of the grain. The lower absorption rate indicated good permeability and a better fumigant effect (Bai *et al.* 2002). Allicin with wheat required a fumigation period of 4 to 6 days.

The results indicate that allicin had a highly toxic effect on three species of stored product insects. Allicin is neither reported to have a toxic to humans and animals, nor does it contribute to environmental pollution. Allicin could be a substitute for existing fumigants to control stored product insects, particularly for the disinfestation of grain where synthetic chemicals are becoming less preferred.

However, allicin's strong odour may limit its application but micro-encapsualtion (Hyun *et al.* 2000; Kavindra *et al.* 2000) may conceal the odour, as it has done with other botanical insecticides. Previous studies have shown its odour can be concealed without reducing its toxicity (Ankri *et al.* 1999). Allicin therefore shows promise as a safe and effective replacement for existing stored product fumigants.

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