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Toxicity of Acetone to Stored-product Insects

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ABSTRACT

In laboratory experiments the toxicity of acetone was investigated against four species of stored-product insects. In empty-space trials, estimates of the lethal concentrations of acetone ($LC_{,}$ 72-h exposure) for 50% mortality against adults of the lesser grain borer, *Rhizopertha dominica* (F.), red flour beetle, *Tribolium castaneum* (Herbst), rice weevil, *Sitophilus oryzae* (L.) and eggs of the angoumois grain moth, *Sitotroga cerealella* (L.) were 33.64, 41.05, 43.90 and 46.11 µl/liter, respectively. At the LC_{95} level, the order of sensitivity was rated to be: *R. dominica* adults > *S. oryzae* adults > *T. castaneum* adults > *S. cerealella* eggs. Penetration tests revealed that acetone vapour could penetrate into the wheat mass and kill concealed insects in interkernel spaces. Comparison of LC_{50} values between empty-space tests and penetration experiments (after 72-h exposure) indicated that the increase in penetration toxicity was 8.52- and 8.09- fold for *R. dominica* and *T. castaneum*, respectively. A similar trend was observed at the LC_{95} level. In the hidden infestation trial, the acetone vapour destroyed all the developmental stages of *S. oryzae* concealed inside the wheat kernels and resulted in a complete control with a concentration of 320 µl/liter for 8 weeks after the exposure. Based on the data collected in this study, acetone should be considered as a potential compound for empty space fumigations. However, due to its sorption characteristics and the application of high doses, acetone may have only limited use as a fumigant under practical conditions.

Keywords: Acetone, Fumigation, Stored-product insect, Wheat.

INTRODUCTION

Numerous investigators have studied the application and effectiveness of fumigants to control stored-product insects (Bell and Wilson, 1995; Rajendran and Muralidharan, 2001). Fumigants are widely used for disinfecting commodities and the treatment of empty stores. Over recent years the removal of some fumigants from the market has been resulted in a wider use of methyl bromide and phosphine (Leesch, 1995).

The consumption of methyl bromide is very extensive throughout the world, but its correct use needs a great level of expertise. In practice, an increase in tolerance to methyl bromide has not been reported among most insects under fumigation conditions. However, methyl bromide is known as an ozone depletor agent and a major threat to the environment (Dunkel and Sears, 1998; Leesch *et al*., 2000; Weller and Morton, 2001).

Phosphine has been used with great effectiveness in a variety of habitats for a long time (Rajendran and Muralidharan, 2001). Conventional use of this compound has shown frequent failure to control insects. Consequently, certain insects have developed a resistance to phosphine (Bell and Wilson, 1995). Furthermore, phosphine may cause chromosomal aberrations in personnel working with this fumigant (Garry *et al*., 1989).

The recent emphasis placed on unacceptable insecticide residues in foodstuffs has prompted considerable thought and research on human health and the environment (Brewer *et al*. 1994). Any compound which

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can reduce the insecticide load in a particular storehouse with adequate effectiveness to control insects can be of utmost importance in a stored-product insect control program. The main challenge is now for alternative substances which are safe enough, readily available, inexpensive, convenient to use, and without substantial disruption of the ecosystem.

Acetone was selected for testing because it is generally recognized as a safe substance to man and the environment, inexpensive, commonly available and convenient to use (Ellenhorn and Barceloux, 1988; Howard, 1991; Tunc *et al*., 1997).

The Purpose of this study was to determine the fumigant action of acetone against stored-product insects.

MATERIALS AND METHODS

The acetone tested was 99.9%, and supplied by Merck Co. Ltd. This compound is a polar and highly volatile liquid (Ellenhorn and Barceloux, 1988). It is absorbed through the skin, but the lungs and kidneys exhale a considerable amount of absorbed acetone in a short period of time (Gossel and Bricker, 1990).

The samples of *T. castaneum*, *S. oryzae*, *R. dominica* and *S. cerealella* were collected from local mills, stores and shops in Urmia (37.39°N 45.4°E), a town in West Azarbijian Province (Iran). The cultures were established and maintained on healthy uncontaminated food at 27 ± 2°C and 60 ± 10% r.h. in glass jars covered with pieces of muslin cloth fixed by rubber bands.

This study was carried out in three stages at the entomology laboratory of Urmia University during the period of 2001-2002. In the first stage, acetone was tested against insects in an empty-space. In the second stage, the effect of acetone was determined by confining the insects under known amount of wheat mass and applying acetone in the air space above the wheat. Finally, in the third stage, acetone was distributed throughout the wheat mass to determine its effectiveness against various life stages of *S. oryzae*, an internal feeder insect.

The insects were reared for two generations before the initiation of experiments. *T. castaneum* was reared on a 50:50 mixture of wheat flour and corn meal. This mixture contained 5% brewer's yeast. The *S. oryzae* , *R. dominica* were reared on soft red winter wheat and the *S. cerealella* on barely.

The following developmental stages of the insects were used in these tests: (i) *T. castaneum* adults, 14 ± 3 days old, (ii) *S. oryzae* and *R. dominica* adults 7 ± 2 days old and (iii) *S. cerealella* eggs at 1 day old. A 1,150-ml tightly closed glass jar was used as a fumigant chamber in empty-space and hidden infestation tests. Penetration trials were conducted in 0.0296 m^3 (ca. 26 kg) capacity vertical welded steel containers. The bioassay procedures were identical in all trials. In all the tests, mortality was recorded after an appropriate exposure period. Insects that did not move when lightly probed or shaken in the light and mild heat, were considered dead. Concentration-mortality data from the replicates were pooled and the concentration-mortality response was determined by probit analysis with an SPSS package (SPSS, 1993).

Empty Space Tests

The adults of *R. dominica, T. castaneum, S. oryzae* and also the eggs of *S. cereallella* were fumigated separately for 48, 72 and 96 h in 1,150-ml glass jars. The adults of *R. dominica*, *T. castaneum* and *S. oryzae* were confined in cages constructed from 40-mesh wires. Each cage contained 30 insects and 3 g of food. Eggs were exposed in small petri dishes. The jars were capped with screwed lids. Blotting-paper strips measuring 2 × 6 cm were attached to the lower side of each lid with adhesive plastic tape. The appropriate amount of acetone was deposited on the blotting-paper strips with an Oxford sampler through a 5-mm diameter hole, located in the center of the lid. Immediately after the acetone was pipetted, the hole in each lid

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was sealed with plastic tape. In each test, the control jar was treated identically except that no acetone was deposited on the blotting-paper.

After exposure, the insects were transferred to clean jars containing a rearing medium and maintained under rearing conditions. Mortality was recorded 4, 7 and 14 days after the termination of the exposure time for *S. cerealella* eggs, *S. oryzae*, *R. dominica,* and *T. castaneum* adults, respectively. Each test was replicated three times on three different days.

Penetration Tests

For each concentration tested, two cages (containing either 30 *T. castaneum* or *R. dominica* adults with 3 g of food) were placed horizontally at the bottom of a steel container. The container was filled with 26 kg of soft red winter wheat with a 13 ± 1% moisture content and with less than 1% foreign materials. The test procedure used was similar to those described for the empty-space tests except for the amount of acetone consumed. Each concentration was replicated three times. The control container was prepared in an identical manner, but no acetone was used. After an exposure period of 72 and 96 h, the insects were transferred to clean glass jars containing food, held at 27 ± 2°C and 60 ± 10% r.h. The mortality rate of *R. dominica* and *T. castaneum* was recorded as after 7 and 14 days following the treatment, respectively.

Hidden Infestation Test

A sample of three hundred g of wheat containing the eggs, larvae and pupae of *S. oryze* was collected from the stock culture and placed in 1,150-ml glass jars. The procedure of applying acetone to blotter-paper and sealing the holes was identical to that described earlier. To distribute acetone throughout the wheat mass, the jar was briefly shaken by hand and tumbled mechanically for 5 minutes. After tumbling, the jar was held under standard conditions for 72 h. Subsequent to the exposure time, the insects and wheat were transferred to a clean jar, held at 27 ± 2°C and 60 ± 10% r.h. for 8 weeks. Under the test conditions, 7-8 weeks were sufficient for eggs of *S. oryzae* to develop to the adult stage. During this period, emerging adults were counted weekly and discarded. Control groups were treated identically but with no acetone deposited onto the blotter-paper.

RESULTS

Empty Space Tests

Concentration-mortality values estimated from the probit analyses of adults and eggs mortality are given in Tables 1 to 3. In all the experiments, acetone was toxic to the insects tested and there was a direct relationship between the exposure time to acetone and the susceptibility of insects. On the ba-

Table 1. Toxicity of acetone to 4 species of stored-product insects exposed 48 h at 27 ± 2°C in 1150-ml jars (Empty-space tests).

Toxicity value	Toxicity (Concentration µl/L)							
	R. dominica adult		*T. castaneum* adult		*S. oryzae* adult		*S. cerealella* egg	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	42.03	192.73	57.77	224.47	68.18	265.92	71.46	273.92
	59.86	115.00	51.21	178.47	60.12	207.50	63.53	190.45
Upper 95% FL	29.45	577.20	65.55	304.10	77.91	373.59	80.76	323.59
Lower 95%FL	2.49 ±0.21		2.80		2.79		3.15	
Slope ± SE			± 0.22		± 0.25		± 0.29	

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Table 2. Toxicity of acetone to 4 species of stored-product insects exposed 72 h at 27 ± 2°C in 1150-ml jars (Empty-space tests).

Toxicity value	Toxicity (Concentration µl/L)							
	R. dominica adult		T. castaneum *adult*		*S. oryzae* adult		*S. cerealella* egg	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	33.64	118.32	41.05	156.21	43.90	152.05	46.11	173.77
Upper	55.29	703.31	77.98	2151.2	75.42	1010.5	83.64	1491.0
95% FL	18.80	67.41	20.97	80.95	26.06	84.72	26.83	92.15
Lower 95%FL	3.01		2.83		3.05		2.86	
Slope ± SE	± 0.24		± 0.23		± 0.23		± 0.22	

sis of LC_{50} values, the sensitivity order of the insects to acetone was measured as: *R. dominica* adults > *T. castaneum* adults > *S. oryzae* adults > *S. cerealella* eggs (Tables 1, 2 and 3). There was a considerable overlap in the 95% fiducial limits of concentration-mortality regression lines. Therefore, no statistically significant difference between the estimated LC_{50} values was observed. Table 1 demonstrates that, at the LC_{95} level, the concentration of acetone required for killing the most tolerant species, (i.e. *S. cerealella*) was 273.92 µl/liter.

Penetration Tests

Results of the fumigation tests showed that acetone penetrated thoroughly in the wheat mass and killed *T. castaneum* and *R. dominica* adults (Tables 4 and 5). There was a direct relationship between acetone concentration and the mortality rate of the insects tested. Based on the LC_{50} values, when acetone was applied to the wheat mass air space the concentration required to achieve 50% mortality after a 72-h exposure time was 8.52 and 8.09 times more than that required for the empty-space tests for *R. dominica* and *T. castaneum*, respectively (Tables 2 and 4). These differences indicated that acetone was sorbed by the wheat mass. A similar trend was observed at the LC_{95} level. The data revealed that there was a noticeable overlap in the fiducial limits of the estimated LC_{50} values (Tables 4 and 5). Therefore, the susceptibility was similar in the tested insects.

Hidden Infestation Test

Table 6 presents the toxicity of acetone on

Table 3. Toxicity of acetone to 4 species of stored-product insects exposed 96 h at 27 ± 2°C in 1150-ml jars (Empty-space tests).

Toxicity value	Toxicity (Concentration µl/L)							
	R. dominica adult		*T. castaneum* adult		*S. oryzae* adult		*S. cerealella* egg	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	29.81	109.26	39.09	159.28	39.56	155.49	39.99	166.03
Upper 95%FL	46.90	524.88	71.64	1802.2	66.64	961.16	64.04	800.20
Lower 95%FL	17.09	63.49	20.48	82.26	23.52	84.82	25.21	92.22
Slope ± SE	2.91 ± 0.24		2.70 ± 0.21		2.77 ± 0.22		2.66 ± 0.21	

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Table 4. Toxicity of acetone to *T. castaneum* and *R. dominica* adults exposed for 72 h under 26-kg wheat mass (Penetration tests).

Toxicity value	Toxicity (Concentration µl/L)			
	T. castaneum adult		*R. dominica* adult	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Effective concentration	332.50	2591.07	286.93	2258.45
Upper 95%FL	4589.43	4835957	2514.56	3030338
Lower 95%FL	181.84	697.43	160.42	463.99
Slope ± SE	1.85 ± 0.23		1.84 ± .22	

Table 5. Toxicity of acetone to *T. castaneum* and *R. dominica* adults exposed for 96 h under 26-kg wheat mass (Penetration tests).

Toxicity value	Toxicity (Concentration µl/L)			
	T. castaneum adult		*R. dominica* adult	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Effective concentration	249.90	1892.84	217.10	1653.63
Upper 95%FL	650.77	39504.26	502.80	26431.52
Lower 95%FL	160.19	701.58	141.19	640.65
Slope ± SE	1.88 ± 0.21		1.86 ± 0.19	

Table 6. The adult emergence from immature stages of the S. oryzae exposed to various concentrations of acetone for 72 h in 1150-ml jars (Hidden infestation test).

Concentration µl/L	Emergence at week								
	1	2	3	4	5	6	7	8	Total
0	10	7	8	11	29	43	81	3	192
40	2	3	0	0	2	0	13	1	21
80	2	5	0	0	0	1	6	0	14
160	1	2	0	0	0	0	6	0	9
320	0	0	0	0	0	0	0	0	0

the *S. oryzae* population concealed in wheat. The results show a good level of effectiveness of acetone as a fumigant where insects are concealed inside the wheat. An inverse relationship between the acetone concentration and the number of *S. oryzae* survivors was observed. None of the adults of *S. oryzae* emerged from the wheat when they had been exposed to acetone at the rate of 320µl/liter. This concentration of acetone was sufficient to kill the different developmental stages of *S. oryzae* inside the wheat kernels. A cohort infested wheat mass that was not treated with acetone yielded 192 adults during the same incubation period.

DISCUSSION

Control of stored-products pest insects is essential wherever grain quality is to be maintained. For the control of these pests, particularly in grain, farmers rely mostly on treatment of contact insecticide to raw cere-

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als (Bond, 1984; Daglish, 1998). Because such treatments may result in the presence of residues in those products that are prepared from treated grain, there should be restrictions in the level of insecticide residues in such products (Bond, 1984; Brewer *et al*., 1994). Therefore, the number of suitable contact insecticides that can be used in the control of stored-product insect is limited (White and Leesch, 1995; Arthur, 1999). For a long time, the main stored -grain protectant insecticide was de-odorized malathion (Daglish, 1995; Arthur, 1999). Unfortunately, most stored-product insects became substantially resistant to this insecticide, and therefore a substitute is needed (Leesch, 1995; Arthur, 1999).

The cost and health risk of fumigation seems to be lower than with traditional methods of preservation (Weller and Morton, 2001). Thus, it appears that fumigation will be the backbone and an indispensable component of stored-product insect control programs in the immediate future. Due to the removal of some fumigants from the market, only methyl bromide and phosphine are now available for use (Leesch, 1995). A good fumigant should show some characteristics in accordance with the fumigation protocol, which ensures an appropriate level of insect control and produces the minimum of hazardous side effects (Bond, 1984). Unfortunately, the two available fumigants fall short of this ideal.

At present, large quantities of stored foodstuffs are fumigated with methyl bromide and phosphine, and so special attention should be paid to the nature of these fumigants. The greatest deficiency in the use of methyl bromide is that, in many instances, the main emphasis has been placed on the methyl bromide fumigation and stock management has been neglected. Therefore, reinfestation occurred soon after the fumigation was completed. Consequently, frequent fumigation was necessary and grain could have bromide residues in excess of the permissible level. Phosphine as a fumigant offers a cost-effective method of insect control (Rajendran and Muralidharan, 2001). Strict controls on detectable concentrations of phosphine are imposed by some organizations (Bond, 1984). Since excessive residue from fumigation is a potential hazard to consumers, methyl bromide and phosphine, are under close scrutiny and will have limited use in the immediate future (Weller and Morton, 2001).

A new approach in fumigation research could include the use of generally recognized safe substances, which are harmless for the use with human food. The application of acetone as a potential fumigant may be an appropriate approach for achieving this objective. Acetone is absorbed through the skin and is distributed throughout the body. The fatal dose of acetone for an average adult lies between 300 and 400 ml, if this amount is ingested in less than an hour (Gossel and Bricker, 1990). Therefore, death from acetone should be uncommon under fumigation conditions. Although acetone is not a novel compound, as yet it is not registered for use as a fumigant. Therefore, there is little published information concerning the toxicity of acetone.

In the present study, acetone was toxic to all insects tested in empty-space tests. This finding would agree with the data collected by Tunç *et al*. (1997) who have demonstrated that acetone was toxic to some insects in empty-space tests. Comparison of empty-space versus penetration toxicities after 72-h exposure indicated that the increase in concentration between the empty-space LC_{50} and the penetration toxicity was nearly 8-fold. Since acetone is polar and miscible with water (Ellenhorn and Barceloux, 1988 and Howard, 1991) its concentration could decrease through sorption by the wheat.

It is well established that a good fumigant must kill all stages of the target insects. Thus, any internal feeder insects such as *S. oryzae* could represent a difficult challenge to any potential fumigant.

Acetone, as a potential fumigant, showed acceptable biological effectiveness and a level of 320 µl/liter resulted in the complete control of *S. oryzae* infestation in jar tests.

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Acetone has good characteristics as a potential fumigant for the control of the tested insects in empty spaces. However, due to high sorption and the application of high doses, acetone may have limited eligibility as a potential replacement for methyl bromide or phosphine under practical conditions.

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سمیت استون به آفات انباری

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چکیده

سمیت استون برای چهار گونه از حشرات آفات انباری در آزمایشگاه مورد بررسی قرار گرفت. در فضای خالی پس از ۷۲ ساعت تدخین مقادیر LC_{50} برای حشرات کامل سوسک دانه غلات, شپشه آرد, شپشه برنج و تخم پروانه دانه خوار غلات به ترتیب ۳۳/۶۴ ، ۴۱/۰۵ ، ۴۳/۹۰ ، ۴۶/۱۱ میکرو لیتر بر لیتر برآورد گردید. در سطح LC_{95} میزان حساسیت حشرات به شرح زیر بود:

تخم پروانه دانه خوار غلات < شپشه آرد < شپشه برنج < سوسک دانه غلات

در بررسی نفوذ استون به داخل توده گندم معلوم گردید که بخار استون می‌تواند به داخل توده گندم نفوذ نموده و حشرات مخفی شده در بین دانه های گندم را از بین ببرد. از مقایسه مقادیر LC_{50} استون در فضای خالی و نفوذ به داخل توده گندم مشخص شد که مقدار LC_{50} برای سوسک دانه غلات ۸/۵ برابر و در مورد شپشه آرد ۸/۰۹ برابر افزایش یافته است. در سطح LC_{95} نیز روند مشابهی دیده شد. در آزمایش نفوذ بخار استون به داخل بذر گندم مشاهده گردید که بخار استون با نفوذ به داخل بذر، کلیه مراحل زیستی شپشه برنج را از بین می برد و با غلظت ۳۲۰ میکرولیتر بر لیتر در مدت ۸ هفته پس از عمل تدخین به طور کامل شپشه برنج را کنترل می کند. بر اساس داده های تحقیق حاضر می‌توان استون را به عنوان یک فومیگان بالقوه در تدخین انبارهای خالی مورد توجه قرار داد لیکن به دلیل مصرف مقدار زیاد و قابلیت جذب توسط گندم, امکان استفاده از استون در انبار های حاوی گندم در عمل محدود می باشد.

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