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Evaluation of Acetone Vapors Toxicity on *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) Eggs

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Abstract: The efficacy of acetone vapors against carefully aged eggs of *Plodia interpunctella* (Hubner) at 17±1 and 27±1°C at different dosage levels of acetone over various exposure times was determined. Acetone was found to be toxic to Indian meal moth eggs. Considerable variation in the susceptibility of different age groups of eggs was apparent in the fiducial limits of the LD_{50} values. An inverse relationship between LD_{50} values and exposure times was observed in age groups of tested eggs. At 27±1°C and 24 h exposure period, eggs aged 1-2 day-old were more tolerant to acetone than other age groups, followed by 0-1 day-old, 2-3 day-old and 3-4 day-old eggs. A similar pattern of susceptibility of eggs was observed at 72 h exposure. In all bioassays, eggs exposed to higher dosages of acetone developed at smaller rate. This was significant for the eggs, which were exposed to the highest dosage for 24 h. Increasing the temperature from 17±1 to 27±1°C greatly increased the efficacy of acetone. At 27±1°C eggs of *P. interpunctella* were killed by less than one-third of the dosage required for control at 17±1°C. Acetone achieved 50% mortality with a dosage of 82.76 mg L^{-1} in 1-2 day-old eggs at 27±1°C. At this temperature hatching was retarded and greatly diminished when eggs aged 1-2 day-old were exposed to 80 mg L^{-1} of acetone for the 24 h exposure period. There was no evidence of a hatch delay longer than the time spent under vapors for eggs exposed at 17±1 or 27±1°C, indicating that some development must have occurred under fumigation.

Key words: Acetone, bioassay, exposure period, Indian meal moth, temperature

INTRODUCTION

Control of stored-products pests has been one of the major tasks for conservators because the damage inflicted to foodstuff is irreversible. A number of insect species pose a potential threat to a variety of stored-products. The Indian meal moth, *Plodia interpunctella* (Hubner) is a cosmopolitan pest attacking a wide range of stored-products of different nutritional values and physical properties. This insect causes serious losses both in quantity and quality of stored foodstuffs (Johnson *et al*., 1992; Hyun and Ryoo, 2000). Fumigants are commonly applied for control of stored-products pests. Because fumigation is often the cheapest and most effective process available, it plays a major role worldwide in preserving commodities (Yonglin *et al*., 1996). Two of the commonly used fumigants are methyl bromide and phosphine. Methyl bromide is now under threat of withdrawal because it apparently depletes the Earth's ozone layer (Dunkel and Sears, 1998; Leesch *et al*., 2000). Phosphine has been used in a variety of habitats for a long time (Rajendran and Muralidharan, 2001). Conventional use of phosphine has been frequent failure to control insects and certain insects have developed resistance to phosphine (Bell and Wilson, 1995). Moreover concerns about the further development of resistance to phosphine has made the search for new alternatives imperative (Leesch, 1995). Under these circumstances evaluation of the innate toxicity of inexpensive, readily available, convenient to use and without substantial disruption of the environment is warranted. According to this criteria acetone as an insect control compound was selected for testing. Acetone is ubiquitous in the environment and readily biodegraded (Howard, 1991). It is widely used in laboratories as solvent in applying insecticides to insects. Acetone is a metabolic product in humans and some other mammals and is a normal constituent of their urine and blood (Ellenhorn and Barceloux, 1988). It is absorbed through the skin, but lungs and kidneys excrete considerable amounts of absorbed acetone in a short period of time (Gossel and Bricker, 1990).

Studies on different insect species agree that the egg is the most difficult stage to kill (Williams and Sprenkel, 1990). These authors also provided the indication of the tolerance changing of eggs to sulfuryl fluoride over

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longer periods in their experiments. Although acetone is not a novel compound, as yet it is not registered for use as a fumigant. Therefore, there is little published information about the toxicity of acetone vapors.

The current study was undertaken to investigate the efficacy of acetone at various temperatures and exposure times on different age groups of eggs of *P. interpunctella*.

MATERIALS AND METHODS

P. interpunctella was collected from local stores and shops, in Urmia (37.39° N 45.4° E), a town in Iran in 2006. Stock cultures were established and maintained in wide-mounted gallon jars. Indian meal moth was reared on a 10:2:1 mix of wheat bran, glycerol and dried yeast powder at 27±1°C and 60±10% r.h. in a 16 h light, 8 h dark lighting regime. All insects were cultured under moderately crowded conditions to ensure proper development of the resultant insects. Insects were reared for two generations before commence of experiments.

Acetone: The test acetone was of 99.99% purity and supplied by Merck Co. Ltd. This compound is polar liquid with density of 0.788 g mL^{-1} at 25°C (Howard, 1991).

Preparation of eggs for experiments: Mixed sex adults were collected from stock cultures, anaesthetized with carbon dioxide before confining them in a plastic sieve by attaching a glass dish. Taping a moist cotton wool pad inside the dish provided drinking water. The sieves were placed over collecting dishes in the rearing room. The eggs laid in the dishes were kept in the same room to arrive at the age required for bioassay. Using a fine sable brush and a binocular microscope, eggs with known age were counted out in batches of 20 on to watch glasses. Eggs with obvious defects were avoided. To commence fumigation each watch glass was placed singly in 1150 mL glass jar, which served as the fumigation chamber. For experiments at 17±1°C the collected eggs were moved to 17±1°C acclimatize for the fumigation which was started following morning. For tests at 27±1°C there was no need for conditioning of eggs prior to fumigation.

Bioassay: Twenty eggs were prepared in each age group for exposure to each dosage. Preliminary dosage-mortality tests were done before each experiment to determine a range of dosages that would produce ca. 20-80% mortality (Robertson and Preisler, 1992). Five dosages between 10 and 160 mg L^{-1} were tested at 27±1°C and five dosages between 30 and 480 mg L^{-1} at 17±1°C. Age groups of eggs were fumigated for 24, 48,72 and 96 h in 1150 mL glass jars separately. The jars were capped with screwed lids. Blotting paper strips measuring 2×6 cm were attached to the lower side of each lid by adhesive plastic tape. The required amount of each dosage of acetone was deposited on the blotting paper strip with an Oxford sampler through a 5 mm diam hole, located in the center of the lid. Immediately after the acetone was pipetted, the hole in each lid was sealed with plastic tape. In each test, the control jar was treated identically except that no acetone was deposited on the blotting paper. At the end of exposure, watch glasses were taken out and eggs were left to air off in the fumigation chamber for 2 h before returning to the rearing room. After returning to 27±1°C, eggs were checked for hatch from three days after exposure until no further hatch was observed and cumulative mortality rates were determined. Each test was replicated three times on three different days. Dosage-mortality data from the replicates were pooled and the dosage-mortality response was determined.

Data analysis: The median lethal dosage (LD_{50}) and LD_{95} of acetone in the term of mg L^{-1} was estimated by subjecting mortality data to the maximum likelihood program of probit analysis using SPSS (1993). This program has a provision for control mortality. Two age groups of eggs were considered significantly different in their susceptibility to acetone if fiducial limits (95%) of LD_{50} of acetone did not overlap.

Results: Dosage-mortality values estimated from the probit analysis of different age groups of egg mortality are given in Table 1 to 8. In all experiments, acetone was toxic to the tested eggs. The toxicity of acetone is greatly increased at 27±1°C. Considerable variation among age groups was apparent in the slopes of regression lines for toxicity test, whether time or concentration was fixed in the tests.

Tests at 27±1°C: At 27±1°C eggs of *P. interpunctella* were much more susceptible than at 17±1°C. There was an inverse relationship between exposure time to acetone and estimated LD_{50} values (Table 1-4). At 27±1°C and 24 h exposure on the basis of LD_{50} values, eggs aged 1-2 days proved more tolerant than other age groups, followed by 0-1 day-old, 2-3 day-old and 3-4 day-old eggs. A similar trend was observed at 72 h exposure period. There was a considerable overlap in 95% fiducial limits of some dosage-mortality regression lines. Therefore, no statistically significant difference between the estimated LD_{50} values was observed. Table 2 presents that at the LD_{50} level, the dosage of acetone required for killing the most tolerant eggs (1-2 day-old) in the shortest exposure (24) h, was 82.76 mg L^{-1}.

Table 1: Toxicity of acetone to 0-1 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 27±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	64.15	570.87	57.69	383.51	46.24	472.94	33.60	461.87
Upper 95% FL	72.32	832.31	64.59	537.45	52.49	712.20	38.10	696.19
Lower 95% FL	57.31	425.12	51.49	296.23	40.65	346.35	29.44	336.33
Slope±SEM	1.73±0.11		2±0.15		1.63±0.12		1.45±0.10	
Number of eggs tested	360		360		360		360	
χ^2	1.34		3.46		3.09		4.95	
p-value	0.72		0.33		0.38		0.18	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 10, 20, 40, 80 and 160 mg L^{-1}

Table 2: Toxicity of acetone to 1-2 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 27±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	82.76	456.46	65.40	444.37	61.14	529.46	38.07	471.09
Upper 95% FL	92.99	651.34	73.39	632.27	68.78	766.26	43.17	710.05
Lower 95% FL	74.08	349.93	58.47	339.31	54.65	396.76	33.42	343.52
Slope±SEM	2.21±0.17		1.97±0.14		1.75±0.12		1.50±0.11	
Number of eggs tested	360		360		360		360	
χ^2	3.84		2.54		2.94		4.74	
p-value	0.28		0.47		0.40		0.19	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 10, 20, 40, 80 and 160 mg L^{-1}

Table 3: Toxicity of acetone to 2-3 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 27±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	44.34	369.87	44.60	367.54	37.20	397.35	28.99	417.72
Upper 95% FL	49.85	523.67	50.62	538.04	42.41	596.36	43.40	2898.04
Lower 95% FL	39.28	282.59	39.01	276.05	32.32	292.28	16.64	188.31
Slope±SEM	1.79±0.13		1.80±0.14		1.60±.012		1.42±0.11	
Number of eggs tested	360		360		360		360	
χ^2	5.05		3.99		4.32		10.15	
p-value	0.17		0.25		0.23		0.01	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 10, 20, 40, 80 and 160 mg L^{-1}

Table 4: Toxicity of acetone to 3-4 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 27±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	30.04	387.88	37.57	365.28	32.23	419.45	28.93	350.26
Upper 95% FL	34.25	576.09	56.25	2311.73	42.18	1175.18	32.82	508.89
Lower 95% FL	26.05	286.06	22.30	176.20	23.73	233.35	25.21	262.20
Slope±SEM	1.48±0.10		1.66±0.13		1.48±0.10		1.52±0.11	
Number of eggs tested	360		360		360		360	
χ^2	3.57		10.52		5.39		4.06	
p-value	0.31		0.02		0.15		0.26	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 10, 20, 40, 80 and 160 mg L^{-1}

Table 5: Toxicity of acetone to 0-1 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 17±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	268.96	7246.32	163.76	5653	187.91	6015	133.34	6015
Upper 95% FL	337.32	3867.20	315.36	†	228.11	14209	230.79	†
Lower 95% FL	223.33	17969	96.32	1486	157.76	3300	75.84	1568
Slope±SEM	1.15±0.11		1.07±0.10		1.09±0.10		0.99±0.11	
Number of eggs tested	360		360		360		360	
χ^2	2.60		8.25		4.78		6.27	
p-value	0.46		0.04		0.19		0.09	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 30, 60, 120, 240 and 480 mg L^{-1}. † Estimated LD_{95} value was too far beyond tested dosage range to be reliable

Table 6: Toxicity of acetone to 1-2 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 17±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	301.17	8769.88	293.28	18731	268.15	7161.86	144.98	8862
Upper 95% FL	382.44	23689.2	375.83	80279	362.76	17966	179.05	27963
Lower 95% FL	248.57	4457.43	240.59	7433	212.42	3812	118.54	4158
Slope±SEM	1.19±0.12		1.11±0.11		0.89±0.10		0.92±0.09	
Number of eggs tested	360		360		360		360	
χ^2	2.93		1.84		0.87		1.10	
p-value	0.40		0.60		0.83		0.78	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 30, 60, 120, 240 and 480 mg L^{-1}

Table 7: Toxicity of acetone to 2-3 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 17±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	232.29	6550.73	180.80	10621	149.47	10057	115.96	5231
Upper 95% FL	588.39	†	228.44	†	186.81	†	140.22	†
Lower 95% FL	137.82	1582	147.12	4768	121.11	4488	95.07	2747
Slope±SEM	1.13±0.11		0.92±0.10		0.89±0.10		0.99±0.10	
Number of eggs tested	360		360		360		360	
χ^2	10.48		1.50		1.49		3.69	
p-value	0.01		0.68		0.68		0.29	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 30, 60, 120, 240 and 480 mg L^{-1}. † Estimated LD_{95} value was too far beyond tested dosage range to be reliable

Table 8: Toxicity of acetone to 3-4 day-old eggs of *Plodia interpuctella* exposed for 24, 48, 72 and 96 h at 17±1°C in 1150 mL jars

	Exposure period (h)							
	24		48		72		96	
	Dosage mg L^{-1}							
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dosage	209.80	14618	163.76	5653	122.12	8865	99.87	5298
Upper 95% FL	270.53	54338	315.36	†	150.05	4111	120.52	13727
Lower 95% FL	169.88	6227	96.32	1486	99.12	†	81.39	2778
Slope±SEM	0.89±0.09		1.06±0.11		0.88±0.09		0.95±0.09	
Number of eggs tested	360		360		360		360	
χ^2	1.33		8.25		3.26		3.71	
p-value	0.72		0.04		0.35		0.29	

Three replicates (20 eggs per replicate) were tested in each of five acetone dosages and control treatment. Acetone quantities used were 30, 60, 120, 240 and 480 mg L^{-1}. † Estimated LD_{95} value was too far beyond tested dosage range to be reliable

Tests at 17±1°C: At 17±1°C and 24 h exposure, the sensitivity order of the age groups of eggs to acetone was measured as 1-2 day-old > 0-1 day-old > 2-3 day-old > 3-4 day-old eggs (Table 5-8). At this temperature and 24 h exposure the dosage required to control 50% of 1-2 day-old eggs was 301.17 mg L^{-1} (Table 6). As at 27±1°C here was considerable overlap in 95% fiducial limits of dosage-mortality regression lines. Therefore, in such cases no statistically significant difference between the estimated LD_{50} values was observed.

DISCUSSION

Fumigation is one of the most successful methods of rapidly controlling insect's infesting stored-products. In evaluating the effectiveness of a fumigant against an insect, it is essential that the dosage recommended be base on the most tolerant stage of the target insect to the fumigant. Studies on different insect species agree that the egg is the most difficult stage to kill (Su and Scheffrahn, 1990; Drinkall *et al*., 1996). Williams and Sprenkel (1990) working with different age groups of *Lyctus brunneus* (Stephens) and *Euvrilletta peltata* (Harris) eggs reported that an intermediate age group proved less susceptibility to Sulfuryl Fluoride (SF) than younger or older eggs. In the present study, the results indicate that the dosage and exposure period required to achieve high mortality was dependent on the egg developmental stage. The most tolerant eggs were aged 1-2 days. In this age group there was sufficient indication that longer exposure period achieved better kill than shorter ones of similar dosage. From this point of view results were in agreement with the findings of Su *et al.* (1989) who studied the toxicity of SF to *Coptotermes formosanus* Shiraki over varied exposure times. They reported that time and dosage was highly interchangeable but there was relatively advantage with longer exposure period.

In the present study, there was no evidence of a hatch delay longer than the time spent under vapors for eggs exposed at 17±1 or 27±1°C, indicating that some development must have occurred under fumigation. This finding would agree with the data collected by Bell (1976) who have demonstrated that the development of stored-products moth's eggs may continue under fumigation period.

A new approach in fumigation research could be the use of less hazardous substances, which are more compatible with environment. The application of acetone as an insect control material may be an appropriate approach to this objective. Little information is available on acetone's vapors efficacy against insect eggs. In view of the wide usage of acetone in toxicological studies, information on its fumigant action against insects could be useful in interpretation of toxicological data. In the current study, acetone vapors was toxic to all age groups of eggs of *P. interpunctella.* This result is in agreement with the finding reported by Tunç *et al.* (1997) who have provided the only efficacy data of acetone vapors against 0-1 day-old eggs of *Ephestia kuehniella* Zeller and *Tribolium castaneum* (Herbst) at 26°C. The mechanism(s) involved in the insecticidal action of acetone vapors are not known. Symptoms like mild skin irritation, or headache due to prolonged inhalation of acetone vapors were reported in industry sittings. Acetone vapor is flammable from 2.6 to 12.8 vol.% in air at 20°C (Howard, 1991). To avoid flammability it can be applied under CO_2-enriched atmospheres, but this would need confirmation by relevant studies. Acetone is a polar liquid and miscible in all proportions with water. Hence, its concentration could decrease through sorption by the stored-products and this could render it ineffective. Further research is, however, needed to check this speculation and evaluate eligibility of acetone as an insect control compound.

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