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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (7): 953-963, 2008
ISSN 1028-8880

Studies on the Toxicity of Acetone, Acrolein and Carbon Dioxide on Stored-Product Insects and Wheat Seed

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Abstract: In laboratory experiments toxicity of acetone, acrolein and carbon dioxide were investigated against 4 species of stored-product insects. In all experiments, acrolein was the most toxic compound to the tested insects. In empty-space trials, estimated LD_{50} values of acrolein for adults of *Tribolium castaneum* (Herbst) (Tenebrionidae), *Rhizopertha dominica* (F.) (Bostrychidae), *Sitophilus oryzae* L. (Curculionidae) and *Oryzaephilus surinamensis* L. (Silvanidae) were 7.26, 6.09, 6.37 and 5.65 μl L^{-1}, respectively. Penetration tests revealed that acetone and acrolein vapors could penetrate into the wheat mass and kill concealed insects in interkernel spaces. Comparison of LD_{50} values of acrolein between empty-space tests and penetration experiments indicated that the increase in penetration toxicity was 4.96, 4.54, 3.64 and 3.43-fold for *T. castaneum*, *R. dominica*, *S. oryzae* and *O. surinamensis*, respectively. The effect of carbon dioxide on the toxicity of acrolein and acetone was synergistic. In the hidden infestation trials, the acrolein vapors destroyed the developmental stages of *S. oryzae* concealed inside the wheat kernels and resulted in a complete control with concentration of 80 μl L^{-1} for 24 h and subsequently observed during 8 weeks after the exposure. Wheat germination and plumule length was reduced following exposure to all doses of acrolein. Acetone and carbon dioxide were harmless to wheat seed viability. The mixture of carbon dioxide with acrolein can be considered as a potential fumigant for replacing methyl bromide or phosphine under ambient storage conditions specifically in empty-space fumigations.

Key words: Acrolein, acetone, fumigation, wheat, stored-product insects

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 the disinfecting of commodities and treatment of empty stores. In the last years the removal of some fumigants from the market has resulted in a wider use of methyl bromide and phosphine (Leesch, 1995).

Methyl bromide is a broad-spectrum pesticide used in the control of pest insects, nematodes, weeds, pathogens and rodents. The consumption of methyl bromide is very extensive throughout the world, but its correct use needs a great level of expertise. Exposure to this chemical will affect not only to the target pests it is used against, but to non target-organisms as well. In practice, the increase in tolerance to methyl bromide has not been reported in most insects in the field. 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; Casanova, 2002).

Phosphine is widely used for insect pest elimination in stored products with great effectiveness in a variety of habitats for a long time (Rajendran and Muralidharan, 2001). However, conventional use of this compound has shown frequent failure to control insects specifically in resistance strains. Consequently, certain insects have developed resistance to phosphine (Bell and Wilson, 1995; Collins *et al*., 2002; Benhalima *et al*., 2004).

The recent emphasis objectionable insecticide residue in foodstuffs has prompted considerable thought and research in the human health and the environment (Brewer *et al*., 1994). Any compound that can reduce the insecticide load in a particular storehouse with adequate effectiveness to control insects may be of utmost importance in stored-product insect control programs. The main challenge is now for alternative substances which are safe enough, readily available, inexpensive, convenient to use and without substantial disruption of

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the environment. According to these criteria acrolein, acetone and carbon dioxide as less hazardous compounds were selected for testing.

Acetone is generally recognized as a less deleterious substance to man and the environment, inexpensive, commonly available and convenient to use (Tunç *et al*., 1997). Acetone is widely used in laboratories as a solvent in applying insecticides to insects. 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).

Acrolein is colorless liquid, with an intensively acrid odor, relatively nonpersistent and does not bioaccumulate in orgamisms. The half-life in aquatic systems ranges from less than one to ≈ four days. Acrolein is not carcinogenic and shows little embryotoxic and teratogenic behavior (Ghilarducci and Tjeerdema, 1995). Because of its high toxicity to insects (Carroll *et al*., 1982) and fast acting characteristics, acrolein could be highly efficacious in fumigation systems.

For preservation of seed grain, it is essential that the seed is tolerant to the fumigant. Seed viability is a good indicator of grain quality (Beraga Jr. *et al*., 2003). Therefore, in this study three measurements related to seed quality were investigated.

Treatment of stored food commodities with CO_2-riched atmospheres has been studied previously (MBTOC, 1998). The treatment meets the demands of the organic market. The technology can be adopted where cheap sources of CO_2 are available and the storage structure is well sealed. CO_2-rich atmosphere has been found suitable for the protection of dried fruits in Turkey (Donahaye *et al*., 1998; Ferizli and Emekci, 2000) and for treating grain elevators in Canada (Macrotte and Tibelius, 1998). CO_2 treatment under elevated temperatures and with low concentrations of phosphine has been recommended as a replacement for methyl bromide in treating flour mills and other food processing facilities in Canada and other developed countries (Mueller, 1998). CO_2 is not carcinogenic and has no adverse effects on the environment. Carbon dioxide treatment requires a long period of 10 days or more. This drawback can be overcome by raising the treatment temperature or by applying the gas under high pressure. Carbon dioxide up to 30% atmospheric composition is tolerable to insects (Leoge *et al*., 1995; Manna *et al*., 1999).

In the absence of suitable alternative fumigants, any loss of phosphine and methyl bromide fumigants will have serious implications to the safety/protection of stored and export food commodities against pest organisms. Hence there is a need to explore for alternative fumigants that are safe to our food and the environment.

The purpose of this study was to determine the fumigant action of acrolein, acetone, carbon dioxide and mixtures of these compounds against stored-products insects and wheat seed viability.

MATERIALS AND METHODS

This study was carried out in four stages at the laboratory of Entomology, Urmia University during the period of 2006-2007. In the first stage, acrolein and acetone were tested against insects in an empty -space. In the second stage, the effect of acrolein, acetone and carbon dioxide were determined by confining the insects under known amount of wheat and applying the compounds in the headspace above the wheat. In the third stage, acrolein and acetone were distributed throughout the wheat mass to determine their effectiveness against various life stages of *S. oryzae*, which has internal immature stages. Finally, in the fourth stage, the effect of the test compounds on wheat seed viability was determined.

Chemicals

Acetone: The test acetone was 99.9% and supplied by Merck Co. Ltd. This compound is polar and a highly volatile and flammable liquid (Howard, 1991).

Acrolein: The test acrolein (2-propanal, acrylaldehyde) was 95% active ingredient with density of 0.8389 g mL^{-1} at 20°C and supplied by Merck-Schuchardt, Mûnchen, Germany. All doses used in this study are expressed as commercial formulations.

Carbon dioxide: The carbon dioxide gas was applied to containers from a vessel of liquid carbon dioxide with appropriate vaporizers and pressure regulators to control the flow rate. To determine the LD_{50} values of carbon dioxide mixture with either acrolein or acetone, the procedure developed by White and Collins (1991) was adopted. The test containers each of 31 L capacity containing wheat and insects were used in these tests. The samples were exposed to different dosages of CO_2 at 27±2°C. Due to on field and applied nature of the research the volume of carbon dioxide in the chamber air was overlooked. Preliminary tests revealed that the atmospheric composition containing ≈ 10% CO_2 is harmless to insects. The atmospheric composition of chamber was modified to contain 10% CO_2 and LD_{50} dosage of acrolein or acetone was introduced to the chamber. The test containers and control group were stored at 27±2°C for 24 h.

Insects: *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (Coleoptera: Curculionidae), *Rhizopertha dominica* (Coleoptera: Bostrychidae) and *Oryzaephilus surinamensis* (Silvanidae) adults were collected from local mills, stores and shops in Urmia (37.39°N 45.40°E), a town in Iran. Cultures were established and maintained on healthy uncontaminated food at 27±2°C and 60±10% r.h. in glass bottles 1.5 L) covered with pieces of muslin cloth fixed by rubber bands. All insects were cultured under moderately crowded conditions to ensure proper development and equal size of the resultant adults.

T. castaneum was reared on a 50:50 mixture of wheat flour and corn meal. This mixture contained 5% brewer's yeast. *S. oryzae* and *R. dominica* were reared on soft red winter wheat and *O. surinamensis* was kept on heat-sterilised broken corn grain and oat.

Bioassays: The following developmental stages of insects were used in these tests: (i) *T. castaneum* adults, 14±3 day old, (ii) *S. oryzae* and *R. dominica* adults 7±2 day old, (iii) *O. surinamensis* 5 day old. Bioassay procedures were identical in all trials. Preliminary dose-mortality tests were done before each experiment to determine a range of doses that would produce ≈ 25-75% mortality at the lowest and the highest doses, respectively (Robertson and Preisler, 1992). In each experiment insects were allowed to recover on their usual media at 27±2°C and 60±10% r.h. In each bioassay mortality was recorded after exposure and recovery period. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead.

An 1150 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.

Empty-space tests: Adults of *T. castaneum*, *S. oryzae*, *R. dominica* and *O. surinamensis* were fumigated for 24 and 48 h in 1150 mL glass jars, separately. The test insects were confined in cages constructed with 40 mesh wire gauze. Each cage contained 30 insects and 3 g of food. 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 appropriate amount of each concentration of acetone or acrolein 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 compound 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 or acrolein was deposited on the blotting paper.

After exposure, the insects were transferred to clean jars containing rearing medium and maintained under rearing conditions. Mortality was recorded 4, 7, 7 and 14 day after termination of the exposure for *O. surinamensis*, *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, four cages (each containing 30 adults of one insect species with 3 g of food) were placed horizontally at the bottom of a steel container with 31 L capacity. The container was filled with 26 kg of soft red winter wheat with 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 consumed acetone, acrolein and carbon dioxide. Each concentration was replicated three times. The control container was prepared in an identical manner without application test compound. After exposure periods of 24 h, the insects were transferred to clean glass jars containing food and held at 27±2°C and 60±10% r.h. Mortality rates of *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum* were recorded after 4, 7, 7 and 14 day following the treatment, respectively.

Hidden infestation tests: A sample of 100 g of wheat containing eggs, larvae and pupae of *S. oryzae* was collected from the stock culture and placed in 1150 mL glass jar. The procedure of applying either acrolein or acetone to blotting paper and sealing the holes was similar to that described earlier. To distribute the test compound in the wheat mass, jars were briefly shaken by hand and tumbled mechanically for 5 min. After tumbling, jars were held under standard conditions for 72 h. After exposure, the insects and wheat were transferred to clean jars and 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, in every week, emerged adults were counted and discarded. Control groups were treated identically except for those with no test compound deposited onto the blotting paper. The experiment was replicated three times and results were pooled.

Germination tests: Germination tests were conducted according to the principles stated in International Seed Testing Association (1999) methods with minor modification. Seeds of wheat were fumigated with acetone, acrolein and carbon dioxide for 24 h in 1150 mL glass jar separately. For each compound fifty treated seeds were soaked with 50 mL of distilled water for 24 h.

Pre-treated seeds were spaced uniformly on sheet paper and placed in a germination cabinet for 8 day at 20°C. Non-fumigated seeds treated identically and served as control standards for comparison. Each experiment was replicated four times on four different days. The number of germinated seeds was counted after 4 and 8 days and the mean plumule length of fifty seedlings was determined at 8 days.

Data analysis: Mortality data from all bioassays were analyzed with SPSS software (SPSS Inc, 1993). Probit analysis was used to estimate LD_{50} and LD_{95} values and the slopes of the regression lines. The values and significance of χ^2 and the 95% CL for resistance ratios were determined according to Robertson and Preisler (1992). Parallel regression lines were also compared using overlapping confidence limits ($p \leq 0.05$) of relative potencies as the criterion to detect significant differences in mortality. In hidden and germination tests, the data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to determine statistical differences between means at $\alpha = 0.05$.

RESULTS

In all experiments acrolein was more toxic than acetone to the tested insects. On the basis of LD_{50} values, the sensitivity order of the insects to acrolein and acetone was measured as*: O. surinamensis* > *R. dominica* > *S. oryzae* > *T. castaneum* adults. Overlap in 95% confidence limits of resistance ratios was detected (Table 1, 2). Therefore, statistically non-significant difference among the estimated LD_{50} values was observed. At the LD_{95} level, the dose of acrolein required for killing the most tolerant species was 31.20 and 21.48 μl L^{-1} at 24 and 48 h exposure periods, respectively (Table 3, 4).

Results showed that acrolein and acetone penetrated thoroughly in the wheat mass and killed the tested insects (Table 5-8). Based on the LD_{50} values, when acrolein was applied to the wheat mass headspace, the dose required to achieve 50% mortality after 24 h exposure time was 3.43, 3.64, 4.54 and 4.96 fold more than that required for the empty-space tests for *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively (Table 3, 7). These differences indicated that acetone was sorbed or breakdown by the wheat mass. In the case of acetone,

Table 1: Toxicity of acetone to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 24 h at 27±2°C in 1150 mL jars (empty-space tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration μl L^{-1} determined for							
Lethal concentration	24.5	78.1	26.2	89.5	25.1	83.2	22.1	69.3
Upper 95 (%) FL	28.8	138.9	31.8	179.1	29.9	157.1	25.3	110.9
Lower 95 (%) FL	21.8	56.4	22.9	61.6	22.1	58.7	19.8	52.1
Slope±SE	3.27±0.48		3.08±0.49		3.17±0.49		3.32±0.43	
No. of insects tested	360.0		360.0		360.0		360.0	
χ^{2a}	1.2		3.1		2.6		1.4	
P	0.7		0.3		0.4		0.6	
RR_{50}[b] (95% CL)	1.1		1.1		1.1			

Three replicates (20 insects per replicate) were tested in each of five acetone concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of $p>0.05$ and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acetone quantities used were 0, 6, 12, 18, 24 and 30 mg L^{-1}

Table 2: Toxicity of acetone to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 48 h at 27±2°C in 1150 mL jars (empty-space tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration μl L^{-1} determined for							
Lethal concentration	16.25	38.25	18.00	40.59	17.06	37.05	15.96	59.6
Upper 95 (%) FL	19.91	83.73	22.21	86.48	21.27	114.04	19.79	95.7
Lower 95 (%) FL	12.43	28.38	14.39	30.07	12.08	27.26	12.34	47.3
Slope±SE	4.42±0.51		4.64 ±0.49		4.88±0.62		4.55±0.46	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	5.37		5.81		6.95		6.36	
p	0.14		0.12		0.07		0.09	
RR_{50}[b] (95% CL)	1.02		1.13		1.06			

Three replicates (20 insects per replicate) were tested in each of five acetone concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of $p>0.05$ and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*) Acetone quantities used were 0, 6, 12, 18, 24 and 30 mg L^{-1}

Table 3: Toxicity of acrolein to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 24 h at 27±2°C in 1150 mL jars (empty-space tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration μl L^{-1} determined for							
Lethal concentration	6.09	24.9	7.26	31.20	6.37	24.0	5.65	21.1
Upper 95 (%) FL	6.97	37.8	8.32	51.01	7.25	35.7	6.44	30.2
Lower 95 (%) FL	5.18	19.1	6.26	23.05	5.45	18.6	4.81	16.7
Slope±SE	2.69±0.31		2.59±0.34		2.85±0.36		2.87±0.26	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	4.15		4.08		3.45		4.05	
p	0.25		0.24		0.32		0.26	
RR_{50}[b] (95% CL)	1.07		1.28		1.12			

Three replicates (20 insects per replicate) were tested in each of five acrolein concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 3, 6, 9, 12 and 15 mg L^{-1}

Table 4: Toxicity of acrolein to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 48 h at 27±2°C in 1150 mL jars (empty-space tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration μl L^{-1} determined for							
Lethal concentration	3.91	17.55	4.60	21.48	4.41	19.78	3.57	17.59
Upper 95 (%) FL	4.68	26.07	5.43	33.34	5.20	29.60	4.36	27.14
Lower 95 (%) FL	3.00	13.73	3.66	16.40	3.50	15.31	2.61	13.55
Slope±SE	2.52±0.35		2.46±0.33		2.52±0.34		2.37±0.35	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	2.32		2.63		3.25		2.90	
p	0.51		0.45		0.36		0.41	
RR_{50}[b] (95% CL)	1.09		1.28		1.23			

Three replicates (20 insects per replicate) were tested in each of five acrolein concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 3, 6, 9, 12 and 15 mg L^{-1}

Table 5: Toxicity of acetone to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 24 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration μl L^{-1} determined for							
Lethal concentration	267.6	2122.0	311.1	1996.0	285.8	1844.0	231.6	1610
Upper 95 (%) FL	509.6	1822.0	674.8	1962.0	565.3	1534.0	367.4	7932
Lower 95 (%) FL	199.5	887.2	225.5	837.1	212.4	803.2	181.7	776
Slope±SE	1.83±0.4		2.03±0.4		2.03±0.46		1.95±0.38	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	2.01		3.33		2.39		0.34	
p	0.57		0.34		0.49		0.95	
RR_{50}[b] (95% CL)	1.15		1.34		1.23			

Three replicates (20 insects per replicate) were tested in each of five acetone concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acetone quantities used were 0, 2, 4, 6, 8, 10 and 12%

similar pattern of toxicity was observed (Table 1 and 5). When carbon dioxide was introduced to the 31 L capacity container containing LD_{50} dosage of acrolein, the estimated LD_{50} value decreased 2.07, 1.32, 2.36 and 1.74 fold for *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively (Table 7 and 10). For acetone, this criteria was 2.15, 2.10, 1.69 and 1.60 fold in the same order (Table 5 and 9).

Table 11 shows the toxicity of acrolein and acetone to *S. oryzae* concealed in wheat, respectively. The results show the effectiveness of acrolein as a fumigant-like compound where insects were concealed inside the

Table 6: Toxicity of acetone to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 48 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration µl L^{-1} determined for							
Lethal concentration	211.4	1325.0	222.8	1310.0	208.1	1145.0	187.9	1126
Upper 95 (%) FL	310.7	5297.0	337.1	5503.0	1815.0	6681.0	257.1	3720
Lower 95 (%) FL	170.5	687.9	178.4	677.1	141.5	411.6	155.2	622
Slope±SE	2.06±0.38		2.13±0.41		2.23±0.42		2.11±0.37	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	3.52		4.21		5.55		2.52	
p	0.32		0.24		0.14		0.47	
RR_{50}[b] (95% CL)	1.12		1.18		1.10			

Three replicates (20 insects per replicate) were tested in each of five acetone concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acetone quantities used were 0, 2, 4, 6, 8, 10 and 12%

Table 7: Toxicity of acrolein to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 24 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration µl L^{-1} determined for							
Lethal concentration	27.69	357.9	36.00	1111.00	23.24	132.40	19.42	136.0
Upper 95 (%) FL	44.95	3718.0	108.20	2424.00	29.27	352.70	24.16	416.0
Lower 95 (%) FL	21.47	139.4	24.58	239.90	19.70	79.53	16.22	77.9
Slope±SE	1.48±0.32		1.10±0.31		2.17±0.36		1.94±0.34	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	2.19		2.45		3.46		2.70	
p	0.53		0.48		0.33		0.44	
RR_{50}[b] (95% CL)	1.42		1.85		1.19			

Three replicates (20 insects per replicate) were tested in each of five acrolein concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 2, 4, 6, 8, 10 and 12%

Table 8: Toxicity of acrolein to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 48 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration µl L^{-1} determined for							
Lethal concentration	15.08	91.57	16.66	85.32	10.95	105.62	9.06	373
Upper 95 (%) FL	17.80	200.24	19.50	170.57	13.50	327.15	12.82	3344
Lower 95 (%) FL	12.61	59.58	14.23	57.68	8.02	61.33	3.73	111
Slope±SE	2.09±0.32		2.31±0.34		1.67±0.31		1.02±0.30	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	3.86		4.13		1.79		3.52	
p	0.28		0.25		0.62		0.32	
RR_{50}[b] (95% CL)	1.66		1.83		1.20			

Three replicates (20 insects per replicate) were tested in each of five acrolein concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p>0.05 and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 2, 4, 6, 8, 10 and 12 %

wheat. An inverse relationship between acrolein dose and the number of *S. oryzae* survivors was observed. No adults of *S. oryzae* emerged from wheat that had been exposed to acrolein at the rate of 80 µl L^{-1}. This dose of acrolein was sufficient to kill different developmental stages of *S. oryzae* inside the wheat kernels. In these tests acetone caused negligible mortality rate on *S. oryzae*. The control groups of wheat that were not treated either with acrolein or acetone yielded 1105 and 930 adults of *S. oryzae* during the same incubation period, respectively.

Table 9: Toxicity mixture of acetone with carbon dioxide to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinamensis* exposed 24 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration µl L^{-1} determined for							
Lethal concentration	158.3	804.9	194.2	1570.0	135.5	630.9	107.70	402
Upper 95 (%) FL	197.5	1992.0	291.4	8328.0	160.6	1262.0	154.60	2729
Lower 95 (%) FL	135.0	498.7	155.0	745.0	117.5	424.7	72.88	235
Slope±SE	2.32±0.38		1.81±0.36		2.46±0.37		2.87±0.35	
No. of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	3.89		1.39		3.28		6.83	
p	0.27		0.71		0.35		0.08	
RR_{50}[b] (95% CL)	1.46		1.80		1.25			

Three replicates (20 insects per replicate) were tested in each of five mixture of acetone with carbon dioxide concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of $p>0.05$ and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Mixture quantities used were 0, 2, 4, 6, 8, 10 and 12%

Table 10: Toxicity of mixture acrolein with carbon dioxide to *Rhizopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae* and *O. surinameusis* exposed 24 h at 27±2°C in 31 L container (penetration tests)

	Toxicity to species							
	R. dominica		*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Toxicity value	Concentration µl L^{-1} determined for							
Lethal concentration	11.69	57.35	20.65	141.18	17.50	116.13	9.34	46.86
Upper 95% FL	13.60	96.65	25.87	419.20	21.13	299.59	11.08	76.77
Lower 95%FL	9.65	41.96	17.35	81.18	14.70	70.59	7.32	34.94
Slope±SE	2.38±0.34		1.97±0.34		2.00±0.32		2.34±0.33	
Number of insects tested	360.00		360.00		360.00		360.00	
χ^{2a}	4.24		4.36		4.14		4.29	
p	0.24		0.23		0.25		0.23	
RR_{50}[b] (95% CL)	1.25		2.21		1.87			

Three replicates (20 insects per replicate) were tested in each of five mixture of acrolein with carbon dioxide concentrations and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of $p>0.05$ and the data fits regression model. [b]Resistance Ration (RR) is equal to LD_{50} each species/LD_{50} of the most susceptible species (*O. surinamensis*). Mixture quantities used were 0, 2, 4, 6, 8, 10 and 12%

Table 11: The adult emergence from immature stages of the *Sitophilus oryzae* exposed to various doses of acetone and acrolein for 24 h in 1150 mL jars (hidden infestation tests)

		Emergence at week								
Compound	Dose (mg L^{-1})	1	2	3	4	5	6	7	8	Mean±SE
Acetone	0	197	156	210	115	76	162	14	0	116.25±28.21[a]
	40	45	92	120	117	46	36	4	1	57.62±16.6 [ab]
	80	52	79	14	0	6	8	0	0	19.87±10.42[b]
	120	39	18	21	0	0	0	1	0	9.87±10.42[b]
	160	5	8	0	0	4	0	0	0	2.13±1.10[b]
	200	0	0	0	0	0	3	1	0	0.50±1.10[b]
Acrolein	0	265	293	242	75	53	159	17	1	138.12±41.4[a]
	40	85	70	33	28	2	2	0	0	27.50±11.91[b]
	50	76	61	29	21	2	3	0	1	24.12±10.46[b]
	60	38	9	0	0	0	1	0	0	6.00±4.70[b]
	70	25	0	0	0	0	0	0	0	3.12±3.12[b]
	80	0	0	0	0	0	0	0	0	0

For each compound means within column with similar letter(s) are not significantly different ($p \geq 0.05$) according to Tukey's test. For each dose and control 4 replicates were used with 100 g of wheat per replicate

The standard error from four replicates of 50 seeds each was less than 1.5% of the mean value in all cases (Table 12). Doses ranging from 40 to 90 µl L^{-1} of acrolein significantly decreased the germination potential in comparison with unfumigated seed. Results from vigor test at 4 day count were unchanged at 8 days count (total germination test). In all cases the standard error in plumule length was less than 3% of the mean value. Plumule length was inversely related to acrolein dose. Acrolein significantly reduced plumule length at all tested

Table 12: Percentage germinability and plumule length of wheat fumigated with acrolein at different doses for 24 h exposure

	Viability	
Dose (mg L^{-1})	Germination rate (%)* (Mean±SEM)	Plumule length (cm) (Mean±SEM)
0	79.29±0.74^{a}	10.67±0.30^{a}
40	76.79±0.62^{ab}	9.35±0.32^{b}
50	74.41±0.68^{bc}	7.62±0.22^{c}
60	72.07±0.62^{c}	6.75±0.08^{cd}
70	46.00±1.37^{d}	6.17±0.07^{cd}
80	44.85±1.46^{d}	5.97±0.11^{de}
90	43.70±0.85^{d}	5.43±0.22^{de}

Means within columns with similar letter(s) are not significantly different ($p \geq 0.05$) according to Turkey's test. *Data were transformed using arcsine square root prior to analysis

Table 13: Percentage germinability and plumule length of wheat fumigated with acetone at different doses for 24 h exposure

	Viability	
Dose (mg L^{-1})	Germination rate (%)* (Mean±SEM)	Plumule length (cm) (Mean±SEM)
0	78.15±0.70^{a}	10.75±0.36^{a}
40	75.62±1.05^{ab}	10.45±0.41^{a}
80	73.44±1.32^{bc}	10.52±0.25^{a}
120	73.08±0.66^{bc}	10.10±0.14^{a}
160	69.75±0.63^{bc}	10.07±0.20^{a}
200	71.61±0.87^{bc}	10.37±0.24^{a}

Means within columns with similar letter(s) are not significantly different ($p \geq 0.05$) according to Turkey's test. *Data were transformed using arcsine square root prior to analysis

Table 14: Percentage germinability and plumule length of wheat fumigated with carbon dioxide at different exposure duration

	Viability	
Time per h	Germination rate (%)* (Mean±SEM)	Plumule length (cm) (Mean±SEM)
0	73.58±0.43^{b}	9.63±1.51^{a}
24	76.20±0.94^{a}	10.72±1.24^{a}
48	76.44±0.36^{a}	11.76±1.58^{a}
72	72.81±0.48^{b}	11.78±1.61^{a}

Means within columns with similar letter(s) are not significantly different ($p \geq 0.05$) according to Turkey's test. *Data were transformed using arcsine square root prior to analysis

doses in comparison with unfumigated seed (Table 12). Acetone and carbon dioxide showed no deleterious effect on wheat seed viability (Table 13, 14).

DISCUSSION

For the control of stored-products pest insects, particularly in grain, farmers rely mostly on the treatment of contact insecticide to raw cereals (Bond, 1984; Daglish, 1998). Because such treatments may result in the presence of residues in those products prepared from treated grain, there are restrictions in the level of insecticide residues allowed 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-products insects are limited (White and Leesch, 1995; Arthur, 1999).

For a long time, the main stored-grain protectant insecticide was de-odorized malathion (Daglish, 1998; Arthur, 1999). Unfortunately, most stored-products insects are substantially resistant to this insecticide and an alternative substitution should be necessary (Leesch, 1995; Arthur, 1999).

Fumigation is one of the most successful methods of rapidly controlling insects infesting stored foodstuffs. The cost and health risk of fumigation seems to be lower than traditional methods of preservation (Weller and Morton, 2001). Thus, it appears that fumigation will be the backbone and indispensable component of stored-products insects control programs in the immediate future. A good fumigant should have some characteristics consistent 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 the present time, large proportions of stored foodstuffs are fumigated with methyl bromide and phosphine. The greatest deficiency in the use of methyl bromide was that in many instances the major reliance has been placed on the methyl bromide fumigation and the stock management was neglected. Therefore, reinfestation occurred soon after the fumigation was completed. Consequently, frequent fumigation was necessary and grain often had bromide residues in excess of the permissible level. Phosphine as a fumigant offers a cost-

effective method of insects control (Rajendran and Muralidharan, 2001). Strict controls on detectable concentrations of phosphine are necessarily imposed by some organizations. 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 be the use of generally recognized less hazardous substances, which are more compatible with environment. The application of acetone, acrolein and carbon dioxide as less hazardous compound, may be an appropriate approach to 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 extremely uncommon under fumigation conditions. Although acetone is not a novel compound, as yet it is not registered for use as a fumigant. However, in view of the wide usage of acetone in toxicological studies, information on its action against insects including as a fumigant could be useful in interpretation of toxicological data. In the current study, acetone was toxic to all tested insects 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 24 h exposure indicated that the increase in the dosage between the empty-space LD_{50} and the penetration toxicity was approximately 10.45, 11.35, 11.86 and 10.88-fold for *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. Since acetone is polar and miscible with water, its concentration could decrease through sorption by the wheat. Apparently, this is a drawback to acetone in speculation on replacing methyl bromide or phosphine.

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* (those in most developmental stages live inside the seed) could represent a difficult challenge to any potential fumigant. Acetone showed negligible biological effectiveness in the control of *S. oryzae* in hidden infestation tests. However, it has merit to be considered as a less hazardous compound for control of the tested insects in empty-spaces.

Acrolein is produced during the combustion of fossil fuels, wood and cooking or processing of fat-containing foods (Rodriguez-Kabana *et al.*, 2003). Acrolein is an intense irritant and its irritancy may limit exposure to this substance. It is retained irreversibly in the respiratory tract after exposure by inhalation (Morris, 1996). Consequently, there is little, if any, distribution to other organs. In the present study, acrolein was very toxic to all tested insects in empty-space tests. This finding would agree with the data collected by Leesch (1995) who has demonstrated that acrolein was toxic to some insects in empty-space tests. Comparison of empty-space versus penetration toxicities after 24 h exposure indicated that the increase in the dose between the empty-space LD_{50} and the penetration toxicity was 3.43, 3.64, 4.54 and 4.96 fold for *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. Since acrolein is moderately soluble in water (Rodriguez-Kabana *et al.*, 2003), its vapors could decrease through sorption by the wheat. Therefore in the presence of wheat, more acrolein is needed for successful fumigation (Table 2 and 7). Moreover, the sorption is undesirable from the standpoints of the likelihood of odour remaining in the grain after fumigation and the problem of potentially unacceptable residue level.

The distinction between live seeds and germinated seeds is important since fumigants may cause injury by retarding germination as well as destruction of germinative capacity. Therefore, decrease in germination rate or plumule length after fumigation was adequate to prove a deleterious effect of acrolein on wheat seed viability

Acrolein, as an insect control compound, showed acceptable biological effectiveness against tested insects and interaction between acrolein and carbon dioxide was synergistic. With retrospect, due to sorption characteristics and deleterious effects on wheat seed viability, acrolein may have only limited use in grain fumigation. Nevertheless, since acrolein is highly toxic to insects and because methyl bromide may not be available for use as a fumigant in immediate future, mixture of acrolein with carbon dioxide could be considered as a potential compound for specifically empty-space fumigations.

Further research is necessary to ascertain other features mixture of acrolein with carbon dioxide specifically its effectiveness against the resistant strains of stored-products insects and the spectrum of activity under different environmental conditions.

ACKNOWLEDGMENTS

The authors wish to thank Urmia University vice president of research for funding this research project (No.:002/K/85). Technical assistance of Hoshang Golizadehi, Naser Vakelie and Hassan Kamyar (Agricultural Faculty, Urmia University) is gratefully acknowledged.

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