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Fumigant Action of Acrolein on Insects and Seed Viability

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Abstract: In laboratory experiments toxicity of acrolein vapors was investigated against 4 species of stored-product insects. In empty-space trials, estimated of the median lethal dosages of acrolein against adults of *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst), were 1.87, 2.35, 3.12 and 6.65 mg L^{-1}, respectively. Penetration tests revealed that acrolein vapors 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 24 h exposure indicated that the increase in penetration toxicity was 6.34, 6.31, 7.17 and 4.54-fold for *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. In the hidden infestation trials, the acrolein vapors destroyed all the developmental stages of *S. oryzae* and *R. dominica* concealed inside the wheat kernels, resulted in a complete control with dosage of 80 mg L^{-1} for 24 h and subsequently observed during 8 weeks after the exposure. Wheat germination rate was diminished by fumigation with acrolein. The plumule length was reduced following exposure to all dosages of acrolein. It is concluded that acrolein could be considered as a potential compound for empty-space fumigations.

Key words: Acrolein, toxicity, fumigation, 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 the disinfesting 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 phosphine and methyl bromide (Leesch, 1995).

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 resistance to phosphine (Collins *et al*., 2002). The consumption of methyl bromide is very extensive throughout the world. 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 (Casanova, 2002). Therefore, considerable research is under way worldwide to find out chemical and non-chemical alternatives to methyl bromide (Isikber *et al*., 2001). In this respect we have been looking for suitable alternative compounds. During the investigation on the toxicity of available compounds on stored-product insects, we observed that acrolein vapor was toxic to tested insects. We thought that acrolein might be a satisfactory replacement for methyl bromide in some situations and well deserved evaluated as a potential fumigant-like insect control agent.

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 effective alternative substances, which are inexpensive, convenient to use and without substantial disruption of the environment. According to these criteria acrolein as a potential insect control compound was selected for testing. Acrolein is colorless liquid, with an intensively acrid odor, relatively nonpersistent and does not bioaccumulate in organisms. The half-life in aquatic systems ranges from less than one to ≈ four days (Bowmer and Higgins, 1976). Acrolein is not carcinogenic and shows little embryo toxic 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 after fumigation (Pomeranz, 1987). Therefore, in this study three measurements related to seed quality were investigated.

The purpose of this study was to determine the fumigant action of acrolein vapors against stored-products insects and wheat seed viability.

MATERIALS AND METHODS

Chemical: The test acrolein (2-propenal, acrylaldehyde) was 95% Active Ingredient (AI) stabilized with ≈ 5% water and 300 ppm hydroquinone. Acrolein is a colorless liquid with density of 0.8389 g mL^{-1} at 20°C and supplied by Merck-Schuchardt, Műnchen, Germany. All dosages used in this study are expressed as commercial formulations.

Insects: *Oryzaephilus surinamensis* (Coleoptera: Silvanidae), *Sitophilus oryzae* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) 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 heat-sterilised food at 27±2°C and 60±10% r.h. inside 1150 mL glass jars covered with pieces of muslin cloth fixed by rubber bands. All insects were cultured under low crowded conditions to ensure proper development and equal size of the resultant adults.

Insects were reared for two generations before 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. *S. oryzae* and *R. dominica* were reared on soft red winter wheat. *O. surinamenis* was kept on heat-sterilised oat.

Bioassays: This study was carried out at Urmia University during the period of 2004-2005. 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* adults 5 day old. An 1150 mL tightly closed glass jar was used as a fumigant chamber in all tests. The insect's adults were assayed using the bioassay technique described by Leesch (1995). Preliminary concentration-mortality tests were done before each experiment to determine a range of dosages that would produce ≈25-75% mortality at the lowest and the highest dosages, respectively (Robertson and Preisler, 1992). In each bioassay mortality was recorded after exposure and recovery period. Since the mortality rates of the tested insects remained unchanged 24 h after exposure, this period of time accepted as exposure period in the bioassays. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead. Mortality data from all bioassays were analyzed with SPSS software (SPSS Inc., 1993). Probit analysis was used to estimate LC_{50} and LC_{95} values and the slopes of the regression lines. The values and significance of χ^2 and the 95% CL for relative toxicity 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 $\alpha = 0.05$.

Empty-space tests: Adults of *O. surinamensis*, S. oryzae, *R. dominica* and *T. castaneum* were fumigated for 24 h in 1150 mL glass jars separately. The insects were confined in cages constructed with 40-mesh wire gauze. Each cage contained 80 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. Acrolein dosages of 0, 2, 4, 6, 8, 10 and 12 mg L^{-1} were used. Each dosage of 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 acrolein was pipetted, the hole in each lid was sealed with plastic tape. In each test, the control jar was treated identically except that no acrolein was deposited on the blotting paper. For each insect species 2800 individuals were used. After exposure, the insects were transferred to clean jars containing rearing medium and maintained at 27±2°C and 60±10% r.h. Mortality was recorded 24 h after termination of the exposure. Each test was replicated five times on five different days and results were pooled.

Penetration tests: Adults of the insects were fumigated for 24 h separately. Acrolein dosages of 0, 5, 10, 15, 20, 25 and 30 mg L^{-1} were used. For each dosage, one cage containing 80 insects with 3 g of food was placed horizontally at the bottom of an 1150 mL glass jar. The jar was filled with 783 g insecticide free 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 acrolein. Each dosage was replicated five times. The control jar was prepared in an identical

manner, but no acrolein was used. For each insect species 2800 individuals were used. After exposure period, the insects were transferred to clean glass jars containing food and held at 27±2°C and 60±10% r.h. Mortality rates of the insects were recorded 24 h after termination of the treatment.

Hidden infestation tests: A sample of 80 g of wheat containing eggs, larvae and pupae of either *S. oryzae* or *R. dominica* was collected from the stock culture and placed in 1150 mL glass jar. The procedure of applying acrolein to blotting paper and sealing the holes were identical to that described earlier. To distribute the acrolein in the wheat mass, each jar was briefly shaken by hand and tumbled mechanically for 5 min. After tumbling, jar was held at 27±2°C for 24 h. After exposure, the insects and food were transferred to a clean jar and held for 8 weeks. Under the test conditions, 7-8 weeks were sufficient for eggs of the insects 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 acrolein deposited onto the blotting paper. The experiment was replicated four times on four different days and results were pooled.

Germination tests: Germination tests were conducted according to the principles stated in International Seed Testing Association (ISTA, 1999) methods with minor modification. Seeds of wheat were fumigated with acrolein for 24 h in 1150 mL glass jar. Fifty acrolein 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 d 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 four and eight days and the mean plumule length of fifty seedlings was determined at eight days.

RESULTS

Empty-space tests: Dosage-mortality values estimated from the probit analyses mortality are given in Table 1. On the basis of LC_{50} values, the sensitivity order of the insects to acrolein was measured as: *O. surinamensis* > *S. oryzae* > *R. dominica* > *T. castaneum* adults. No overlap in 95% confidence limits of LC_{95} was detected. Therefore, statistically significant difference among the estimated LC_{50} values was observed. At the LC_{95} level, the dosage of acrolein required for killing the most tolerant species was 29.56 mg L^{-1}.

Penetration tests: Results of the fumigation tests showed that acrolein penetrated thoroughly in the wheat mass and killed the tested insects (Table 2). There was a direct relationship between acrolein dosage and mortality rate of the tested insects. Based on the LC_{50} values, when acrolein was applied to the wheat mass headspace, the dosage required to achieve 50% mortality after 24 h exposure time was 6.34, 6.31, 7.17 and 4.54 times more than that required for the empty-space tests for *O. surinamensia*, *S. oryzae*, *R. dominica* and *T. castaneum*, respectively (Table 1 and 2). These differences indicated that acrolein was sorbed or breakdown by the wheat mass.

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

	Toxicity to species							
	Dosage mg L^{-1} determined for							
	O. surinamensis adult		*S. oryzae* adult		*R. dominica* adult		*T. castaneum* adult	
Toxicity value	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal dosage	1.87	14.32	2.35	13.81	3.12	24.36	6.65	29.56
Upper 95% CL	2.01	16.92	2.59	15.83	3.42	78.54	6.98	34.75
Lower 95%CL	1.74	12.38	2.11	12.33	2.80	44.24	6.33	25.81
Slope ± SE	1.86±0.07		2.14±0.11		1.84±0.11		2.53±0.12	
No. of insects tested	2800		2800		2800		2800	
χ^{2a}	3.46		4.07		4.72		5.22	
p-value	0.48		0.39		0.31		0.27	
RT_{50}[b] (95% CL)	-		1.26(0.707-1.00)		1.67(0.506-0.735)		3.56(0.221-0.354)	

Five replicates (80 insects per replicate) were tested in each of six acrolein dosages and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p are>0.05 and the data fits regression model. [b]Relative Toxicity (RT) is equal to LC_{50} each species/LC_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 2, 4, 6, 8, 10 and 12 mg L^{-1}

Table 2: Toxicity of acrolein to *Oryzaephilus surinamensis*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* exposed 24 h at 27±2°C under 783 g wheat mass (penetration tests)

	Toxicity to species							
	Dosage mg L^{-1} determined for							
	O. surinamensis adult		*S. oryzae* adult		*R. dominica* adult		*T. castaneum* adult	
Toxicity value	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal dosage	11.87	91.75	14.85	174.59	22.38	161.75	30.23	173.03
Upper 95% CL	12.70	116.55	16.05	255.70	24.24	224.69	33.43	245.93
Lower 95%CL	11.03	75.64	13.73	130.08	20.85	125.02	27.83	132.07
Slope ± SE	1.85 ± 0.12		1.54 ± 0.10		1.91 ± 0.13		2.17±0.15	
No. of insects tested	2800		2800		2800		2800	
χ^{2a}	5.41		1.57		4.63		2.72	
p-value	0.25		0.81		0.33		0.61	
RT_{50} [b]	-		1.25 (0.715-0.868)		1.89 (0.484-0.597)		2.54 (0.326-0.415)	
(95% CL)								

Five replicates (80 insects per replicate) were tested in each of six acrolein dosages and control treatment. [a]Pearson's χ^2 goodness-of-fit tests: all values of p are > 0.05 and the data fits regression model. [b] Relative Toxicity (RT) is equal to LC_{50} each species/LC_{50} of the most susceptible species (*O. surinamensis*). Acrolein quantities used were 0, 5, 10, 15, 20, 25 and 30 mg L^{-1}

Table 3: The adult emergence from immature stages of the *Sitophilus oryzae* and *Rhyzopertha dominica* exposed to various dosages of acrolein for 24 h at 27±2°C in 1150 mL jars (hidden infestation tests)

		Emergence at week								
Species	Dosage (mg L^{-1})	1	2	3	4	5	6	7	8	Mean±SE
S. oryzae	0	239	283	227	72	48	141	16	2	128.50±38.79[a]
	50	69	53	32	25	4	3	0	1	26.71±10.06[b]
	60	35	9	0	0	0	4	0	0	16.00±9.60[b]
	70	33	0	0	0	0	3	0	0	18.00±14.99[b]
	80	0	0	0	0	0	0	0	0	0.00
	90	0	0	0	0	0	0	0	0	0.00
R. diminica	0	38	35	47	67	105	79	137	223	91.37±22.49[a]
	50	9	13	35	0	1	0	1	0	11.80±6.24[b]
	60	5	9	24	0	0	0	0	0	12.66±5.77[b]
	70	2	5	0	0	0	0	0	0	3.5 0±1.49[b]
	80	0	0	0	0	0	0	0	0	0.00
	90	0	0	0	0	0	0	0	0	0.00

For each species means within column with similar letters are not significantly different ($p \geq 0.05$) according to Tukey's test. For each dosage and control 4 replicates were used with 80 g of wheat per replicate

Table 4: Percentage germinability and plumule length of wheat fumigated with acrolein at different dosages for 24 h exposure

	Viability	
Dosage (mg L^{-1})	Germination rate (%)*	Plumule length (mm)
0	78.50±0.60[a]	87.04±2.29[a]
50	75.24±0.33[ab]	73.28±2.02[b]
60	72.55±0.41[b]	67.88±1.58[bc]
70	46.14±0.70[c]	63.44±1.87[cd]
80	44.13±1.16[cd]	59.72±2.04[cde]
90	43.28±0.62[cd]	56.44±1.86[de]
100	42.26±0.95[d]	54.40±1.67[e]

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

Hidden infestation tests: Table 3 presents the toxicity of acrolein to *S. oryzae* and *R. dominica* populations concealed in wheat, respectively. The results show the effectiveness of acrolein as a fumigant-like compound where insects were concealed inside the wheat. An inverse relationship between acrolein dosage and the number of *S. oryzae* and *R. dominica* survivors was observed. No adults of *S. oryzae* and *R. dominica* emerged from wheat that had been exposed to acrolein at the rate of 80 and 90 mg L^{-1}. These dosages of acrolein were sufficient to kill different developmental stages of *S. oryzae* and *R. dominica* inside the wheat kernels. The control groups of wheat that were not treated with acrolein yielded 1028 and 731 adults of *S. oryzae* and *R. dominica* during the same incubation period, respectively.

Germination tests: The germination rate of wheat after exposure to acrolein is shown in Table 4. The standard error from four replicates of 50 seeds each was less than 3% of the mean value in all cases. Dosages ranging from 50 to 100 mg L^{-1} significantly decreased the germination potential in comparison with unfumigated seed. Results from vigour test at four-day count were unchanged at

eight-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 dosage. Acrolein significantly reduced plumule length at all tested dosages in comparison with unfumigated seed.

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, some farmers rely mostly on the treatment of contact insecticide to raw cereals. 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 numbers of suitable contact insecticides that can be used in the control of stored-products insects are limited (White and Leesch, 1995).

Fumigation is one of the most successful methods of rapidly controlling insects infesting stored foodstuffs. 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.

A new approach in fumigation research could be the use of effective substances, which are more compatible with environment. The application of acrolein as an insect control material may be an appropriate approach to this objective. Acrolein is produced during the combustion of fossil fuels, wood and cooking or processing of fat-containing foods (Lipari *et al*., 1984). 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. Although acrolein 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 acrolein vapors.

In the present study, acrolein was very toxic to all tested insects in empty-space. Leesch and Sukkestad (1980) determined the toxicity of methyl bromide to cigarette beetle and confused flour beetle adults in similar experiments. The LD_{50} obtained in that study for methyl bromide in empty-space tests against the cigarette beetle and confused flour beetle adults were 2.66 and 3.17 mg L^{-1}, respectively. In current study the estimated median lethal dosages (Table 1) indicate that apparently acrolein is somewhat as toxic as methyl bromide.

Comparison of empty-space versus penetration toxicities of acrolein after 24 h exposure indicated that the increase in the dosage between the empty-space LC_{50} and the penetration toxicity was from 4.54 up to 7.17-fold. 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.

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.

It is well established that a good fumigant must kill all stages of the target insects with acceptable dosage in a short period of time. 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. Acrolein, as an insect control compound, showed acceptable biological effectiveness against *S. oryzae* and *R. dominica* in hidden infestation tests. However, 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, acrolein could be considered as a potential compound for empty-space fumigations.

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