African Journal of Biotechnology Vol. 10(32), pp. 6133-6144, 4 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.080
ISSN 1684-5315

Full Length Research Paper

Effects of nitrogen and phosphine mixtures on stored-product insects' mortality

Golam Reza Sadeghi*, Ali Asgar Pourmirza and Mohammad Hasan Safaralizade

Department of Plant Protection, Agricultural Faculty, Urmia University, Urmia, Iran.

Accepted 29 April, 2011

A study to determine the effect of nitrogen mixed with phosphine on controlling stored-grain insects was conducted in the storehouse. Adults of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (F.), *Callosobrochus maculatus* (F.) and 3th larvae of *Plodia interpunctella* (Hubner) were exposed the mixture of nitrogen and phosphine. After exposure periods of 24 h, the insects were transferred to clean jars containing food and held at 27±2°C and 65 ±5% R H. Experiments were performed in different heights (30, 35, 40, 45 and 100 cm) and nutrition materials (rice, wheat and date), in penetration tests and empty-space tests. In empty-space trials, the highest mortality was for *S. oryzae* and *P. interpunctella*. In the penetration tests, treatment with high-pressure nitrogen and phosphine under different height and foodstuff resulted in different rates of mortality. The mixture of nitrogen and phosphine in the interaction between height and diet (height × diet) was not significant for *T. castaneum, R. dominica*, *C. maculatus* and *P. interpunctella,* but for *S. oryzae*, it was significant. The influence of nitrogen gas and phosphine in the date was more than it was in rice and wheat. The mixture of nitrogen with phosphine can be a suitable fumigant for decreasing phosphine under ambient storage conditions in penetration and empty-space fumigations.

Key words: Fumigant, Insect, nitrogen, phosphine, *Sitophilus oryzae*, *Tribolium castaneum*, *Rhizopertha dominica*, *Callosobrochus maculatus*, *Plodia interpunctella.*

INTRODUCTION

Stored products of agricultural are attacked by more than 1200 species of pests (Rajendran, 2002). In recent years, the number of fumigants available for use against stored-product insects has been decreased because of the removal of fumigants such as carbon disulphide and ethylene dibromide and only two fumigants, methyl bromide and phosphine are in use (Leesch, 1995). Methyl bromide depletes the ozone layer (Cassanova, 2002). Application of methyl bromide will be abolished in the developed countries in immediate feature (UNEP, 1998). Phosphine is an appropriate fumigant, but because of slowness in its function, insects' resistance to it has been developed in various countries (Zettler, 1993). Mills shows the constant use of phosphine as the main result for the increase in the insects' resistance to this fumigant (Mills, 2001; Mills and Pacho, 1996). Resistance to phosphine has been observed in *Sitophilus oryzae*, *Tribolium castaneum* and *Rhizopertha dominica* (Chimbe and Galley, 1996; Collins et al., 2002). The resistance of stored-grain insects to phosphine was reported following a worldwide survey carried out by the Food and Agriculture Organization (FAO) of the United Nations in 1972 and 1973 (Champ and Dyte, 1976), which detected resistance in 33 of the 82 countries surveyed, involving 82 of the 849 populations tested (Athie et al., 1998). Due to the Montreal protocol, pesticide resistance and the increased demand for organic grains, food manufacturers and grain handlers around the world are looking for novel ways to control insects and pathogens in stored commodities (Zettler et al., 1989; Zettler and Cuperus, 1990). Exposure of insects to toxic concentrations of atmospheric gases has been practiced for centuries and has been promoted in recent years as a biorational substitute for chemical fumigations (Navarro, 2006).

The cost of gases needed for controlled atmospheres may also be a hindrance to adoption. Carbon dioxide has been used as a viable alternative to phosphine for the control of insects attacking stored products (Jay, 1986). CO_2 is efficient only when concentrations higher than

*Corresponding author. E-mail: rsadeghi1357@yahoo.com Tel: +989125732940. Fax: +982733341956.

40% are maintained for long periods. Exposure periods longer than 14 days are required to kill the insects when the concentration of CO in the air is below 40% (Kashi, 1981). CO_2 due to the technology for the generation of low O_2 and high CO_2 burner gas through cleaned effluent from an exothermic gas-burning generator (Storey, 1973) is expensive and must be available in large supply for certain applications. N_2 for use in low O_2 treatments is less expensive and can be generated from ambient air, in which it is close to 80% concentration, via membrane-adsorption technology (Phillips and Throne, 2010). Ozonation is a powerful oxidant that reduces or inhibits mold spore development and kills stored product insects, therefore serving as a non-chemical alternative for stored grain protection (EPA, 1999; Mendez et al., 2003). Treatment of stored product food commodities with nitrogen has been studies previously (Rajendran, 2005). Gunasekaran and Rajendran (2005) demonstrated that O_2, N_2 and CO_2 were toxic to some stored–product insects. Comprehensive data on the toxicity of nitrogen against the rusty grain beetle, *Cryptolestes furrugineus* (Stephens) which is the major pest of stored spices and spice products, is limited (Li et al., 2009). Nitrogen is a common normally colourless, odourless, tasteless and mostly diatomic non-metal gas. It has five electrons in its outer shell, so it is trivalent in most compounds and nitrogen constitutes 78% of earth's atmosphere and is a constituent of all living tissues (Croswell, 1996). Nitrogen is an essential element for life, because it is a constituent of DNA and, as such, is part of the genetic code (Jahn et al., 2005). Nitrogen molecules occur mainly in air, in water and soils, nitrogen can be found in nitrates and nitrites. All of these substances are a part of the nitrogen cycle and there are all interconnected (Bothe et al., 2007).

The application of fumigant mixtures has been recognized as a means of overcoming the disadvantages of using a single fumigant. A combination of fumigants is advisable, because none of the common fumigants, used singly, possesses the ideal characteristics (Navarro, 1986). 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 organism. 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 effect of N_2 mixed with phosphine on the mortality of stored-products insects to reduce the appropriate amount of phosphine.

MATERIALS AND METHODS

This study was carried out in two stages at the fumigation store of entomology, Urmia University during the period of 2009 to 2010. In the first stage, nitrogen and phosphine were tested against insects in an empty- space. In the second stage, the effect of nitrogen and phosphine were determined by confining the insects under different heights and nutrition materials.

Chemicals

Phosphine

The test phosphine tablets were 1 g PH_3 active ingredient and 2 g aluminum oxide with 3 g weight. All doses used in this study are expressed as commercial formulations.

Nitrogen

The nitrogen gas was applied to containers from a vessel of liquid nitrogen with appropriate vaporizers and pressure regulators to control the flow rate. The fumigation store had the following internal dimensions of 4.7 m long, 2.8 m wide and 2.9 m high (volume about 38 m^3) containing bins with different height (30, 35, 40, 45 and 100 cm) and 20 cm diameter were placed in the middle of the fumigation chamber of rice, wheat and date and used for filling the bins.

Insects

Sitophilus oryzae (Coleoptera: Curculionidae), *Triobolium castaneum* (Coleoptera: Tenebrionidae), *Rhizopertha dominica* (Coleoptera: Bostrychidae), *Callosobrochus maculatus* (Coleoptera: Bruchidae) and *Plodia interpunctella* (Lepidoptera: Pyralidae) adults were collected from local mills, stores and shops in Urmia (37.39ºN 45.40ºE), a town in Iran. Cultures were established and maintained on healthy uncontaminated food at 27±2°C and 65±5% 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. *S. oryzae* and *R. dominica* were reared on Soft kernel wheat (Padin et al., 2002; Bell et al., 1977); the culture medium comprised whole-wheat flour with 5% yeast for *T. castaneum* (Childs and Overby, 1983). *C. maculatus* was reared on chickpeas (Keita et al., 2000) and *P. interpunctella* was reared on diet of 80% ground wheat 10% glycerin, 5% brewer's yeast and 5% honey (Rafaeli and Gileadi, 1995).

Bioassays

The following developmental stages of insects were used in these tests: (1) *S. oryzae* and *R. dominica* adults 7±2 day old; (2) *T. castaneum* adults of 14±3 day old; (3) *C. maculatus* adults of 3 day old and (4) 3th larvae of *P. interpunctella*. Preliminary dose-mortality tests were done before each experiment to determine a range of doses that would produce 25 to 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±5% 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. Empty-space and penetration tests were conducted in 38 m^3 capacity chamber.

Empty-space tests

Adults (mixed-sex) of *S. oryzae, T. castaneum*, *R. dominica*, *C. maculatus* and 3th larvae of *P. interpunctella* were fumigated for 24 h in the fumigation chamber separately. The test insects were confined in cages constructed with 40 mesh wire gauze. Each cage

Table 1. Variance analysis of different treatments of the five experimented insects mortality in the empty-space tests.

Source	df	Mean square	F	ρ
Between groups	4	929.583	41.797	0.000**
Within groups	10	22.240		
Total	14			

n.s *P* is not significant; **p* is significant at 0.05 level;***p* is significant at 0.01 level.

Table 2. Arcsin $\sqrt{x}$ average mortality of insects in the empty-space tests*.

Insect	Mean of mortality percentage		
	Group 1	Group 2	Group 3
R. dominica	42.1206		
T. castaneum		65.9540	
C. maculatus		73.4030	73.4030
S. oryzae			84.7380
P. interpunctella			84.7380
ρ	1.000	0.360	0.086

In each column mean that letters are different at a 5 percent level with one another are significant differences.

contained 20 insects and 3 g food. The appropriate amount of phosphine tablets (Three-quarters of the recommended phosphine dose = 28.5 tablets) were placed in several parts of the chamber, then the door of the chamber were entirely closed and 3 kg of N_2 was injected with a hose which was made between the injection gate of the chamber and the N_2 cylinder. Immediately after the N_2 injection, the injection gate was closed. In each test, the control insects were treated identically except that none were exposed to phosphine and N_2. After exposure periods of 24 h, the insects were transferred to clean jars containing food and were held at 27±2°C and 65 ±5% R.H. Mortality rates of *S. oryzae, T. castaneum*, *R. dominica*, *C. maculatus* adult and 3th larvae of *P. interpunctella* were recorded 24 h after the termination exposure (Pourmirza and Tajbakhsh, 2008).

Penetration tests

The penetration tests were carried out in the earlier mentioned chamber (38 m^3). Experiments were performed in different heights (30, 35, 40, 45 and 100 cm) and nutrition materials (rice, wheat and date). For each experiment, five cages for the five experimental species [Adults (mixed-sex) of *S. oryzae,* T. *castaneum*, *R. dominica*, *C. maculatus* and 3th larvae of *P. interpunctella*, each containing 20 adults of one insect species with 3 g food] were placed horizontally at the bottom of PVC bins with different heights. Each bin was filled by the three mentioned nutrition materials separately.

The procedure used was similar to those described for the empty- space tests (in penetration tests, 4 kg N_2 was used for injection). Each experiment was replicated three times for three days. The control case was prepared in identical manner without the application of the test compounds. After exposure periods of 24 h, the insects were transferred to clean jars containing food and were held at 27±2°C and 65±5% R.H. Mortality rates of *S. oryzae, T. castaneum*, *R. dominica*, *C. maculatus* adult and 3th larvae of *P. interpunctella* were recorded after 24 h after the termination exposure. The appropriate amount of phosphine tablets in these trials were half the recommended dose (19 tablets) (Pourmirza and Tajbakhsh, 2008).

Data analysis

Mortality data from all the bioassays were analyzed with SPSS software (SPSS Inc, 1993). In all the experiments, the data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to determine the statistical differences between the means at α= 0.05.

RESULTS AND DISCUSSION

Table 1 shows that the F values of the insects had significant difference in the empty-space test; in this space the highest mortality was for *S.oryzae* and *P. interpunctella* (Table 2) and the lowest mortality was observed in *R. dominica* (Table 2 and Figure 6). Treatment with nitrogen and phosphine in the empty-space test resulted in different rates of mortality, for example, for *S. oryzae* and *P. interpunctella*, mortality percentage was observed significantly different with other insects, while significant difference was not achieved within *T. castaneum* and *C. maculatus* (Table 3).

Table 4 shows that the F values of height were significant for *S. oryzae, T. castaneum, R. dominica* and *C. maculatus,* but for *P. interpunctella*, it was not significant. This table shows that the F values of the diet were significant for *S. oryzae, T. castaneum* and *R. dominica,* but not for *C. maculatus* and *P. interpunctella.*

Table 3. Multiple Comparisons of insects in empty-space test.

Insect	Insect	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
S. oryzae	*T. castaneum*	18.78400*	3.85058	0.005	6.1114	31.4566
	R. dominica	42.61734*	3.85058	0.000	29.9448	55.2899
	C. maculatus	11.33494	3.85058	0.086	-1.3376	24.0075
	P.interpunctella	.00000	3.85058	1.000	-12.6726	12.6726
T. castaneum	*S. oryzae*	-18.78400*	3.85058	0.005	-31.4566	-6.1114
	R. dominica	23.83334*	3.85058	0.001	11.1608	36.5059
	C. maculatus	-7.44906	3.85058	0.360	-20.1216	5.2235
	P.interpunctella	-18.78400*	3.85058	0.005	-31.4566	-6.1114
R. dominica	*S. oryzae*	-42.61734*	3.85058	0.000	-55.2899	-29.9448
	T. castaneum	-23.83334*	3.85058	0.001	-36.5059	-11.1608
	C. maculatus	-31.28240*	3.85058	0.000	-43.9550	-18.6098
	P. interpunctella	-42.61734*	3.85058	0.000	-55.2899	-29.9448
C. maculatus	*S. oryzae*	-11.33494	3.85058	0.086	-24.0075	1.3376
	T. castaneum	7.44906	3.85058	0.360	-5.2235	20.1216
	R. dominica	31.28240*	3.85058	0.000	18.6098	43.9550
	P. interpunctella	-11.33494	3.85058	0.086	-24.0075	1.3376
P. interpunctella	*S. oryzae*	.00000	3.85058	1.000	-12.6726	12.6726
	T. castaneum	18.78400*	3.85058	0.005	6.1114	31.4566
	R. dominica	42.61734*	3.85058	0.000	29.9448	55.2899
	C. maculatus	11.33494	3.85058	0.086	-1.3376	24.0075

*p is significant at 0.05 level.

The interaction between height and diet (height × diet) were not significant for *T. castaneum, R. dominica*, *C. maculatus* and *P. interpunctella,* but for *S .oryzae* it was significant in $p < 0.01$.

Treatment with high-pressure nitrogen and phosphine under different height and foodstuff may result in different rates of mortality; for example, at 100 cm, mortality percentage for *S. oryzae* and *R. dominica* was observed significantly different from other heights (Tables 5 and 7), while the same level of control was achieved within 100 cm with 30, 35, 40 and 45 cm in *T. castaneum*, *C. maculatus* and *P. interpunctella* (Tables 6 and 8). The results showed that for each of the bins, nitrogen and phosphine mixture achieved almost complete mortality against *P. interpunctella* in all the heights (Table 9). The results showed that there was a significant difference in the mortality between 30 cm with other heights in *C. maculatus* (Table 8).

Figures 1, 3 and 5 shows that the influence of nitrogen gas and phosphine in the date was more than that of rice and wheat, because the highest mortality of *S. oryzae*, *R. dominica* and *P. interpunctella* was observed in the bins that contained date. The lowest mortality rate of *R. dominica* and *C. maculatus* occurred in the wheat reservoirs (Figures 3 and 4), but for *T. castaneum* and *P. interpunctella,* the lowest mortality rate was related to rice which had less influence of nitrogen gas and phosphine (Figures 2 and 5).

For the control of stored-product pest insects, particularly on grain, farmers mostly rely on the treatment by using contact insecticide on 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 (Pourmirza and Tajbakhsh, 2008). Therefore, the number of suitable contact insecticides that can be used in the control of stored-product insects are limited (White and Leesch, 1995; Arthur, 1999). Fumigation is one of the most successful methods for the rapid control of insects infesting foodstuffs (Weller and Morton, 2001). Presently, large proportions of stored foodstuffs are fumigated with methyl bromide and phosphine. In many instances, the major reliance has been placed on the methyl bromide fumigation and the stock management was neglected. Phosphine as fumigant offers a cost effective method of insects' control (Rajendran and Muralidharan, 2001). Strict controls on detectable concentrations of phosphine are necessarily

Table 4. Variance analysis of different treatments of five experimented insects mortality in penetration tests.

S. V	*S. oryzae*				*T. castaneum*				*R. dominica*				*C. maculatus*				*P. interpunctella*			
	df	Mean square	F	Ω2	df	Mean square	F	Ω2	df	Mean square	F	Ω2	df	Mean square	F	Ω2	df	Mean square	F	Ω2
Height(a)	4	373.73	48.72**	0.037	4	230.13	25.19**	0.052	4	342.13	17.05**	1.46	4	541.28	31.63**	0.146	4	95.50	1.60n.s	0.074
Diet(b)	2	210.84	27.48**	0.01	2	58.84	6.44*	0.006	2	92.53	4.61*	0.197	2	21.36	1.24n.s	0.002	2	163.44	2.75n.s	0.063
height × diet (ab)	8	70.71	9.22**		8	15.32	1.67n.s		8	13.33	.66n.s		8	9.92	0.58n.s		8	12.12	0.20n.s	
Total	14				14				14				14				14			
Ω2a/Ω2b				3.7				8.6				7.45				73				1.17

n.s *p* is not significant; **p* is significant at 0.05 level; ** *p* is significant at 0.01 level; Ω^2a/Ω^2b is equal to the ratio of height superiority to nutrient in mortality.

Table 5. Multiple comparisons of height for *S. oryzae.*

High (cm)	High (cm)	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
30	35	.0000	1.30559	1.000	-3.7951	3.7951
	40	1.2522	1.30559	0.871	-2.5429	5.0473
	45	11.2144*	1.30559	0.000	7.4193	15.0096
	100	15.3038*	1.34577	0.000	11.3918	19.2157
35	30	.0000	1.30559	1.000	-3.7951	3.7951
	40	1.2522	1.30559	0.871	-2.5429	5.0473
	45	11.2144*	1.30559	0.000	7.4193	15.0096
	100	15.3038*	1.34577	0.000	11.3918	19.2157
40	30	-1.2522	1.30559	0.871	-5.0473	2.5429
	35	-1.2522	1.30559	0.871	-5.0473	2.5429
	45	9.9622*	1.30559	0.000	6.1671	13.7573
	100	14.0515*	1.34577	0.000	10.1396	17.9634
45	30	-11.2144*	1.30559	0.000	-15.0096	-7.4193
	35	-11.2144*	1.30559	0.000	-15.0096	-7.4193
	40	-9.9622*	1.30559	0.000	-13.7573	-6.1671
	100	4.0893*	1.34577	0.037	0.1774	8.0012
100	30	-15.3038*	1.34577	0.000	-19.2157	-11.3918
	35	-15.3038*	1.34577	0.000	-19.2157	-11.3918
	40	-14.0515*	1.34577	0.000	-17.9634	-10.1396
	45	-4.0893*	1.34577	0.037	-8.0012	-0.1774

**p* is significant at 0.05 level.

Table 6. Multiple comparisons of height for *T. castaneum.*

High (cm)	High (cm)	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
30	35	3.3473	1.42483	0.158	-0.7856	7.4802
	40	7.7728*	1.42483	0.000	3.6400	11.9057
	45	10.3273*	1.42483	0.000	6.1944	14.4602
	100	12.3445*	1.42483	0.000	8.2117	16.4774
35	30	-3.3473	1.42483	0.158	-7.4802	.7856
	40	4.4256*	1.42483	0.031	.2927	8.5584
	45	6.9800*	1.42483	0.000	2.8471	11.1129
	100	8.9973*	1.42483	0.000	4.8644	13.1301
40	30	-7.7728*	1.42483	0.000	-11.9057	-3.6400
	35	-4.4256*	1.42483	0.031	-8.5584	-.2927
	45	2.5544	1.42483	0.396	-1.5784	6.6873
	100	4.5717*	1.42483	0.024	.4388	8.7046
45	30	-10.3273*	1.42483	0.000	-14.4602	-6.1944
	35	-6.9800*	1.42483	0.000	-11.1129	-2.8471
	40	-2.5544	1.42483	0.396	-6.6873	1.5784
	100	2.0173	1.42483	0.623	-2.1156	6.1501
100	30	-12.3445*	1.42483	0.000	-16.4774	-8.2117
	35	-8.9973*	1.42483	0.000	-13.1301	-4.8644
	40	-4.5717*	1.42483	0.024	-8.7046	-.4388
	45	-2.0173	1.42483	0.623	-6.1501	2.1156

**p* is significant at 0.05 level.

Table 7. Multiple comparisons of height for *R. dominica.*

High (cm)	High (cm)	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
30	35	4.6589	2.11157	0.206	-1.4791	10.7968
	40	6.0456	2.11157	0.055	-0.0924	12.1835
	45	11.0144*	2.11157	0.000	4.8765	17.1524
	100	17.4464*	2.17655	0.000	11.1195	23.7732
35	30	-4.6589	2.11157	0.206	-10.7968	1.4791
	40	1.3867	2.11157	0.964	-4.7513	7.5246
	45	6.3556*	2.11157	0.040	.2176	12.4935
	100	12.7875*	2.17655	0.000	6.4607	19.1143
40	30	-6.0456	2.11157	0.055	-12.1835	0.0924
	35	-1.3867	2.11157	0.964	-7.5246	4.7513
	45	4.9689	2.11157	0.157	-1.1691	11.1068
	100	11.4008*	2.17655	0.000	5.0740	17.7277
45	30	-11.0144*	2.11157	0.000	-17.1524	-4.8765
	35	-6.3556*	2.11157	0.040	-12.4935	-0.2176
	40	-4.9689	2.11157	0.157	-11.1068	1.1691
	100	6.4319*	2.17655	0.045	.1051	12.7588

Table 7 Contd. Multiple comparisons of height for *R. dominica.*

100	30	-17.4464*	2.17655	0.000	-23.7732	-11.1195
	35	-12.7875*	2.17655	0.000	-19.1143	-6.4607
	40	-11.4008*	2.17655	0.000	-17.7277	-5.0740
	45	-6.4319*	2.17655	0.045	-12.7588	-0.1051

**p* is significant at 0.05 level.

Table 8. Multiple comparisons of height for *C. maculatus.*

High (cm)	High (cm)	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
30	35	8.7378*	1.94994	0.001	3.0697	14.4059
	40	13.5622*	1.94994	0.000	7.8941	19.2303
	45	16.3656*	1.94994	0.000	10.6974	22.0337
	100	20.8228*	2.00995	0.000	14.9802	26.6653
35	30	-8.7378*	1.94994	0.001	-14.4059	-3.0697
	40	4.8244	1.94994	0.125	-0.8437	10.4926
	45	7.6278*	1.94994	0.004	1.9597	13.2959
	100	12.0850*	2.00995	0.000	6.2424	17.9276
40	30	-13.5622*	1.94994	0.000	-19.2303	-7.8941
	35	-4.8244	1.94994	0.125	-10.4926	0.8437
	45	2.8033	1.94994	0.609	-2.8648	8.4714
	100	7.2606*	2.00995	0.009	1.4180	13.1031
45	30	-16.3656*	1.94994	0.000	-22.0337	-10.6974
	35	-7.6278*	1.94994	0.004	-13.2959	-1.9597
	40	-2.8033	1.94994	0.609	-8.4714	2.8648
	100	4.4572	2.00995	0.202	-1.3853	10.2998
100	30	-20.8228*	2.00995	0.000	-26.6653	-14.9802
	35	-12.0850*	2.00995	0.000	-17.9276	-6.2424
	40	-7.2606*	2.00995	0.009	-13.1031	-1.4180
	45	-4.4572	2.00995	0.202	-10.2998	1.3853

**p* is significant at 0.05 level.

Table 9. Multiple comparisons of height for 3th larvae of *P. interpunctella.*

High (cm)	High (cm)	Mean difference	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
30	35	1.8644	3.63320	0.985	-8.6740	12.4029
	40	4.2122	3.63320	0.774	-6.3263	14.7507
	45	6.7167	3.63320	0.366	-3.8218	17.2551
	100	7.8122	3.63320	0.226	-2.7263	18.3507
35	30	-1.8644	3.63320	0.985	-12.4029	8.6740
	40	2.3478	3.63320	0.966	-8.1907	12.8863
	45	4.8522	3.63320	0.672	-5.6863	15.3907
	100	5.9478	3.63320	0.486	-4.5907	16.4863

Table 9 Contd. Multiple comparisons of height for 3th larvae of *P. interpunctella.*

40	30	-4.2122	3.63320	0.774	-14.7507	6.3263
	35	-2.3478	3.63320	0.966	-12.8863	8.1907
	45	2.5044	3.63320	0.957	-8.0340	13.0429
	100	3.6000	3.63320	0.857	-6.9385	14.1385
45	30	-6.7167	3.63320	0.366	-17.2551	3.8218
	35	-4.8522	3.63320	0.672	-15.3907	5.6863
	40	-2.5044	3.63320	0.957	-13.0429	8.0340
	100	1.0956	3.63320	0.998	-9.4429	11.6340
100	30	-7.8122	3.63320	0.226	-18.3507	2.7263
	35	-5.9478	3.63320	0.486	-16.4863	4.5907
	40	-3.6000	3.63320	0.857	-14.1385	6.9385
	45	-1.0956	3.63320	0.998	-11.6340	9.4429

*p is significant at 0.05 level.

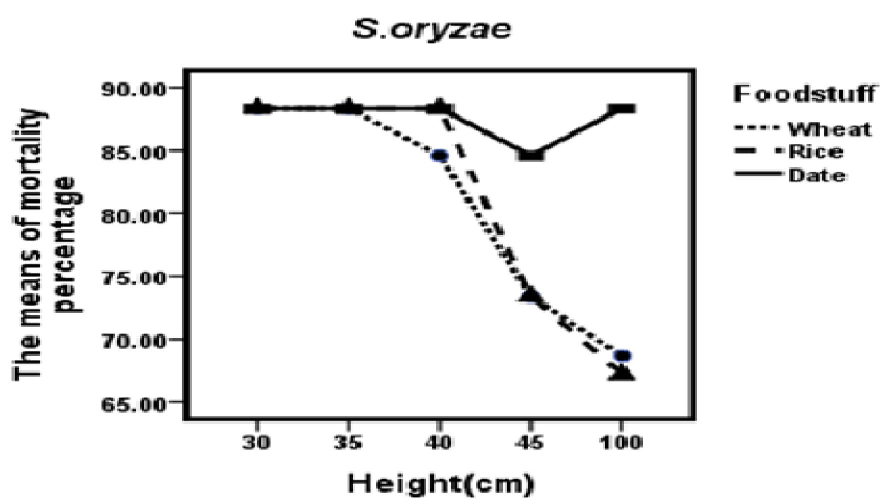

Figure 1. The comparison of mortality of *S. oryzae* in the different height and foodstuffs.

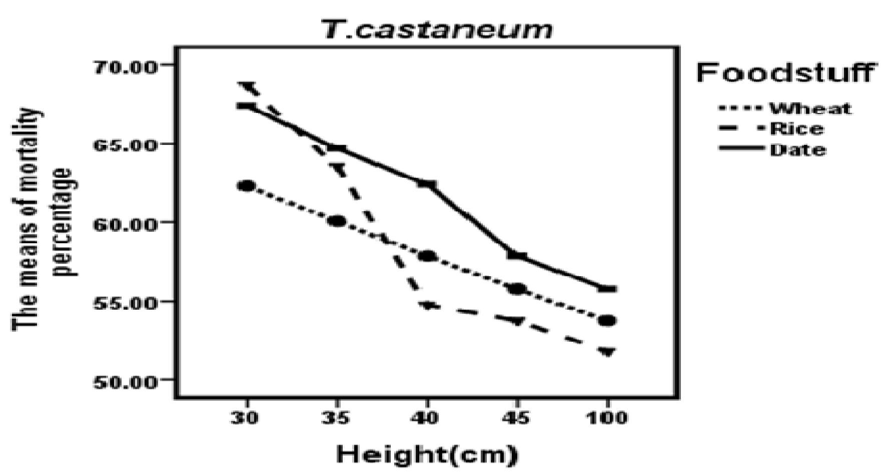

Figure 2. The comparison of mortality of *T .castaneum* in the different height and foodstuffs.

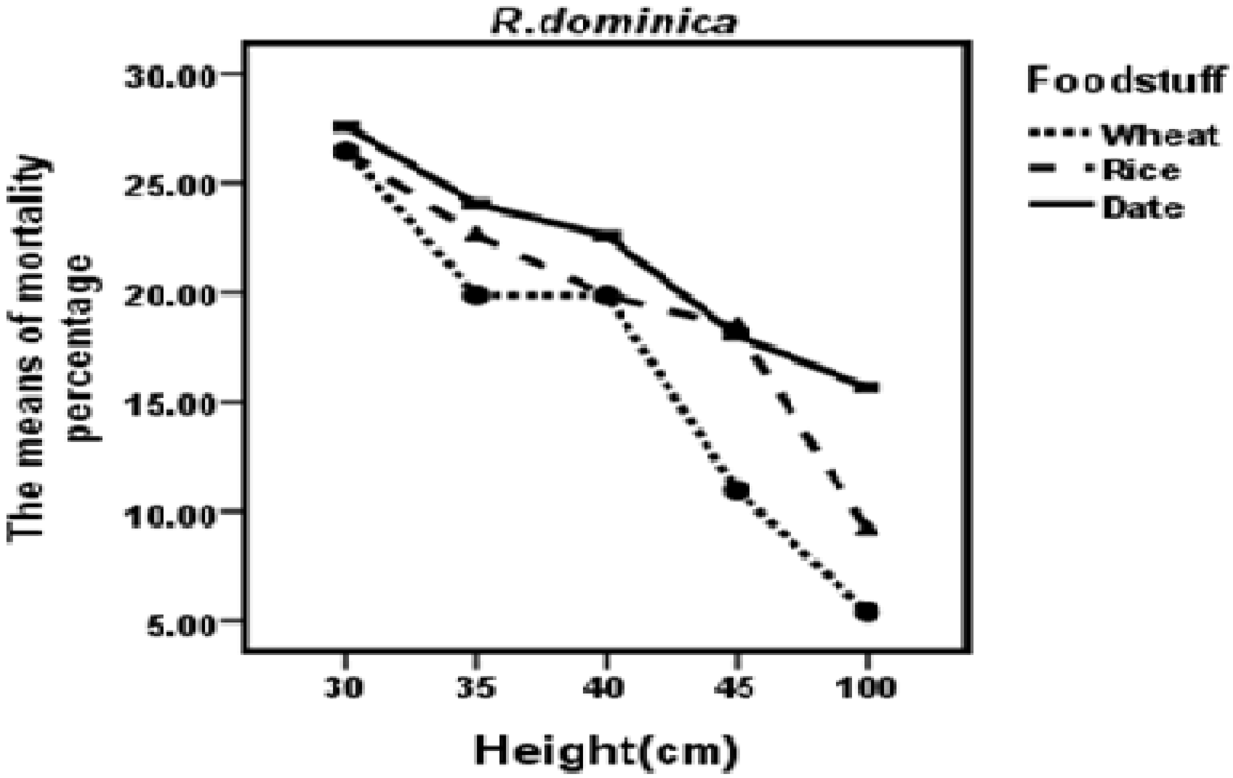

Figure 3. The comparison of mortality of *R. dominica* in the different height and foodstuffs.

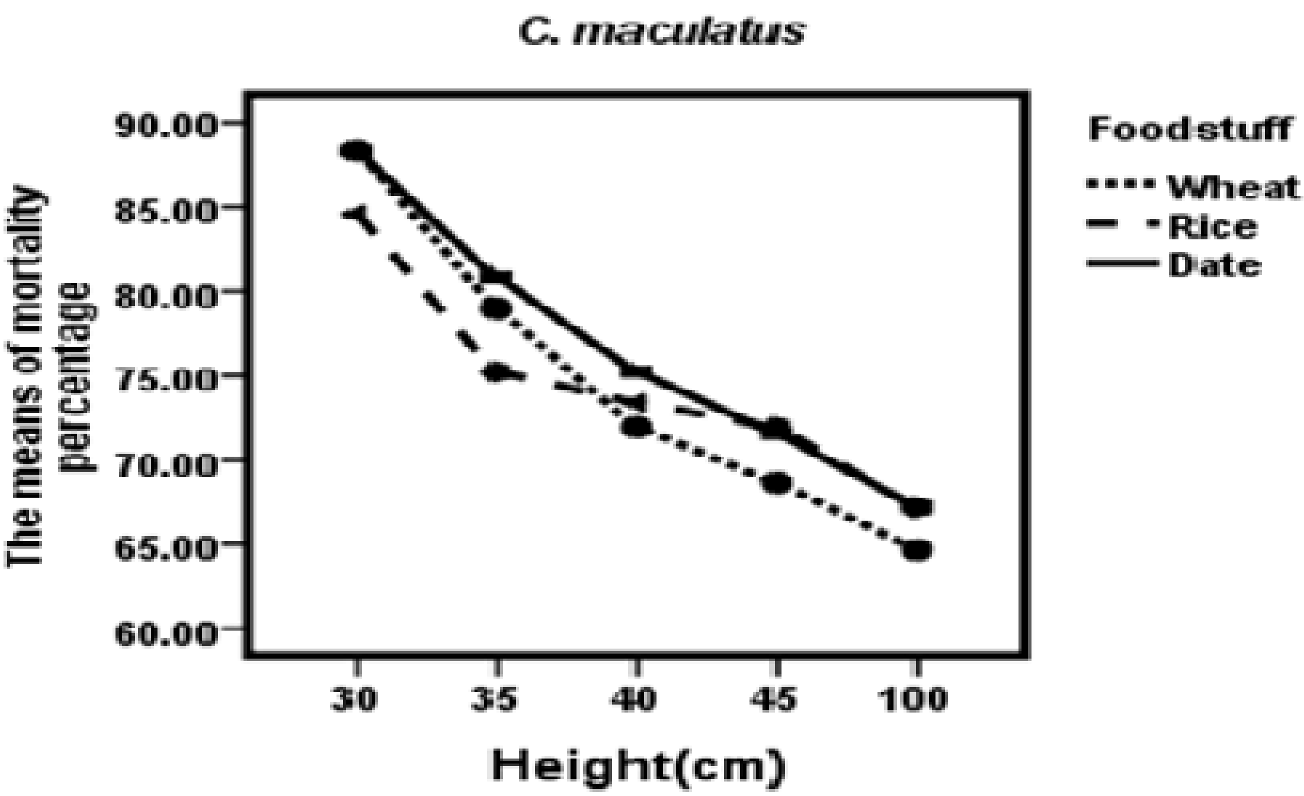

Figure 4. The comparison of mortality of *C. maculatus* in the different height and foodstuffs.

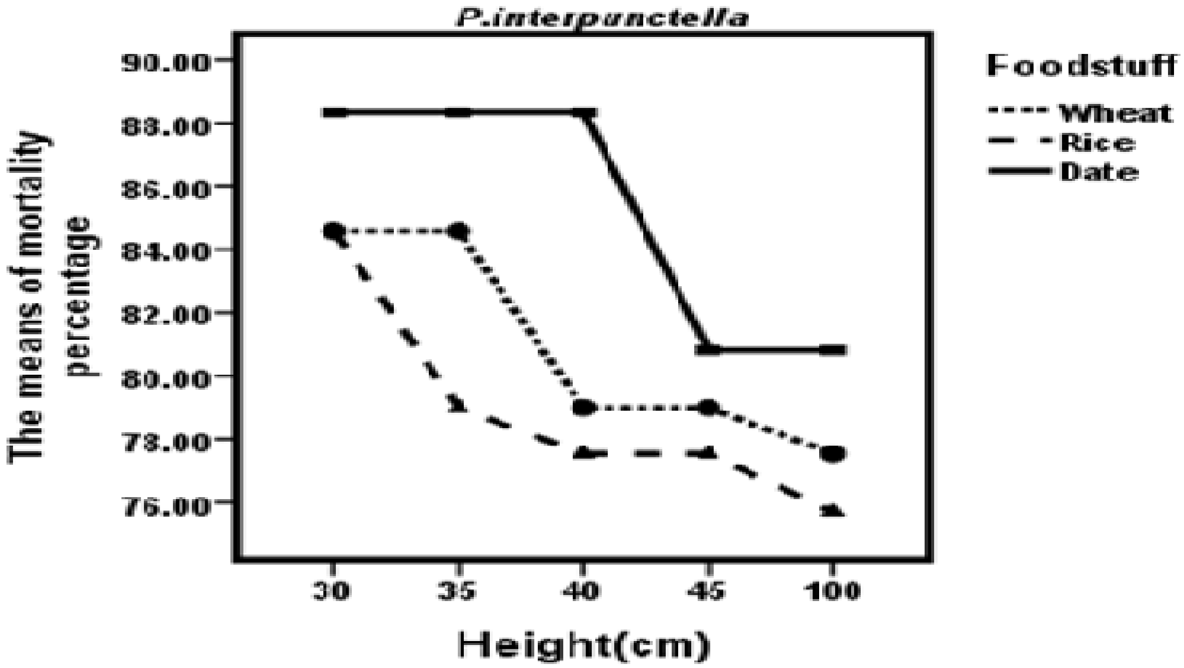

Figure 5. The comparison of mortality of *P. interpunctella* in the different height and foodstuffs.

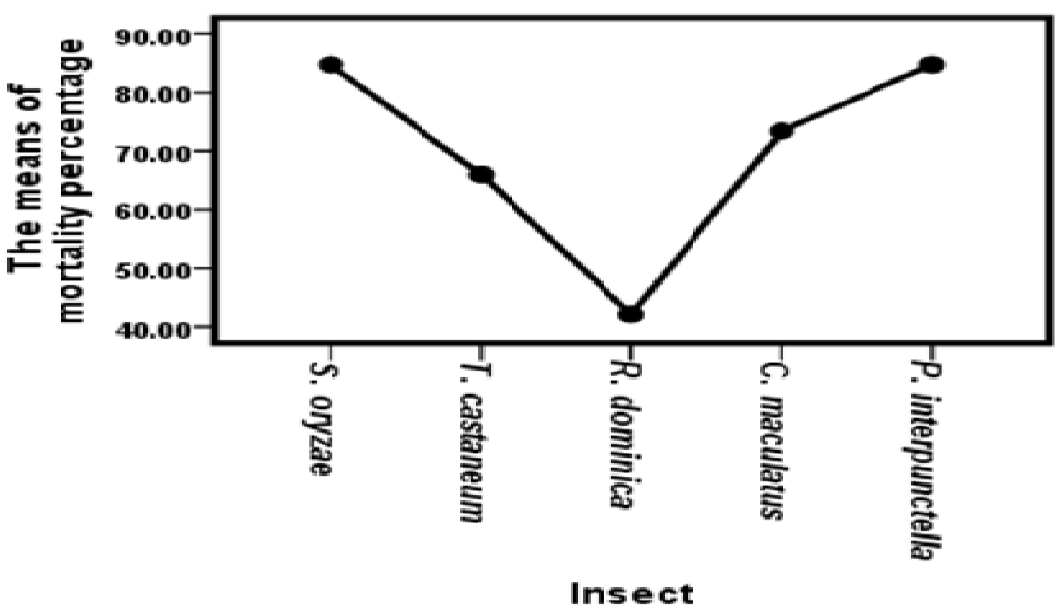

Figure 6. The comparison of mortality of insects in the empty-space test.

imposed by some organizations. Since excessive residue from fumigation is a potential hazard to consumers, phosphine is under close scrutiny and will have limited use in the immediate future (Pourmirza and Tajbakhsh, 2008). A new approach in insect control researches could be the use of less hazardous substances, which are more compatible with the environment. The application of gas and phosphine as a less hazardous compound may be an appropriate approach to this objective. Trials have been conducted on the use of nitrogen as a fumigant to replace phosphine in the control of insects damaging stored products. The use of N_2 rich atmospheres showed promising results in disinfesting food commodities in small storage facilities (Bennett, 2003).

Bennett (2003) recorded a high percentage of mortality on *S. oryzae*, *R. dominica*, *Trogoderma granarium*

Everts. and *T. castaneum* after six days exposure to biogas in PVC bins. In this study however, the use of nitrogen gas with phosphine as its main components, achieved good results in the control of stored pests, because the results showed that in the stored-product infested with *C. maculatus* and *T. castaneum,* there was significant mortality. *S. oryzae* and *P. interpunctella* recorded up to 100% mortality after 24 h of exposure to nitrogen and phosphine mixture in PVC bins. The addition of N_2 to phosphine caused an increase in the mortality of the population of the five species as also observed by Athie et al. (1998) with the addition of CO_2 to phosphine. The mortality data for adults of *S. oryzae*, *C. mculatus*, *T. castaneum* and *P. interpunctella* in this study agrees with those of Li et al. (2009), who treated the *C. furrugineus* only with liquid nitrogen in bins which were filled with hard red spring wheat. This effect of gas in mixture with phosphine was also indicated by Desmarchelier and Wohlgemuth (1984) and El-Lakwah et al. (1991). In this study, N_2 was very effective in mortality to all the tested insects in the empty space and penetration tests. This finding agrees with the data collected by Gunasekaran and Rajendran (2005) which demonstrated that O_2, N_2 and CO_2 were toxic to some stored-product insects.

Due to the problem of insect resistance to phosphine and the lack of viable alternatives for control methods and also because synergistic interaction implies that the risk-reduced phosphine dose can be used with N_2 for its effectiveness against strains of stored-product insects and the spectrum of activity under different environmental conditions, N_2 treatment under suitable temperatures and with low concentrations of phosphine is recommended as a replacement for methyl bromide in treating stored products and other food and it is desirable for management of stored-product insects in conventional small stores with common available facilities.

ACKNOWLEDGEMENT

The authors thank the Faculty of Agriculture, Urmia University, for providing the facilities for this research.

REFERENCES

Arthur FH (1999). Evaluation of an encapsulated formulation of cyfluthrin to control *Sitophilus oryzae* (L.) on stored wheat. J. Stored Prod. Res., 35: 159-166.

Athie I, Gomez RAR, Bolonhezi S, Valentini ST, De castro MFPM (1998). Effects of carbon dioxide and phosphine mixtures on resistant populations of stored-grain insects. J. Stored Prod. Res., 34: 27-32.

Bell CH, Hole BD, Evans PH (1977). The occurrence of resistance to phosphine in adult and egg stages of strain of *Rhizopertha dominica* F. Coleoptera. J. Stored Prod. Res., 13: 91-94.

Bennett S (2003). Stored product insects, Available on: http://www.the-piedpiper.co.uk/th7.htm. (Accessed 27 August 2010).

Bothe H, Ferguson SJ, Newton WE (2007). Biology of the nitrogen cycle. Elsevier. p. 283.

Cassanova JL (2002). An overview of the scientific aspect of ozone depletion and their impact on environment, pp. 23-27. In: Batchhelor TA, Bolivar JM (eds.), Proceeding of International Conference on Alternative to Methyl Bromide, 5-7 March 2002, Sevilla, Spain.

Champ BR, Dyte CE (1976). Report of the FAO Global Survey of Pesticide Susceptibility of Stored Pests, p. 297. FAO, Rome.

Childs DP, Overby JE (1983). Mortality of the cigarette beetles in high-carbon dioxide atmospheres. J. Econ. Entomol., 76: 456–544.

Chimbe CM, Galley DJ (1996). Evaluation of material from plants of medicinal importance in Malawi as protectants of stored grain against insects, Crop Prod., 15: 289-294.

Collins PJ, Daglish GJ, Bengeston M, Lambkin TM, Pavic H (2002). Genetics of resistance to phosphine in *R. dominica*. J. Econ. Entomol., 95: 862-869.

Croswell K (1996). Alchemy of the Heavens. Anchor. ISBN 0-385-47214-5. Available on: http:// kencroswell. com/ alchemy.html. (Accessed 30 August 2010).

Desmarchelier JM, Wohlgemuth R (1984). Response of several species of insects to mixtures of phosphine and carbon dioxide. In Proceedings of the International Symposium on Practical Aspects of Controlled Atmosphere and Fumigation in Grain Storages, pp. 75-81. Perth, Australia.

El-Lakwah F, Meuser F, Abdel GA, Wohlgemuth R, Darwish A (1991). Efficiency of phosphine alone and mixtures with carbon dioxide against Angoumis grain moth *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae). J. Plant Diseases Prod. 98: 92-102.

EPA (1999). Alternative disinfectants and oxidants guidance manual, Publication: 815 R 99014, United States Environmental Protection Agency, Washington, D. C.

Gunasekaran N, Rajendran S (2005). Toxicity of carbon dioxide to drugstore beetle *Stegobium paniceum* and cigarette beetle *Lasioderma serricorne*. J. Stored Prod. Res., 41: 283-294.

Jahn GC, Almazan LP, Pacia JB (2005). Effect of nitrogen fertilizer on the intrinsic rate of increase of the rusty plum aphid, *Hysteroneura setariae* (Thomas) (Homoptera: Aphididae) on rice (*Oryza sativa* L.). Environ. Entomol., 34 (4): 938–943.

Jay E (1986). Factors affecting the use of carbon dioxide for treating raw and processed agricultural products. In Proceedings of the GASGA Seminar on Fumigation Technology in Developing countries, pp. 172-189. Slough, Berkshire.

Kashi KP (1981). Controlling pests in stored grain with carbon dioxide. Span, 24: 69-71.

Keita SM, Vincent C, Schmit JP, Ramaswamy S, Belanger A (2000). Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Products Res., 36: 355-364.

Leesch JG (1995). Fumigant action of acrolein on stored product insects. J. Econ. Entomol., 88: 326-330.

Li H, Paliwal J, Jayas DS, White NDG (2009). Disinfestation of wheat using liquid nitrogen aeration. Proceedings of World Academy of Science, Engineering Tech., 37: 29-31.

Mendez F, Maier DE, Mason LJ, Woloshuk CP (2003). Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. J. Stored Prod. Res., 39: 33-44.

Mills KA (2001). Phosphine resistance-where to now? In Proceeding. International Conference on Controlled Atmospheres and Fumigation in Stored Products, 29 October-3 November 2000. Fereso. CA.

Mills KA, Pacho IA (1996). Resistance to phosphine in stored product insects and a strategy to prevent its increase, In: Proceeding XX International Congress of Entomology (Firenze Italy August 25-31).

Navarro S (1986). Gaseous fumigant mixtures with special reference to mixture of carbon dioxide with phosphine or methyl bromide. In Proceedings of the GASGA Seminar on Fumigation Technology in Developing Countries, pp. 150-158. Slough, Berkshire.

Navarro S (2006). Modified atmospheres for the control of stored-product insects and mites. See Ref. 41, pp. 105–45.

Padin S, Dalbello G, Fabrizio M (2002). Grain loss closed by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthosceloides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. J. Stored Prod. Res., 38: 69-74.

Phillips TW, Throne JE (2010). Biorational approaches to managing stored-product insects. The annual review of entomology is online at www.ento.annualreviews.org.

Pourmirza AA, Tajbakhsh M (2008). Studies on the toxicity of aceton, acrolein and carbon dioxide on stored-product insects and wheat seed. Pak. J. Biol. Sci., 11: 953-963.

Rafaeli A, Gileadi C (1995). Factors affecting pheromone production in the stored product moth, *plodia interpunctella*: A preliminary study. J. Stored Prod. Res., 31: 243-247.

Rajendran S (2002). Postharvest pest losses. In: Pimentel. D. (Ed.). Encyclopedia of pest Management. Mareel Cekker. Inc New York, pp. 654-656.

Rajendran S, Muralidharan N (2001). Performance of phosphine in fumigation of bagged paddy rice in indoor and outdoor stores. J. Stored Prod. Res., 37: 351-358.

Robertson JL, Preisler HK (1992). Pesticide Bioassays with Arthropods. CRC Press, Boca Ratone, pp. 35-48.

SPSS (1993). *SPSS for Windows User's Guide Release 6*. SPSS Inc. Chicago.

Storey CL (1973). Exothermic inert-atmosphere generators for control of insects in stored wheat. J. Econ. Entomol., 66: 511–514.

UNEP (1998). United Nations Environment Programme, Montreal Protocol on Substance that Deplete the Ozone Layer, 1998: Assessment of Alternatives to Methyl Bromide. Methyl Bromide Alternatives Committee. Nairobi, Kenya.

Weller GL, Morton R (2001). Fumigation with carbonyl sulfide: A model for the interaction of concentration, time and temperature. J. Stored Prod. Res., 37: 383-398.

White NDG, Leesch JG (1995). Chemicals Control. In: Integrated Management of Insects in Stored Products, Subramanyam, Bh. and D. Hagstrum(Eds.). Dekker, New York, pp. 287-330.

Zettler JL (1993). Phosphine resistance in stored product insects. In: Navarro, S. and Donahaye, E. (eds.), Proceedings International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, June 1992, Jerusalem, Caspit Pres Ltd., pp. 449-460.

Zettler JL, Cuperus GW (1990). Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhizopertha dominica* (Coleoptera: Bostrichidae) in wheat. J. Econ. Entomol., 83: 1677-1681.

Zettler JL, Halliday WR, Arthur FH (1989). Phosphine resistance in insects infesting stored peanuts in the Southeastern United States. J. Econ. Entomol., 82: 1508-1511.