African Journal of Biotechnology Vol. 11(23), pp. 6377-6382, 20 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1971
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

The impact of carbon dioxide in stored-product insect treatment with phosphine

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Accepted 23 May, 2011

In laboratory experiments, toxicity of carbon dioxide and carbon dioxide - phosphine mixture was investigated against 4 species of stored-product insects. In empty-space trials, estimates of the median lethal doses of carbon dioxide against adults of *Oryzaephilus surinamensis* (L.), *Lasioderma serricorne* (F.) and eggs of *Plodia interpunctella* (Hűbner) and *Cadra cautella* (Walker) were 7.67, 12.10, 5.76 and 7.25 mg/L, respectively. Penetration tests revealed that, carbon dioxide vapors could penetrate into the foodstuff mass and kill concealed insects in foodstuff spaces. Comparison of LD_{50} values between empty-space tests and penetration experiments after 24 h exposure indicated that, the increase in penetration toxicity was 3.61, 2.90, 4.02 and 3.03-fold for *O. surinamensis, L. serricorne*, *P. interpunctella* and *C. cautella,* respectively. In the hidden infestation trials, the carbon dioxide-phophine mixture destroyed developmental stages of tested insects concealed inside the foodstuff spaces. These results to a complete control with CO_2 – phosphine mixtures for 24 h and subsequently, observed during 7, 10 and 11 weeks after the exposure. It is concluded that, combination of carbon dioxide with reduced dose of phosphine could be considered as a viable alternative to methyl bromide under field conditions due to much wider margin of safety for operational personnel and substantiate fewer undesirable environmental side effects.

Key words: *Oryzeaphilus surinamensis*, Indian meal moth, fumigation, bioassay.

INTRODUCTION

Fumigation plays a key role in control and management of infestation stored commodities worldwide. Therefore, 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 stores. In recent years, the removal of some fumigants from the market has resulted in a wider use of methyl bromide and phosphine (Leesch, 1995).

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).

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Phosphine gas (PH_3) 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). This gas has a garlic odor which causes severe gastrointestinal symptoms and it is not carcinogenic (Green et al., 1984). Alminum phosphide releases posphine gas upon contact with moisture, leading to fatalities (EPA, 1999). The explosion limits of phosphine-air mixture at 1 atm pressure and 0.39% (v/v) water vapor at 30°C is 1.98% v/v (30.1 g per m^3 of phophine concentration). In most fumigation, the concentration of phosphine is unlikely to exceed this limit (Green et al., 1984). Phosphine penetrates well into commodities and can be removed rapidly by aeration after treatment. It has a low degree of sorption by most commodities and normal fumigation practice ensures that, the residues produced are well below 0.01 g t^{-1}, the current Codex alimentarius limit for processed cereals. The exposure period often needs to

be prolonged for effective action against all developmental stages of pests, typically 5 to 15 days, depending on the temperature. The mode of action is very particular. Herbert et al. (2004) set out the principles involved and clarified the sites and reactions. It appears that phosphine acts on the respiratory chain (Singh et al., 2006; Dua and Gill, 2004).

The recent emphasis on 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 inexpensive, convenient to use and without substantial disruption of the environment. According to these criteria mixture of carbon dioxide (CO_2) and sub-lethal dose of phosphine (PH_3) as a potential insect control agent was selected for testing. The mixture is almost insoluble in water, non-flammable and has a high vapor pressure (boiling at 30°C).

Carbon dioxide enhances the penetration and distribution of phosphine through foodstuff mass (Leesch, 1992). Furthermore, carbon dioxide poses little hazardous impact on human and environmental entity. CO_2 is a non-flammable gas with toxicity value of 5000 ppm v/v (UIG, 2008). Because of its accelerating respiration rate, CO_2 could be highly efficacious and useful synergist in fumigation systems.

The objectives of trials were threefold: (1) To find out the synergistic effect of carbon dioxide and sub-lethal dose of phosphine mixture; (2) to reduce the dose of fumigant; (3) to reduce the exposure period.

MATERIALS AND METHODS

Chemicals

Phosphine

Phosphine is a toxic gas to all insect life stages. This colorless gas has a distinct garlic or carbide odor that is noticeable at very low concentrations. Fire or an explosion can occur if phosphine is liberated too rapidly from commercial formulation of aluminum phosphide. To reduce this risk, some formulations give off carbon dioxide, which helps retard the risk of fire or explosion. It has a threshold limit value (TLV) of 0.3 ppm. Phosphine was generated from tablets containing aluminum phosphide and ammonium carbonate. Each tablet weighs 3 g and release 1 g of phosphine. Although, this gas is about 20% heavier than the air, it has a high molecular activity and so does not tend to stratify. Chemical and physical properties of phosphine are as follow: molecular weight 34; boiling point at 1 atm -87.74°C; specific gravity 1.2; flammability limits in air, v/v % 13.5 to 14.5 and solubility in water, v/v % 3.4 (EPA, 1999).

Carbone dioxide

Carbone dioxide (CO_2) is a ubiquitous gas which is found in small proportion in the atmosphere. It is produced from the combustion of coal or hydrocarbons, the fermentation of liquids and the breathing of humans and animals. CO_2 is assimilated by plants which in turn produce oxygen. This gas has a slightly irritating odor, is colorless, non-flammable and heavier than air with toxicity value of 5000 ppm under fumigation conditions (UIG, 2008). Initial trials using the CO_2 cylinder were conducted at the store to give confidence in handling the set up system.

Insects

Oryzaephilus surinamensis (Coleoptera: Silvanidae), *Lasioderma serricorne* (Coleoptera: Anobiidae), *Plodia interpunctell* (Lepidoptera: Pyralidae) and *Cadra cautella* (Lepidoptera: Pyralidae) 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-sterilized food at 27 ± 2°C and 60 ± 10% r.h. inside 900 ml glass rearing jars covered with pieces of muslin cloth fixed by rubber bands. *O. surinamensis* and *L. serricorne* were reared on a medium consisting of white flour with 5% brewers' yeast (19/1, w/w). *P. interpunctella* and *C. cautella* were reared on wheat germ, rolled oats and brewer's yeast (9:9:2 = w/w/w).

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.

Bioassays

This study was carried out at three stages at the entomology laboratory of Urmia University during the period of 2008 to 2009. The following developmental stages of insects were used in these tests: (1) *O. surinamesis* and *L. serricorne* adults, 5 ± 1 day old; (2) *P. interpunctella* and *C. cautella* eggs 2 days old. In laboratory experiments, fumigation chambers were 51 L highly durable, round-bottom standard gas cylinders of stainless steal, fitted with gas tight lids. Each chamber is provided at the top surface with carbon dioxide inlet line having a control valve and flow regulator.

The CO_2 gas source was a 30 k pressurized cylinder which was supplied by a local company. The cylinder was equipped with valves, pressure sensor and connected to fumigation tubing. The gas pressure can be controlled by regulating inflow of feed rate. A constant flow of carbon dioxide was delivered into the fumigation site through the gas outlet line. In field study, the fumigation store had the following internal dimensions: 4.7 m long, 2.8 m wide and 2.9 m high (volume about 38 m^3). Two gases mixture was tested: 1 mg/l PH_3 in air, 0.5 mg/l PH_3 in air plus 8% CO_2 and 0.33 mg/l PH_3 in air plus 24% CO_2. Before the commencement of each test, controller was adjusted to deliver the required amount of CO_2. 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 to 75% mortality at the lowest and the highest doses, respectively (Robertson et al., 2007). Untreated control groups were kept next to the treated insects.

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. Mortality data from all bioassays were analyzed with the Statistical Package for the Social Science (SPSS) software (SPSS Inc., 1993). Probit analysis (Finney, 1971) 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 potency ratios were determined according to Norusis (2008). 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.

Empty-space tests

Adults of *O. surinamensis* and *L. serricorne* and eggs of *P. interpunctella* and *C. cautella* were fumigated for 24 h in 51 L stainless steel container separately. The insects were confined in cages constructed with 40-mesh wire gauze. Each cage contained either 50 unsexed adult insects with 3 g of rearing medium or 15 eggs without food and was placed horizontally in the container. The containers were capped with stainless steel screwed lids. For each dose, calculated amount of CO_2 was introduced through rubber tubing via a 5 mm diameter hole, at the top of the container. Immediately after that, CO_2 was pumped; the hole in each lid was sealed with stainless steel gas-tight cap. In each test, the control container was treated identically except that no gas was pumped into the container.

After exposure, the insects were transferred to clean jars containing rearing medium and maintained under rearing conditions. Mortality was recorded 24 h after termination of the exposure. Each test was replicated three times on different days and results were pooled.

Penetration tests

The insects were fumigated for 24 h separately. For each dose, one cage containing either 50 unsexed insects with 3 g of food or 15 eggs without food was placed horizontally at the bottom of each 51-L stainless steel container. The container was filled with 3 dry measures of insect's rearing medium. The test procedure used was similar to those described for the empty-space tests except for the amount of consumed carbon dioxide. The control container was prepared in an identical manner, but no gas was used. After exposure period, the insects were transferred to a clean glass jar containing 3 g of food, held at 27 ± 2°C and 60 ± 10% r.h. Mortality rates of the insects were recorded 24 h after termination of the treatment. The trial was replicated three times on different days and results were pooled.

Hidden infestation test

A sample of 300 g of rearing medium containing eggs, larvae and pupae of *O. surinamensis, L. serricorne, P. interpuctella* and *C. cautella* were collected separately from the stock cultures and placed in 1,150 ml glass jars. The jars were placed horizontally at the floor of the store. For each trial calculated, the amount of phostoxin was placed in a glass container, 10 ml of sulfuric acid was delivered on it and immediately the store was left and the door was sealed. The procedure for applying CO_2 was similar to that described earlier, but through a 5-mm diameter hole located in the center of the door. After exposure, each jar was held under standard conditions for appropriate period of time. Under the test conditions, 7, 10, 7 and 11 weeks were sufficient for eggs of the *O. surinamensis*, *L. surricorne, P. interpunctella* and *C. cautella* to develop to adult stage, respectively. Due to subsequent exposure, the insects and food were transferred to a clean jar and held at 27 ± 2°C and 60 ± 10% r.h. for 7, 11 and 11 weeks. During this period of incubation, in each week, emerged adults were counted and discarded to prevent any post-fumigation oviposition. Control group jars were placed inside a gas-tight container and treated identically except for those with no gas which was introduced to jars. The experiment was replicated three times on different days and results were pooled.

RESULTS

Empty-space tests

Dose-mortality values estimated from the probity analyses mortality are given in Table 1. On the basis of LD_{50} values, the sensitivity order of the insects to carbon dioxide was measured as: *P. interpunctella* > *C. cautella*a > *O. surinamensis* > *L. serricorne*. For *L. serricorne* overlap in 95% confidence limits of potency ratios was not detected with other insects. Therefore, statistically significant difference among the estimated LD_{50} values was observed. At the LD_{95} level, the dose of CO_2 required to kill the most tolerant species was 256.01 mg/l.

Penetration tests

Results of the fumigation tests showed that, carbon dioxide vapors penetrated thoroughly in bulk commodity and diffused rapidly through it and killed the tested insects (Table 2). There was a direct relationship between CO_2 dose and mortality rate of the tested insects. Based on the LD_{50} values, when CO_2 was applied to the rearing medium mass headspace, the dose required to achieve 50% mortality after 24 h exposure time was 3.61, 2.90, 4.02 and 3.03 times more than that required for the empty-space tests for *O. surinamensia, L. serricorne, P. interpunctella,* and *C. cautella,* respectively (Tables 1 and 2). These differences indicated that, CO_2 was absorbed by the rearing medium mass.

Hidden infestation tests

Table 3 presents the toxicity of CO_2-phosphine mixtures on all tested insects populations concealed in foodstuff mass. The results show the effectiveness of mixture as a fumigant-like compound where insects were concealed inside the foodstuff. Only a few number of adults of tested insects emerged from insect's foods that had been exposed to mixture at the rate of either 0.5 PH_3/l + 8% CO_2 or 0.33 PH_3/l + 24% CO_2. These doses of mixture were sufficient to kill different developmental stages of all insects inside the foodstuff mass. The control groups of foodstuff that were not treated with CO_2-phosphine mixture yielded 2936, 4113, 3337 and 4152 adults of *O. surinamensis, L. serricorne, P. interpunctella* and *C. cautella* during the same incubation period, respectively.

DISCUSSION

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

Table 1. Toxicity of CO_2 to adults of *O. surinamensis*, *L. serricorne* and eggs of *P. interpunctell* and *C. cautella* exposed 24 h at 27 ± 2°C in 51-L stainless steel container (empty-space tests).

Toxicity value	Toxicity to species							
	O. surinamensis		*L. serricorne*		*P. interpunctella*		*C. cautella*	
	Dose mg/l determined for							
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dose	7.67	82.03	12.10	256.01	5.76	43.75	7.25	71.00
Upper 95% CL	9.02	173.33	16.76	1102.41	7.25	127.21	9.80	332.72
Lower 95%CL	6.65	50.63	9.47	112.17	4.52	25.48	5.64	35.06
Slope ± SE	1.59 ± 0.18		1.24 ± 0.17		1.86 ± 0.32		1.66 ± 0.33	
Number of insects tested	900		900		270		270	
$\chi^{2\,a}$	0.54		0.21		0.58		0.34	
P	0.91		0.97		0.90		0.95	
RR_{50} [b] (95% CL)	1.33		2.10				1.26	

Three replicates were tested in each of five CO_2 doses 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]resistance ration (RR) is equal to LD_{50} each species / LD_{50} of the most susceptible species (*P. interpuctella*). CO_2 quantities used were 0, 4, 6, 8, 10 and 12%.

Table 2. Toxicity of CO_2 to adults of *O. surinamensis, L. serricorne* and eggs of *P. interpunctella* and *C. cautella* exposed 24 h under foodstuff mass (penetration tests).

Toxicity value	Toxicity to species							
	O. surinamensis		*L. serricorne*		*P. interpunctella*		*C. cautella*	
	Dose mg/l determined for							
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal dose	27.75	86.49	35.16	161.28	23.19	73.15	22.01	77.67
Upper 95% CL	30.18	131.62	42.86	389.63	26.41	172.59	25.27	220.28
Lower 95%CL	25.86	67.00	31.26	101.38	19.94	51.57	18.26	52.60
Slope ± SE	3.33 ± 0.42		2.48 ± 0.41		329 ± 0.72		3.00 ± 0.71	
Number of insects tested	900		900		270		270	
$\chi^{2\,a}$	0.88		0.19		0.18		0.20	
P	0.82		0.97		0.98		0.97	
RR_{50} [b] (95% CL)	1.26		1.60		1.05			

Three replicates were tested in each of five CO_2 doses 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]resistance ration (RR) is equal to LD_{50} each species / LD_{50} of the most susceptible species (*C. cautella*). CO_2 quantities used were 0, 10, 15, 20, 25 and 30%.

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 CO_2 with reduced dose of PH_3 mixture as an insect control material may be an appropriate approach to this objective. CO_2 is produced during the combustion of fossil fuels, wood and breathing of humans and animals. Carbon dioxide is retained irreversibly in the respiratory tract after exposure by inhalation and subsequently expels by exhalation (UIG, 2008). Therefore, death from CO_2 should be extremely uncommon under fumigation conditions. Although, CO_2 is not a novel compound, as it is yet to be registered for use as a fumigant in practical sense, there is therefore, paucity of published information about the direct toxicity of carbon dioxide to insects.

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 from 2.90 up to 4.02-fold. Since, the CO_2 is slightly soluble in water (Green et al., 1984),

Table 3. The adult emergence from immature stages of the *O. surinamensis, L. serricorne, P. interpunctella* and *C. cautella* exposed to various doses of CO_2 and CO2- PH_3 mixtures for 24 h in 39 m^3 store (hidden infestation tests).

Species	Treatment	Emergence at week											
		1	2	3	4	5	6	7	8	9	10	11	Total
O. surinamensis	Control	288	856	581	338	463	304	106	-	-	-	-	2936
	CO_2 8%	21	96	82	4	0	43	82	-	-	-	-	328
	PH_3 1 mg/l	2	0	0	0	0	0	0	-	-	-	-	2
	PH_3 0.5 mg/l CO_2 8%	0	0	0	0	0	0	0	-	-	-	-	0
	PH_3 0.33 mg/l CO_2 24%	0	0	0	0	0	0	0	-	-	-	-	0
L. serricorne	Control	309	1250	347	276	453	625	219	162	262	210	-	4113
	CO_2 8%	70	252	45	14	5	183	36	75	89	97	-	866
	PH_3 1 mg/l	0	0	1	1	3	0	0	0	0	0	-	5
	PH_3 0.5 mg/l CO_2 8%	0	2	0	1	2	0	1	1	4	3	-	14
	PH_3 0.33 mg/l CO_2 24%	0	0	0	0	1	3	0	0	0	0	-	4
P. interpuctella	Control	312	685	236	285	124	663	1032	-	-	-	-	3337
	CO_2 8%	32	75	29	38	15	80	133				-	402
	PH_3 1 mg/l	1	0	0	0	2	1	0	-	-	-	-	4
	PH_3 0.5 mg/l CO_2 8%	0	0	0	2	2	3	1	-	-	-	-	8
	PH_3 0.33 mg/l CO_2 24%	0	1	1	0	0	0	3	-	-	-	-	5
C. cautella	Control	422	643	325	467	398	443	774	185	162	141	192	4152
	CO_2 8%	73	114	41	34	37	62	155	12	25	20	8	581
	PH_3 1 mg/l	0	0	0	0	0	2	1	0	1	1	0	5
	PH_3 0.5 mg/l CO_2 8%	0	0	0	0	1	2	5	0	0	0	0	8
	PH_3 0.33 mg/l CO_2 24%	1	1	0	0	0	0	4	0	1	0	0	7

its concentration could decrease through sorption by the foodstuff mass. Therefore, in the presence of foodstuff, more CO_2 is needed for successful fumigation.

At the current study, fumigant mixture needed for the fumigation was pumped into the site of fumigation at one time instead of the conventional method of placing required tablets at different level in the store. This indicates that, the exposure period can be reduced to 24 h (1 day) from 120 h (5 days). Mueller (1998) reported that, increased carbon dioxide concentration accelerates respiration of the insects thereby rendering them more susceptible to phosphine. Likewise, Ren et al. (1998) found that, when insects were exposed to greater than 20% (v/v) of carbon dioxide, intake of phosphine was more than doubled. Admittedly, combination of lower levels of phosphine and higher level of carbon dioxide for a period of 24 h exposure is to be considered as a potential for replacing methyl bromide application. Phosphine-carbon dioxide mixture is non-flammable. Hence, in the application of very high doses of CO_2 and phosphine mixture, which are expected to produce volume of phosphine, vapors in air near flammability range and the risk of fire could be ruled out entirely.

Synergism of PH_3 using CO_2 has been reported a number of times (Athie et al., 1998; Mueller, 1998). However, these reports have little relevance to field applications as their tests were only for exposure period 72 h usually on adults and only on PH_3 susceptible strains. Due to this, no real comparison can be made between their results and present trials outcomes. Results of present study showed that, the impact of carbon dioxide on phosphine's toxicity is pronounced and synergistic

effect. Likewise, more recently, Yokoyama (2010) reported complete control of Hessian fly by phosphine-carbon dioxide fumigation. This conclusion fortifies the strength of current study. Another salient and important feature of using carbon dioxide and phosphine mixture is that, it penetrates foodstuff mass efficiently which is a significant benefit in situations where insect infestations are located deep within foodstuff-packed cracks. If a control agent lacks these penetrating capabilities, re-infestation of other area occurs very rapidly, severely undermining the value of a fumigation treatment. Since PH_3–CO_2 mixture is highly toxic to insects and because methyl bromide may not be available for use as a fumigant in immediate future, such mixture could be considered as less hazardous fumigant in management programs for stored-product insects due to much wider margin of safety for operational personnel and considerably fewer undesirable environmental side effects.

ACKNOWLEDGEMENTS

The authors are grateful to Abas Samadi and Mehdie Yasie (Urmia University) for helpful comments on an earlier draft of the manuscript. Technical assistance of Golamreza Sadeghie, Mojtaba Ganie Jahromie and Hoshang Jafar Golizadehie in rearing insects and conducting the experiments is gratefully acknowledged.

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