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Combined effect of ozone mixed with carbon dioxide on the mortality of five stored-product insects

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ABSTRACT

A study to determine the effect of ozone mixed with carbon dioxide on controlling stored-grain insects was conducted in the storehouse. Adults of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica*(F.), *Oryzephilus surinamensis*(L.) and 3th larvae of *Plodia interpunctella*(Hubner) were exposed to the mixture of ozone and carbon dioxide. 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, 40, 50 and 100 cm) and nutrition materials (date, wheat and rice), in penetration tests and empty-space tests. In empty-space trials, the highest mortality was for *P. interpunctella*. In penetration tests, treatment with high-pressure ozone and carbon dioxide under different height and foodstuff may result in different rates of mortality. The mixture of ozone and carbon dioxide in the interaction between height and diet (heigh×diet) are not significant for the *S. oryzae, T. castaneum, R. dominica*and *P. interpunctella*but for *O. surinamensis* is significant. The influence of ozone gas and carbon dioxide in the date is more than rice and wheat. The mixture of ozone with carbon dioxide can be as suitable fumigant for decreasing phosphine and methyl bromide under ambient storage conditions in penetration and empty-space fumigations.

Keywords: Fumigant, foodstuff, phosphine, methyl bromide.

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), thus application of it 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 *S. oryzae*, *T. castaneum* and *R. dominica* (Chimbe and Galley, 1996; Collins *et al*., 2002). 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 40% are maintained for long periods. Exposure periods longer than 14 d are required to kill the insects when the concentration of CO_2 in the air is below 40% (Kashi, 1981). Ozone is a triatomic form of oxygen (O_3) and is referred to as activated oxygen, or allotropicoxygen. Ozone can be generated by electrical charges in air and is currently used in the medical industry as a disinfection technique against microorganisms and viruses, as a means of reducing odor, and for removing taste, colour, and environmental pollutants in industrial applications (Kim *et al*., 1999). In 1997, ozone was recognized as being generally safe (GRAS) for food contact applications in the United States (Graham *et al*., 1997; U.S. Food and Drug Administration, 1997). Since that time, interest in developing ozone applications in the food industry has increased, although some regulatory issues regarding ozone use for this purpose have not been resolved. Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. An attractive aspect of ozone is that it decomposes rapidly (within about 50 min) to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. At low concentrations ozone protects clean surfaces from subsequent fungal contamination and growth, although higher doses are required to kill fungi on contaminated surfaces (Rice *et al*., 1982). Five ppm ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* Link and *Fusarium moniliforme* Sheldon (Mason *et al*., 1997). Ozone in its gaseous form has also been shown to have potential to kill insect pests in commodities (Erdman, 1980; Mason *et al.,* 1997; Kells *et al.,* 2001). High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* Motschulsky, and the confused flour beetle *Tribolium confusum* du Val, and the larval stage of the Indian meal moth, *Plodia interpunctella* (Hubner) exposed to low ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980; Kells *et al*., 2001). Erdman (1980) also observed mortality of larvae of *T. confusum* and the red flour beetle, *Tribolium castaneum* (Herbst) when exposed to a 45 ppm ozone environment. Leesch (2003) tested ozone as a toxicant to stored-product insects in the hope of killing insects at low dosages in short periods of time. In his study, even high concentrations of 200–500 ppm (v/v) required many hours to kill the insects exposed. Other than these studies, little has been done to determine susceptibility of stored-product insects to ozone treatments. The purpose of this study was to determine the effect of O_3 mixed with CO_2 on the mortality of stored-products insects for reduce the appropriate amount of gaseous ozone.

MATERIALS AND METHODS

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

Ozone

Gaseous ozone was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT provided by the company Ozomax Inc. (http://www.ozomax.com), Canada) with 5 g / h of output from purified extra dry

oxygen (O_2) feed gas. The generator is capable of producing 13.88 mg/L ozone at an O_2 flow rate of 6 L/min at room temperature.

Carbon dioxide

The CO_2 atmospheres were obtained by replacement of a volume of atmospheric air taken from the desiccators with the same volume of CO_2 to obtain concentration of 30%.

Each desiccator was fitted with one valve which permitted the withdrawal or introduction of gas.

Fumigation chamber

The fumigation chamber had the following internal dimensions: 4.7 m long, 2.8 m wide and 2.9 m high (volume about 38 m^3) containing bins with different height (30, 40, 50 and 100 cm) and 20 cm in diameter were placed in middle of fumigation chamber, of date, wheat and rice were used for filling the bins.

Test insects

Sitophilus oryzae (Coleoptera: Curculionidae), *Triobolium castaneum* (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *Oryzephilus surinamensis*(Coleoptera: Silvanidae) and 3th larvae of *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±2ºC 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), *O. surinamensis* was reared on oat (Tunçbilek, 1997) 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: (i) *S. oryzae* and *R. dominica* adults 7±2 day old, (ii) *T. castaneum* adults, 14±3 day old, (iii) *O. surinamensis*adults 3 day old and (iiii) 3th larvae of *P. interpunctella.* 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±2ºC 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*, *O. surinamensis* 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 contained 20 insects and 3 g food, then door of chamber were entirely closed and 90mg/l of gaseous ozone was injected with a hose which was made relation between injection gate of ozone generator and then 30% of camber's value was filled with carbon dioxide which was introduced into the fumigation chamber from CO_2 cylinders which their weight was 5 Kg. Immediately after the O_3 and CO_2 injection the injection gate was closed. In each test, the control insects were treated identically except that no expose to O_3 and CO_2. 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. Mortality rates of, *S. oryzae, T. castaneum*, *R. dominica*, *O. surinamensis* adult and 3th larvae of *P. interpunctella* were recorded 24h after termination exposure.(Pourmirza and Tajbakhsh, 2008).

Penetration tests

The penetration tests were carried out in the above mentioned chamber (38 m^3). Experiments were performed in different heights (30, 40, 50 and 100 cm) and nutrition materials (date, wheat and rice). For each experiment five cages for five experimented species (Adults (mixed-sex) of *S. oryzae, T. castaneum*, *R. dominica*, *O. surinamensis* 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 3 mentioned different nutrition materials separately. The procedure used was similar to those described for the empty- space tests (in penetration tests used of 120mg/l O_3 for injection with 30% Co_2). Each experiment was replicated three times in three days. The control case was prepared in identical manner without application test compounds. 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. Mortality rates of *S. oryzae, T. castaneum*, *R. dominica*, *O. surinamensis* adult and 3th larvae of *P. interpunctella* were recorded after 24 h after termination exposure.

Data analysis

Data analyzed using of Variance after arcsine transforming them. Data were subjected to Univariate analysis using SPSS (SPSS Inc 1993). Data of experiment were analyzed by a completely randomized design using factorial arrangements of treatments. The analysis of data was performed on each dependent variable using the treatments were compared for significance with ANOVA. Mean separation was determined using the Tukey's test.

RESULTS AND DISCUSSION

Table 1 showed that, F values of insects are significant difference in empty- space test, in this space the highest mortality was for *S. oryzae* and *P. interpunctella* (Table 2) and lowest mortality showed in *R. dominica*(Table 2 and figure 6). Treatment with ozone and carbon dioxide in empty- space test may result in different rates of mortality, for example, for *P. interpunctella* mortality percentage was observed significantly different with other insects except *S. oryzae*, while significant difference no achieved within *O. surinamensis* with *S. oryzae*, *T. castaneum*and*R.dominica* (Table 3).

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

Source	df	Mean Square	F	*ρ*
Between groups	4	302.350	17.948	.000**
Within Groups	10	16.84		
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 empty- space tests* In each column mean that letters are different at a 5 percent level with one another are significant differences

Mean of mortality percentage			
	Groups		
Insect	1	2	3
R. dominica	57.8593928		
O. surinamensis	66.1449829	66.1449829	
T. castaneum	68.6640184	68.6640184	
S. oryzae		75.2410395	75.2410395
P. interpunctella			84.5902002
ρ	.055	.121	.108

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

Insect					95% Confidence Interval	
	Insect	Mean Difference	Std. Error	Sig.	Lower Bound	Upper Bound
	T. castaneum	6.57702108	3.35120640	.348	-4.4520745	17.6061166
S. oryzae	*R. dominica*	17.38164665*	3.35120640	.003	6.3525511	28.4107422
	O. surinamensis	9.09605653	3.35120640	.121	-1.9330390	20.1251521
	P.interpunctella	-9.34916074	3.35120640	.108	-20.3782563	1.6799348
	S. oryzae	-6.57702108	3.35120640	.348	-17.6061166	4.4520745
T. castaneum	*R. dominica*	10.80462557	3.35120640	.055	-.2244700	21.8337211
	O. surinamensis	2.51903545	3.35120640	.939	-8.5100601	13.5481310
	P.interpunctella	-15.92618182*	3.35120640	.005	-26.9552774	-4.8970863
	S. oryzae	-17.38164665*	3.35120640	.003	-28.4107422	-6.3525511
R. dominica	*T. castaneum*	-10.80462557	3.35120640	.055	-21.8337211	.2244700
	O. surinamensis	-8.28559011	3.35120640	.173	-19.3146857	2.7435054
	P. interpunctella	-26.73080739*	3.35120640	.000	-37.7599029	-15.7017118
	S. oryzae	-9.09605653	3.35120640	.121	-20.1251521	1.9330390
O. surinamensis	*T. castaneum*	-2.51903545	3.35120640	.939	-13.5481310	8.5100601
	R. dominica	8.28559011	3.35120640	.173	-2.7435054	19.3146857
	P. interpunctella	-18.44521727*	3.35120640	.002	-29.4743128	-7.4161217
	S. oryzae	9.34916074	3.35120640	.108	-1.6799348	20.3782563
P.interpunctella	*T. castaneum*	15.92618182*	3.35120640	.005	4.8970863	26.9552774
	R. dominica	26.73080739*	3.35120640	.000	15.7017118	37.7599029
	O. surinamensis	18.44521727*	3.35120640	.002	7.4161217	29.4743128

*p is significant at 0.05 level.

Table 4 shows that, F values of height are significant for *S. oryzae, T. castaneum, R. dominica, O. surinamensis* and *P. interpunctella*. This table showed F values of diet are significant for all of the insects. The interaction between height and diet (heigh× diet) are not significant for *S. oryzae*, *T. castaneum, R. dominica*and *P. interpunctella*but but for *O. surinamensis* is significant in $\rho < 0.05$.

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

	S. oryzae			*T. castaneum*			*R.dominica*			*O. surinamensis*			*P.interpunctella*		
S. V	df	Mean square	F	df	Mean square	F	df	Mean square	F	df	Mean square	F	df	Mean square	F
Height(a)	3	411.50	97.95**	3	288.53	89.73**	3	284.45	91.29**	3	390.73	82.43**	3	1215.4	110.9**
Diet(b)	2	203.78	48.51**	2	60.99	18.97*	2	60.48	19.41**	2	122.72	25.82**	2	87.67	8.00**
height × diet(ab)	6	70.71	1.66n.s	6	4.96	1.54n.s	6	3.63	1.16n.s	6	14.49	3.05*	6	5.69	.52n.s
Total	11			11			11			11			11		

n.sp is not significant.
*p is significant at 0.05 level.
**p is significant at 0.01 level.

Treatment with high-pressure ozone and carbon dioxide under different height and foodstuff may result in different rates of mortality, for example, at 100 cm mortality percentage for all of the insects was observed significantly different with other heights (Tables 5, 6, 7, 8 and 9), Results showed that each of bins, ozone and carbon dioxide mixture to achieve highly mortality against *P. interpunctella*in all foodstuffs (Figure 6). Results showed that there is a significant difference in mortality between different heights in *O. surinamensis*and *P. interpunctella*(Tables 8 and 9).

Table 5: Multiple Comparisons of height for *S. oryzae*

high (cm)	high (cm)	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	40	2.3281441	.96618564	.102	-.3371842	4.9934725
30	50	7.4002370*	.96618564	.000	4.7349087	10.0655654
	100	15.2721975*	.96618564	.000	12.6068692	17.9375259
	30	-2.3281441	.96618564	.102	-4.9934725	.3371842
40	50	5.0720929*	.96618564	.000	2.4067645	7.7374212
	100	12.9440534*	.96618564	.000	10.2787251	15.6093817
	30	-7.4002370*	.96618564	.000	-10.0655654	-4.7349087
50	40	-5.0720929*	.96618564	.000	-7.7374212	-2.4067645
	100	7.8719605*	.96618564	.000	5.2066322	10.5372889
	30	-15.2721975*	.96618564	.000	-17.9375259	-12.6068692
100	40	-12.9440534*	.96618564	.000	-15.6093817	-10.2787251
	100	-7.8719605*	.96618564	.000	-10.5372889	-5.2066322

*p is significant at 0.05 level.

Table 6: Multiple Comparisons of height for *T. castaneum*

high (cm)	high (cm)	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	40	-1.3935997	.84529746	.372	-3.7254448	.9382455
30	50	6.2819147*	.84529746	.000	3.9500696	8.6137598
	100	10.7756474*	.84529746	.000	8.4438022	13.1074925
	30	1.3935997	.84529746	.372	-.9382455	3.7254448
40	50	7.6755144*	.84529746	.000	5.3436692	10.0073595
	100	12.1692470*	.84529746	.000	9.8374019	14.5010922
	30	-6.2819147*	.84529746	.000	-8.6137598	-3.9500696
50	40	-7.6755144*	.84529746	.000	-10.0073595	-5.3436692
	100	4.4937327*	.84529746	.000	2.1618875	6.8255778
	30	-10.7756474*	.84529746	.000	-13.1074925	-8.4438022
100	40	-12.1692470*	.84529746	.000	-14.5010922	-9.8374019
	100	-4.4937327*	.84529746	.000	-6.8255778	-2.1618875

*p is significant at 0.05 level.

Table 7: Multiple Comparisons of height for *R. dominica*

high(cm)	high(cm)	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	40	.7135357	.83210289	.826	-1.5819107	3.0089822
30	50	7.9900752*	.83210289	.000	5.6946287	10.2855216
	100	11.5204541*	.83210289	.000	9.2250077	13.8159006
	30	-.7135357	.83210289	.826	-3.0089822	1.5819107
40	50	7.2765394*	.83210289	.000	4.9810930	9.5719859
	100	10.8069184*	.83210289	.000	8.5114719	13.1023649
50	30	-7.9900752*	.83210289	.000	-10.2855216	-5.6946287
	40	-7.2765394*	.83210289	.000	-9.5719859	-4.9810930
	100	3.5303790*	.83210289	.002	1.2349325	5.8258254
100	30	-11.5204541*	.83210289	.000	-13.8159006	-9.2250077
	40	-10.8069184*	.83210289	.000	-13.1023649	-8.5114719
	100	-3.5303790*	.83210289	.002	-5.8258254	-1.2349325

*p is significant at 0.05 level.

Table 8: Multiple Comparisons of height for *O. surinamensis*

high (cm)	high (cm)	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
30	40	5.4566098*	1.0262955	.000	2.6254616	8.2877579
	50	11.5865704*	1.0262955	.000	8.7554223	14.4177186
	100	14.8495413*	1.0262955	.000	12.0183932	17.6806895
40	30	-5.4566098*	1.0262955	.000	-8.2877579	-2.6254616
	50	6.1299607*	1.0262955	.000	3.2988125	8.9611088
	100	9.3929316*	1.0262955	.000	6.5617834	12.2240797
50	30	-11.5865704*	1.0262955	.000	-14.4177186	-8.7554223
	40	-6.1299607*	1.0262955	.000	-8.9611088	-3.2988125
	100	3.2629709*	1.0262955	.020	.4318228	6.0941191
100	30	-14.8495413*	1.0262955	.000	-17.6806895	-12.0183932
	40	-9.3929316*	1.0262955	.000	-12.2240797	-6.5617834
	100	-3.2629709*	1.0262955	.020	-6.0941191	-.4318228

*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	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
30	40	9.3217103*	1.5600103	.000	5.0182519	13.6251687
	50	16.4800913*	1.5600103	.000	12.1766329	20.7835497
	100	27.5238479*	1.5600103	.000	23.2203895	31.8273063
40	30	-9.3217103*	1.5600103	.000	-13.6251687	-5.0182519
	50	7.1583811*	1.5600103	.001	2.8549227	11.4618394
	100	18.2021376*	1.5600103	.000	13.8986792	22.5055960
50	30	-16.4800913*	1.5600103	.000	-20.7835497	-12.1766329
	40	-7.1583811*	1.5600103	.001	-11.4618394	-2.8549227
	100	11.0437565*	1.5600103	.000	6.7402982	15.3472149
100	30	-27.5238479*	1.5600103	.000	-31.8273063	-23.2203895
	40	-18.2021376*	1.5600103	.000	-22.5055960	-13.8986792
	100	-11.0437565*	1.5600103	.000	-15.3472149	-6.7402982

*p is significant at 0.05 level.

Figures 1, 2, 3, 4 and 5 shows the influence of ozone and carbon dioxide in the date is more than rice and wheat, because the most mortality of *S. oryzae*, *T. castaneum*, *R. dominica, O. surinamensis*and and *P. interpunctella* in bins that contain date were observed. The lowest mortality rate of *S. oryzae* and *P. interpunctella* occurred in reservoirs rice (Figures 1 and 5).

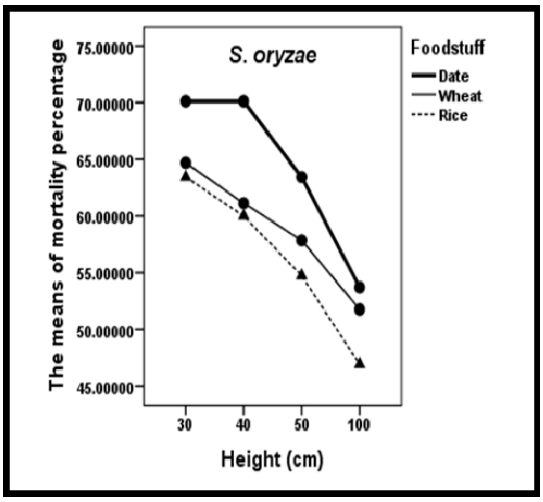

Fig 1: The comparison of mortality of *S. oryzae* in different height and foodstuffs

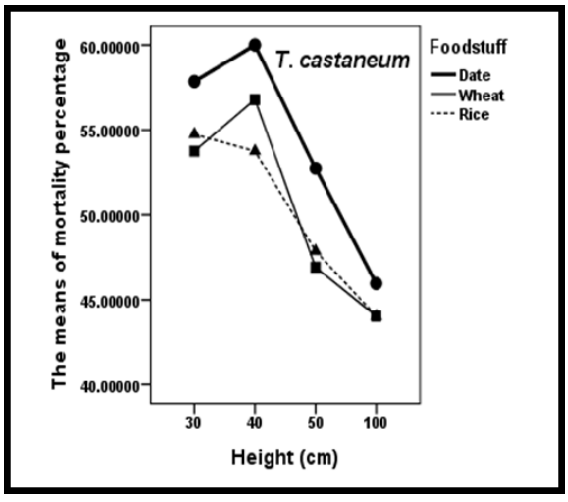

Fig 2: The comparison of mortality of *T .castaneum* in different height and foodstuffs

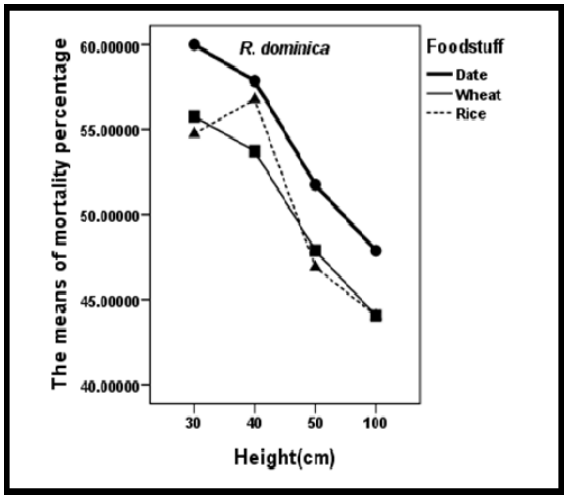

Fig 3: The comparison of mortality of *R. dominica* in different height and foodstuffs

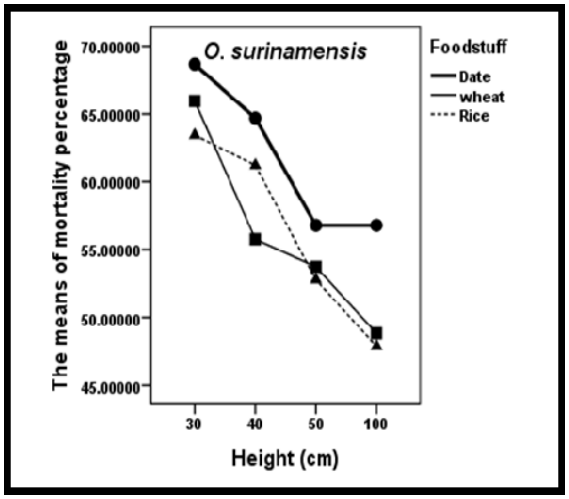

Fig 4: The comparison of mortality of *O. surinamensis* in different height and foodstuffs

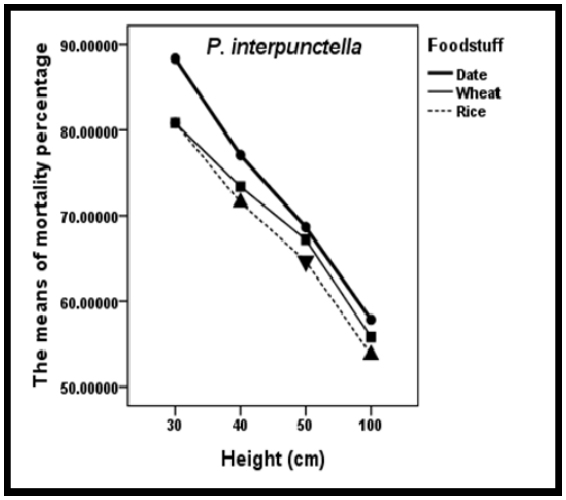

Fig 5: The comparison of mortality of *P.interpunctella* in different height and foodstuffs

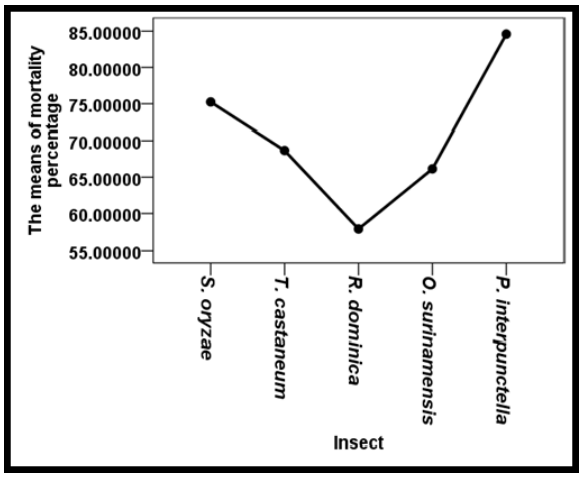

Fig 6: The comparison of mortality of insects in empty- space test.

Several chemicals have been proposed as replacements for methyl bromide since its listing as an ozone-depleting chemical (UNEP, 1995). Among those that have shown some promise are methyl iodide, carbonyl sulfide and sulfuryl fluoride (Vikane or ProFume) (Zettler *et al*., 1997, 1999), and propylene oxide (Navarro *et al*., 2004; Isikber *et al*., 2006). In addition, ozone in its gaseous form has been considered to have potential to kill pests in commodities (Erdman, 1980; Kells *et al*., 2001; Mendez *et al*., 2003; Leesch, 2003). However, since many factors influence fumigation, including equipment performance and environmental conditions, defining the abilities and limitations of ozone penetration of the commodity and efficacy against insects are important aspects in planning strategies for fumigation of commercial facilities. Thus, it has been important to increase the penetration of ozone to achieve maximum mortality. To achieve this goal, in the current study, it was attempted to increase the ozone penetration by adding the carbon dioxide. Our toxicity data for empty space

ozone and carbon dioxide mixture treatments indicated a remarkable difference in susceptibility between insects in this research. Empty space ozone and carbon dioxide mixture treatment resulted in highly kill of adults *P. interpunctella* and *S. oryzae*, whilst very low mortalities of adult *R. dominica* were observed. These results support those of Leesch (2003) who tested the toxicity of gaseous ozone to different stages of *P. interpunctella* and adults of *T. confusum* and found that life stages (apart from eggs) of *P. interpunctella* were more or less susceptible to laboratory treatment with ozone at 300 ppm for a 4-h exposure period. Our toxicity data indicated that *R. dominica* and *T. castaneum* was generally more tolerant to ozone and carbon dioxide mixture treatment than other insects. Leesch (2003) found that adults of *T. confusum* were much more tolerant of exposure to ozone than adults of *P. interpunctella*. Our findings on the efficacy of ozone and carbon dioxide as a fumigant against *S. oryzae* and *P. interpunctella* may be compared with several studies on the efficacy of ozone to control insect pests of stored grain. The results obtained by kells *et al.* (2001) indicated that high mortality was achieved for adults of the maize weevil, *S. zeamais*, and the red flour beetle, and the larval stage of the Indian meal moth, *P. interpunctella* exposed to 50 ppm ozone for 3 days. In laboratory studies, Mason *et al.* (1997) reported that 5 ppm of ozone resulted in 100% mortality of adult saw-toothed grain beetle, *O. surinamensis*and confused flour beetle after exposure times of 3 and 5 days, respectively. In these studies the exposure time was much longer than that tested here, though the ozone concentrations were much lower.

Therefore the differences in the efficacy of ozone and carbon dioxide mixture against insects can be caused by differences of ozone concentration, exposure period or ozone application method. Ozone and carbon dioxide mixture flush treatment at 30cm height resulted in almost highly mortality of *T. castaneum* and *R. dominica*, whereas these insects placed in 100cm were hard to kill. For *P. interpunctella*, larvae placed in the deep 100 cm were easily killed. These results indicated that ozone with carbon dioxide, like chemical fumigants, could penetrate into the commodity enough to kill the insects. The results of our study indicate that gaseous ozone needs to be re-flushed intermittently to keep the required concentration and control the insects. Initial movement of ozone through the grain was impeded by a phenomenon described as the ozone demand of the medium (Kim *et al*., 1999). Our data show that ozone and carbon dioxide mixture has potential for the control of storage pests though there are differences between species in the levels of exposure required. 3th Larvae of *P. interpunctella* is generally much more susceptible to gaseous ozone and carbon dioxide mixture than other insects. We have also shown that ozone and carbon dioxide mixture has not an initial problem in being able to penetrate through the commodity. This ability redound to kill insect pests at deeper levels.

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