EFFECTS OF NITROGEN AND CARBON DIOXIDE MIXTURES ON THE MORTALITY OF FIVE STORED-PRODUCT INSECTS

*Reza Sadeghi** and *Arsalan Jamshidnia*

Department of Entomology and Plant Pathology, College of Abouraihan,
University of Tehran, Iran.

Correspondence address:
College of Abouraihan, University of Tehran.
Tel.:00989125732940
Fax.:002763040909
e-mail: rsadeghi@ut.ac.ir

Abstract: A study to determine the effect of nitrogen 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.), *Oryzaeohilus surinamensis* (L.) and 3[th] larvae of *Plodia interpunctella* (Hubner) were exposed the mixture of nitrogen 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 depths (30, 40, 50 and 100 cm) and nutrition materials (rice, wheat and date), in penetration tests. In penetration tests, treatment with high-pressure nitrogen and carbon dioxide under different depth and foodstuff may result in different rates of mortality. The mixture of nitrogen and carbon dioxide in the interaction between depth and diet (depth ×diet) are not significant for the *S. oryzae, T. castaneum, R. dominica* and *P. interpunctella* but for *O. surinamensis* is significant. The influence of nitrogen gas and carbon dioxide in the date is more than rice and wheat. The mixture of nitrogen with carbon dioxide can be as suitable fumigant for decreasing phosphine under ambient storage conditions in penetration fumigation.

Key words: carbon dioxide, foodstuff, fumigant, nitrogen.

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 *Rhyzopertha 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 1972-1973 (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 40% are maintained for long periods. Exposure periods longer than 14 d are required to kill the insects when the concentration of CO, in the air is below 40% (Kashi, 1981). CO_2 due to exists 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). Carbon dioxide (CO_2) is an "organic" fumigant that does not produce harmful residues and is relatively safe to use. It is effective at killing insects in all stages of their life cycles through asphyxiation. When the energy-transfer (production of ATP) is blocked and the respiration is accelerated, the insects have to use their stored energy sources and the exhaustion of those resources directly lead to the death of the insects. CO_2 is the only fumigant that can be used for long-term storage of products, and is the only fumigant that can be used to control insect pests in organic product storage. It has been demonstrated that high concentrations of CO_2 are effective at controlling various insect pests (Sekhon et al., 2010). 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 percent 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 the 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).

MATERIALS AND METHODS

Culturing of test insects

The culture medium comprised whole-wheat flour with 5% yeast for *T. castaneum* (Childs and Overby, 1983), and Soft kernel wheat was used for rearing *S. oryzae* and *R. dominica* (Padin et al., 2002; Bell et al., 1977). *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). Cultures were maintained at room temperatures (27±2°C) and humidity (65 ±5%).

Experimental materials

In present study carbon dioxide and N_2 was used as fumigant materials. N_2 was provided by six kilograms cylinders containing liquid N_2. 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. Experimental chamber had 38 m^3 capacities. Bins with different depth (30, 40, 50 and 100 cm) and 20 cm in diameter were placed in middle of fumigation chamber. Rice, wheat and date were used for filling the bins.

Penetration tests

The penetration tests were carried out in the above mentioned chamber (38m^3). Experiments were performed in different depths (30, 40, 50 and 100cm) and nutrition materials (rice, wheat and date). For each experiment, five cages with adults (mixed-sex) of *S. oryzae, T. castaneum*, *R. dominica*, *O. surinamensis* and 3th larvae of *P. interpunctella*, each containing 20 adults of one species with 3g food were placed horizontally at the bottom of PVC bins. Each bin was filled by 3 mentioned different nutrition materials separately. In penetration tests used of 4Kg N_2 and 30% of cambers' value was filled with carbon dioxide which was introduced into the fumigation chamber from CO_2 cylinders which their weight was 5 Kg. After the N_2 and CO_2 injection the gate was closed.

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^{0}C and 65 ±5% RU. Mortality rates of *S. oryzae, T. castaneum*, *R. dominica*, *O. surinamensis* adults and 3th larvae of *P. interpunctella* were recorded 24 h after termination exposure.

Data analysis

Data analyzed using of Variance after their arcsine transforming. Data were subjected to Univariate analysis using SPSS (SPSS Inc, 1993). A completely randomized design using factorial arrangements of treatments was used to analyze data of experiments. 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 DISCUSSIONS

Table 1 shows that, F values of depth are significant for *S. oryzae, T. castaneum, R. dominica* and *O. surinamensis* and *P. interpunctella*. F values of diet are significant for *S. oryzae, T. castaneum* and *R. dominica* and *O. surinamensis* but not for *P. interpunctella.* The interaction between depth and diet (depth×diet) are not significant for *S.oryzae, T. castaneum, R. dominica* and *P. interpunctella* but for *O. surinamensis* is significant in $\rho < 0.05$.

Treatment with high-pressure nitrogen and carbon dioxide under different depth and foodstuff may result in different rates of mortality, for example at 100cm, mortality percentage for all insects was observed significantly different with other depths (Table 2). Results showed that there is no significant difference in mortality between 30cm with 40cm in *T. castaneum* and *R. dominica***.**

Figures 1, 2, 3, 4 and 5 showed the influence of nitrogen gas 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 *P. interpunctella* in bins that contain date were observed. The lowest mortality rate of *S. oryzae*, *T. castaneum*, *R. dominica, O. surinamensis* occurred in reservoirs rice (Figures 1, 2, 3 and 4) but for *P. interpunctella* the lowest mortality rate was related to wheat that shows less influence of nitrogen gas and carbon dioxide this nutrient (Figure 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, 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). At the present time, large proportions of stored foodstuffs are fumigated with methyl bromide and phosphine. Methyl bromide was that 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 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, 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 as a less hazardous compound may be an appropriate approach to this objective. Trials have been conducted on the use of nitrogen and carbon dioxide 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). In the present study however, the use of nitrogen gas with carbon dioxide as its main components, may achieve good results in the control of stored pests, because the results showed that in stored- product infested with *S. oryzae, T. castaneum, R. dominica, O. surinamensis* and *P. interpunctella* there is significantly mortality after 24 hours exposure to nitrogen and carbon dioxide mixture in PVC bins. The addition of N_2 to carbon dioxide caused an increase in the mortality of the population of five species as also observed by Athie et al. in 1998 with the addition of CO_2 to phosphine. The mortality data for adults of *S. oryzae*, *Osurinamensis*, *T. castaneum* and *P. interpunctella* in the present study agrees with those of Li et al., (2009) who treated the *Cryptolestes furrugineus* (Stephens) only with liquid nitrogen in bins which were filled with hard red spring wheat. In our study, N_2 was very effective in mortality to all tested insects in penetration tests. This finding would agree with the data collected by Gunasekaran and Rajendran (2005) which has demonstrated that O_2, N_2 and CO_2 were toxic to some stored–product insects.

CONCLUSION

Due to the problem of insect resistance to phosphine and the lack of viable alternatives for control methods, further research is necessary to ascertain other features mixture of nitrogen with carbon dioxide specifically its effectiveness against strains of stored-product insects and the spectrum of activity under different environmental conditions.

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Table 1. Variance analysis of different treatments of five experimented insects mortality in penetration tests

S. V	*S. oryzae*			*T. castaneum*			*R. dominica*			*O. surinamensis*			*P. interpunctella*		
	df	Mean square	F	df	Mean square	F	df	Mean square	F	df	Mean square	F	df	Mean square	F
Depth (a)	3	255.60	27.25**	3	291.97	53.62**	3	338.32	31.00**	3	388.29	60.02**	3	578.36	39.07**
Diet (b)	2	122.29	13.04**	2	107.82	19.80**	2	57.58	5.27*	2	74.26	11.48**	2	20.02	1.35 n.s
depth × diet (ab)	6	2.18	.23 n.s	6	3.75	.69 n.s	6	7.02	.64 n.s	6	31.71	4.90*	6	2.66	.18 n.s
Total	11			11			11			11			11		

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

Table 2. Multiple Comparisons of depth for insects

data Tukey HSD							
Insect	(Depth) a(cm)	(Depth) a(cm)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
S. oryzae	30	40	4.00088	1.443571	.049*	.01863	7.98313
		50	5.06824	1.443571	.009*	1.08599	9.05048
		100	12.74745	1.443571	.0001**	8.76521	16.72970
T. castaneum	30	40	-.25945	1.100008	.995n.s	-3.29394	2.77504
		50	6.97114	1.100008	.0001**	3.93665	10.00563
		100	11.45153	1.100008	.0001**	8.41704	14.48603
R. dominica	30	40	3.98415	1.557243	.076n.s	-.31168	8.27997
		50	8.97972	1.557243	.0001**	4.68390	13.27555
		100	14.13884	1.557243	.0001**	9.84302	18.43467
O. surinamensis	30	40	8.22334	1.199000	.0001**	4.91577	11.53091
		50	10.55913	1.199000	.0001**	7.25156	13.86671
		100	15.77620	1.199000	.0001**	12.46863	19.08377
P. interpunctella	30	40	5.89271	1.813715	.017*	.88938	10.89604
		50	11.49954	1.813715	.0001**	6.49621	16.50287
		100	18.79261	1.813715	.0001**	13.78928	23.79594

*. The mean difference is significant at the 0.05 level.

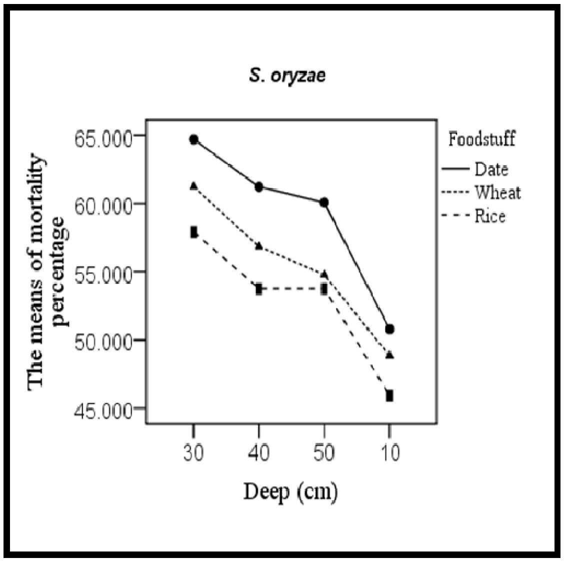

Figure 1. The comparison of mortality of *S. oryzae* in different depths and foodstuffs

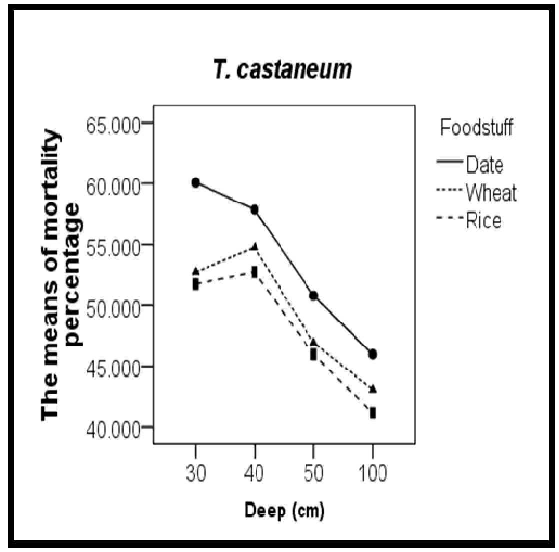

Figure 2. The comparison of mortality of *T. castaneum* in different depths and foodstuffs

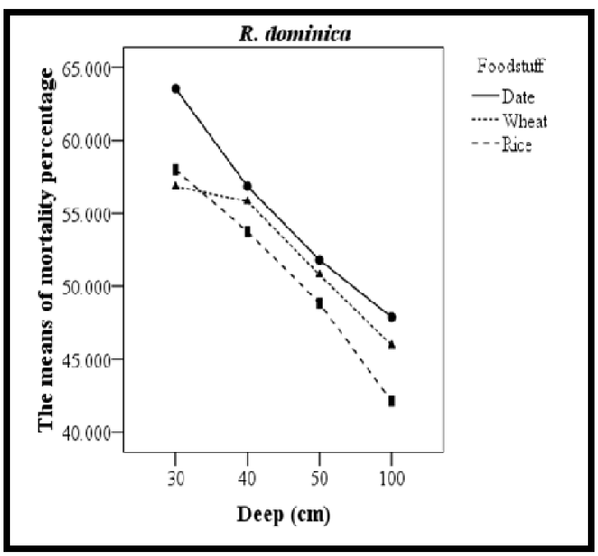

Figure 3. The comparison of mortality *R. dominica* in different depths and foodstuffs

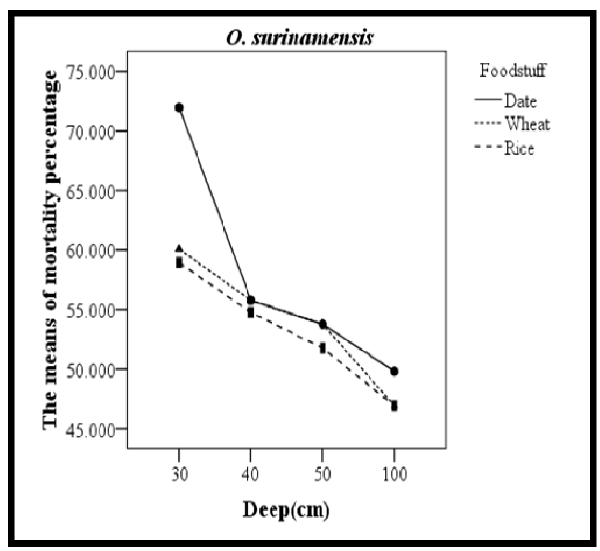

Figure 4. The comparison of mortality of *O. surinamensis* in different depths and foodstuffs

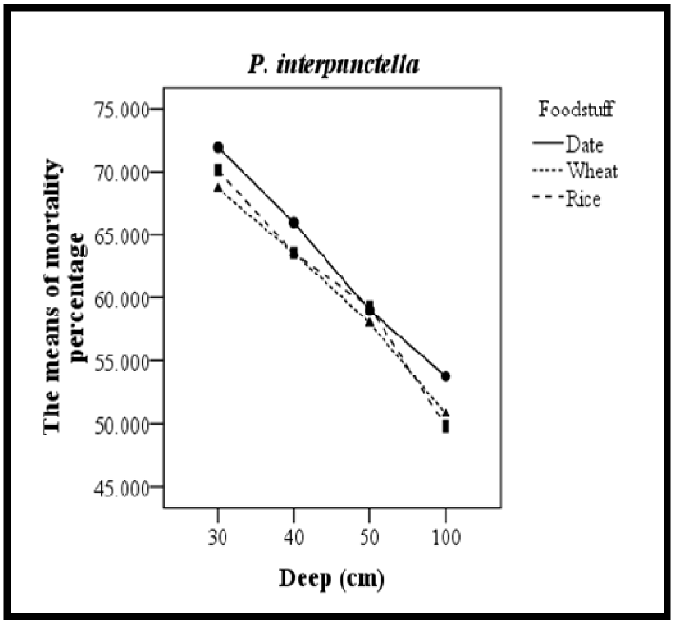

Figure 5. The comparison of mortality *P. interpunctella* in different depths and foodstuffs