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EFFICACY OF PURE CARBON DIOXIDE GAS AGAINST SOME INSECT SPECIES OF STORED PRODUCTS AT DIFFERENT TEMPERATURES

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ABSTRACT: Laboratory studies were carried out to investigate the efficacy of the pure gas carbon dioxide against the developmental stages and adults of four insect species of stored products at four degrees of temperature viz. 20, 25, 30 and 35°C. The results showed that carbon dioxide was more effective at the highest temperature than at the lowest one. The Angoumois grain moth, *Sitotroga cerealella* (Olivier) was the most sensitive species compared with the other three species,(the cowpea beetle, *Callosobruchus maculatus* (F), the lesser grain borer, *Rhizopertha dominica* (F.) and the red flour beetle, *Tribolium castaneum* (Herbst)).The adult stage of the tested species proved to be the most susceptible stage to pure carbon dioxide gas at the various temperatures. Time of exposure was an important factor from the mortality point of view, the mortality percentages increased as the exposure period increased at various temperatures.

Key words: Carbon dioxide, temperature, insect of stored products, control.

INTRODUCTION

Attention has been focused on the problems arising from application of chemical pesticides for pest control by the authorities concerning the public health and environment (WHO and EPA) who emphasized upon using of alternative methods for pest control. Carbon dioxide (CO_2) is one of the normal components of the atmosphere. Many studies have been conducted to control the stored product pests by using this gas singly or in mixtures with

1997; and Hashem, 2000). Now, carbon dioxide is used in several countries for the treatment of stored products particulary grain bulk.The action of CO_2, as a modified atmosphere treatment, lies in its availibility, relative convenience, and safety of application beside the fact that it does not leave toxic residues (Jay, 1980 and Johnson, 1981).

This study aims to evaluate the efficacy of pure carbon dioxide against some insect species of stored products at different degrees of temperature.

MATERIALS AND METHODS

Test Insects

Four known species of stored product insects were chosen for the present study. These insect species were: the cowpea beetle, *Callosobruchus maculatus* (F.), the lesser grain borer, *Rhizopertha dominica* (F.), the red flour beetle, *Tribolium castaneum* (Herbst), and the Angoumois grain moth, *Sitotroga cerealella* (Oliveir). The original cultures of these insects were started by batches of adults which had been collected from the local warehouses at Zagazig region and rearcd under the laboratory condition for several generations out of pesticides exposure.

Rearing Technique

Rearing technique of the tested insects was the same as described by Miller et al. 1969) the USDA Stored Product nsects Rescarch and Development laboratory, Savannah, Georgia.

1. Vessels of insect exposure to gases.

a- Wire gauze cages (exposure unit)

Copper wire gauze of 60 mesh/inch was used for making the cages of insect exposure to gases. These cages were cylindrical in shape (4 cm diameter x 7 cm hight) supported at the bottom with a plastic base and at the top with plastic ring. The cages were covered with muslin cloth secured by rubber bands.

b- Exposure chambers

The known urine collection plastic bags were used as gas exposure chambers for experiment of CO_2 bioassay against the tested insects. These bags are ractangular in shape 18 x 23 cm and 2000 ml capacity, each bag has two tubes (hoses) at both ends inlet and out let.These bags were firstly used for this purpose by us.

2. Exposure procedures

Batches of 50 individuals of the different developmental stages

were put separately in the vessels of gas exposure. Three replicates of each developmental stage were put in the plastic bags of urine collection through a lateral cut and the bags were tightly sealed using hand pressing sealer. The air inside the bags was allowed to get out by pressing gently on them and then the bags were connected to a CO_2 gas cylinder. The gas was purged very slowly inside the bags for about 20-30seconds to ensure complete exchange of the air inside the bag with the tested gas without any pressure. Treated bags were tightly closed and kept in an incubator at the tested temperatures namely, 20, 25, 30and 35 ± 1° C for different periods of gas exposure; 24, 48, 96, 168, 216 and 240 hours At the end of the exposure periods, the bags were aerated and the wire gauze cages were taken out and examined immediately for recording the death of adults stage. The immature stages of the tested insects *S. cerealella, C. maculatus* and *R.. dominica and eggs of T. castaneum* were maintained in an incubator at the tested temperatures continued until adult emergence was completely ceased.. Mortalities of the larvae and pupae of *T.castaneum* were determined after 24 hrs from aeration of the samples. The data were statistically analyzed accoording toFinney (1971).

RESULTS AND DISCUSSION

Data concerning the susceptibility of the different developmental stages and adults of the four tested insect species to pure carbon dioxide gas in closed area are presented in Tables 1 to 4. The obtaind results could be discussed as follows:

The Cowpea Beetle, *C. maculatus* (F.).

The results presented in Table 1 show generally that cumulative percentage mortality of different stages of *C.maculatus* raised markedly as the exposure period to gas was increased at various temperatures.

At 20°C. and after 24 hrs exposure period, adults was the most sensitive stage recording the highest percent mortality (66.33%) followed by egg stage (45.33%) while the larval and pupal stages; were the least sensitive ones (38.66 and 39.0%, respectively). Cumulative mortality in the second day and the subsequent days took the same trend of the first day recording 100% mortality in the seventh day for adult stage, in the

Table 1: Cumulative mortality percentage of developmental stages of *C. maculates F.* exposed to pure carbon dioxide in closed vessels at different degrees of temperature

Tested stage	Exposure period(in hours)									Temperature degrees
	24	48	96	168	216	240	LT_{50}	LT_{90}	Slope	
Eggs	45.33	65.66	89.66	94.66	100	-	28.93	101.22	2.47	20°C
Larvae	38.66	67.00	83.00	91.33	100	-	32.32	122.56	2.214	
Pupae	39.0	57.66	80.00	88.66	94.0	100	35.69	147.34	2.813	
Adults	66.33	93.33	99.66	100	-	-	18.96	40.75	3.8581	
Eggs	61.66	79.33	87.33	96.33	100	-	17.29	88.29	1.810	25°C
Larvae	58.66	71.66	88.00	96.66	100		22.59	91.36	2.112	
Pupae	56.0	77.33	85.66	88.33	100		18.78	119.58	1.594	
Adults	83.66	88.33	96.00	100	-		6.50	42.65	1.568	
Eggs	80.33	88.00	95.33	99.33	100	-	8.48	48.08	1.7016	30°C
Larvae	69.00	78.66	92.66	99.66	100	-	16.15	64.74	2.125	
Pupae	62.66	79.66	79.66	99.00	100	-	19.88	56.29	2.835	
Adults	87.66	97.66	100	100	-	-	10.32	26.12	3.177	
Eggs	91.66	97.66	98.33	100	-	-	2.62	19.07	1.489	35°C
Larvae	87.33	93.00	100	-	-	-	8.18	30.57	2.239	
Pupae	78.66	95.00	97.36	-	-	-	9.87	38.55	2.167	
Adults	94.00	99.66	100	-	-	-	6.73	17.30	2.801	

ninth day for egg and larval stages and in tenth day for pupal stage, LT_{50} values were 18.96, 28.93, 32.32 and 35.69 hrs for adults, egg, larval and pupal stages, respectively. Slope values of the reggression lines were relatively similar for eggs, larvae and pupae and ranged between 2.47-2.81 while it was increased to 3.85 for that of the adult stage indicating a high degree of homogeny for the susceptibility of adults to the tested gas than the other stages.

At 25°C, the relative sensitivity of the developmental stages to the gas remained similar to that of the previous temperature, but the percent mortality was increased than that of 20°C. Mortality increased gradually as the exposure period was increased reaching 100% after 7 days for adults and after 9days for the other stages. LT_{50} values slightly decreased than that of 20 °C. indicating a positive effect of temperature. Mortality of insects obviously increased at the high temperature (30 and 35°C) for the same period of exposure (Table 1). Complete mortality of adults took place after a short period (4 days only) at these two temperatures and after 7 days for the other three stages. At 35°C. the results showed also that time required to obtain 50% mortality of the developmental stages was clearly shortened at 35 °C to be 2.62 hrs for egg stage, 6.73 hrs for adults stage, 8.18 hrs for larval stage and 9.87 hrs for pupal stage.

Slope values of the regression lines, a relation ship between time of exposure to gas and % mortality, revealed that eggs were the most heterogenous stage for the susceptibility to gas than the other stages while adult stage was the most homogenous.

The Red Flour Beetle, *T. castaneum*

Data recorded in Table 2 reveal clearly that at 20 °C the adult stage was the most sensitive to CO_2 gas than the other stages. The order of stage sensitivity to gas was adults, larvae, pupae and eggs.

Table 2:Cumulative mortality percentage of developmental stages of *T. castaneum* exposed to pure carbon dioxide in closed vessels at different degrees of temperature

Tested stage	Exposure period(in hours)									Temperature degrees
	24	48	96	168	216	240	LT_{50}	LT_{90}	Slope	
Eggs	43.66	64.66	71.33	84.33	97.00	100	32.267	163.574	1.8179	20°C
Larvae	70.00	77.30	89.30	96.30	100	-	14.952	71.475	1.8861	
Pupae	58.33	73.66	78.00	86.66	93.33	100	16.534	18.511	1.3822	
Adults	78.66	99.33	100	-	-	-	16.646	29.784	5.7217	
Eggs	49.00	70.00	79.00	97.00	100	-	27.104	110.124	2.1049	25°C
Larvae	84.00	92.66	98.00	100	-	-	24.010	50.059	4.1638	
Pupae	72.66	91.66	94.66	99.00	100	-	11.571	49.542	2.2913	
Adults	90.00	95.00	100	-	-	-	6.032	25.581	2.4262	
Eggs	65.00	76.66	87.66	100	-	-	16.670	82.968	1.8388	30°C
Larvae	90.00	99.00	100	-	-	-	10.558	24.050	3.5848	
Pupae	77.66	92.66	96.66	100	-	-	10.462	41.241	2.1511	
Adults	91.00	95.00	100	-	-	-	5.167	24.129	1.914	
Eggs	83.66	97.66	100	-	-	-	11.913	29.220	3.227	35°C
Larvae	95.33	98.00	100	-	-	-	3.659	16.358	1.975	
Pupae	88.66	95.66	100	-	-	-	7.673	27.987	2.2805	
Adults	94.00	97.00	100	-	-	-	3.704	16.194	1.8539	

The percent mortality of adult after 24 hrs of exposure was 78.66% and increased to reach 99.33% and 100% after 48 and 96 hrs of exposure, respectively. larval stage recorded 70% mortality after 24 hrs exposure and the larval mortality increased gradually to reach 100 % after 9 days of exposures. The mortality of pupal stage recorded 58.33% after 24 hrs expossure and increased gradually to reach 100% after ten days.

Eggs of *T.castaneum* were the least sensitives; complete mortality of eggs exposed to the gas was attained after ten days. The data indicate that the time needed to obtain 50% mortality was 32.26, 14.95, 16.53 and 16.64 hours for eggs, larvae, pupae and adults, respectively. The slope values of the toxicity line indicated that adults stage was the most homogenous for its sensitivity to the CO_2 gas compared with the other stages.

At 25°C data showed that susceptibility of different stages, to CO_2 took the same trend as mentioned before. Complete mortality occurred at shorter periods for all stages than that recorded for 20°C. Mortality percentages of all tested stages clearly increased compared with that at 20°C. Mortality of the treated stages increased in the sub. sequent days to reach 100% after 4, 7, 9, and 10 days for adults, larvae, eggs and pupae, respectively. The time needed to kill 50% was 27.10, 24.01, 11.57 and 6.03 hours for eggs, larvae, pupae and adults, respectively. The slope values showed that larval stage was more homogeneous for the susceptibility to CO_2 gas than the other stages.

At 30°C, data in Table 2 show that adults and larvae were still the most susceptible stages recording t'.e highest levels of mortality after 24 hrs (91 and 90%, respectively). Percent kill of the all tested stages increased at this temperature compared with the previous one at any time of the exposure period. Complete kill of adults and larvae was attained after 4 days for both stages and after 7 days for eggs and pupae. Efficacy of the gas against the insects increased in general as the temperature. increased to 35°C . After 24 hrs the larval and adult mortality were very close and these stages were the most sensitive to the gas than pupae and eggs. Data showed that a complete mortality occurred after 96 hours for all tested stages.

The Lesser Grain Borer, *R.dominica*

Data in Table 3 indicate that the developmental stages of *R. dominica* exhibited different levels of susceptibility to CO_2 after any period of exposure at 20°C. Adults

Table 3: Cumulative mortality percentage of developmental stages of *R. dominica* exposed to pure carbon dioxide in closed vessels at different degrees of temperature

Tested stage	Exposure Period(in hours)									Temperature degrees
	24	48	96	168	216	240	LT_{50}	LT_{90}	Slope	
Eggs	56.66	76.00	84.0	93.66	100	-	20.665	107.572	1.778	20°C
Larvae	59.66	73.33	79.66	92.00	100	-	19.585	125.676	1.587	
Pupae	41.00	58.66	74.33	88.33	93.0	100	35.744	139.983	2.161	
Adults	78.66	88.33	100	-	-	-	15.150	44.338	2.748	
Eggs	63.33	94.00	98.66	99.66	100	-	19.673	34.608	1.792	25°C
Larvae	70.33	77.00	90.66	97.33	100	-	16.135	78.970	1.7152	
Pupae	51.33	62.66	81.33	93.00	100	-	27.836	126.106	1.953	
Adults	80.33	91.66	100	-	-	-	11.690	37.963	2.505	
Eggs	87.33	97.33	98.33	100	-	-	6.026	28.066	1.9184	30°C
Larvae	84.00	92.66	99.66	100	-	-	8.691	35.040	2.1164	
Pupae	75.00	85.33	91.00	98.66	100	-	10.169	63.178	1.6155	
Adults	95.33	100	-	-	-	-	-	-	-	
Eggs	92.66	98.33	100	-	-	-	6.862	21.829	2.5507	35°C
Larvae	94.33	97.33	100	-	-	-	3.704	18.194	1.8539	
Pupae	89.33	96.66	100	-	-	-	7.509	26.240	2.3585	
Adults	100	100	100	-	-	-	-	-	-	

were the most succeptible while pupae were the least. Larvae and eggs were very close and occupied an intermediate position in their susceptibility to the gas Cumulative mortality of insccts. Increased in the subsequent days by similar rates approximately to reach 100% in the fourth, ninth and tenth day for adults, eggs, larvae and pupae, respectively.

At 25°C., the same trend of mortality percentag occurred, but mortality percentag increased than that at 20°C after 24 hrs of exposure to the gas and in the subsequent periods also. Complete mortality of eggs, larvae and pupae was attained after 9 days of exposure and after 4 days only for the adults. Mortality of insects by the gas pronouncedly increased as the temp. increased.

Adults were completely died after 2 days of exposure to gas at 30°C and after one day at 35°C. Sinsitivity of eggs and larvae was relatively very close at 30°C and 35°C and completely killed after 4 days and 7 days of exposure to gas, respectively. It seems clearly from the results of Table 3 that pupal stage was the most tolerant stage at any degree of temp. And long lasted to kill. In general mortality rate of insect was very high after the first day of exposure specially at the higher temperature. Survived insects after the first day of exposure were killed during the subsequent days but in decreasing rates.

The Angoumois Grain Moth, *S.cerealella*

It was observed generally from the results in Table 4 that the developmental stages (eggs, larvae and pupae) of this insect species were less sensitive to CO_2 gas than the previous species *(R.dominica)*. Inversely, adult stage of this insect was more sensitive to the gas than that of *R. dominica* at any degree of temperature. At 20°C. and after 24 hrs exposure, mortality of the developmental stages of *S cerealella* was relatively low specially the pupal stage (30.66%), while it was 35.64, 44.66 and 95.33% for eggs larvae and adults, respictevely. Cumulative mortality of the different stages increased rapidly in the subsequent days to reach 100% in the 4^{th} day for adults, in the 7^{th} day for eggs and in the 9^{th} day for larvae and pupae, respectively.

Table 4: Cumulative mortality percentage of developmental stages of *S. cerealella* exposed to pure carbon dioxide in closed vessels at different degrees of temperature

Tested stage	Exposure period (in hours)									Temperature degrees
	24	48	96	168	216	240	LT_{50}	LT_{90}	Slope	
Eggs	35.66	62.00	87.00	100	-		24.531	97.196	2.8515	20°C
Larvae	44.66	69.66	91.33	98.33	100		28.276	86.353	2.6432	
Pupae	30.66	62.00	86.33	98.66	100		36.916	100.082	2.9588	
Adults	95.33	98	100	-	-		3.659	16.358	1.9702	
Eggs	44.66	69.33	97.66	100	-		28.389	69.884	3.3459	25°C
Larvae	49.33	77.00	90.66	100	-		22.216	79.219	2.3210	
Pupae	55.33	70.00	88.00	98.66	100		24.127	87.516	2.2902	
Adults	90.60	98.66	100	-	-		7.801	23.127	2.7154	
Eggs	71.66	93.00	100	-	-		16.872	39.525	3.4665	30°C
Larvae	75.00	94.00	100	-	-		15.272	37.104	3.3242	
Pupae	71.00	85.66	95.35	100	-		13.879	56.790	2.0940	
Adults	100	100	-	-	-		-	-	-	
Eggs	91.66	97.33	100	-	-		6.621	23.329	2.3431	35°C
Larvae	85.33	98.66	100	-	-		12.306	28.248	3.5512	
Pupae	88.33	92.00	98.33	-	-		6.114	29.994	1.8552	
Adults	100	100	-	-	-		-	-	-	

At 25°C., the percentages of insect mortality were increased than that at 20°C. after 24 hrs of exposure to the gas. This trend was also observed in the subsequet days. Complete mortality of eggs and larvae occurred after seven days of exposure, while pupal stage reached complete mortality after nine days. Complete kill of adults was recorded at the fourth day of exposure. At 30°C., adults completely died after one day only of exposure to the gas. Egg and larval stages reached 100% mortality after four days of exposure to the gas, pupal stages reached complete mortality after seven days of exposures.

At 35°C the percentages of insect mortality were increased than that of 30°C after 24 hrs of exposure as well as the subsequent days for all the tested stages except adult stage which completely died after one day of exposure to the gas. Egg and Larval stages reached complete mortality at the fourth days of exposure, while the pupals were completely killed after the seventh day.

It seems generally from the results that all stages of *S. cerealella* were more sensitive to CO_2 gas than that of the other three tested species specially the adult stage.

It could be concluded generally that temperature affected the efficacy of pure CO_2 gas in closed space against the different developmental stages of the tested insect species. Efficacy of the gas increased as the temperature increased from 20°C to 35°C. The shortest period of complete kill occurred at 35°C.

Developmental stages of the tested insect differed in their susceptibility to the gas where adult stage was the most sensitive while the pupal stage was the least one. The highest percent. of cumulative mortality of insects occurred in the first day of exposure, then the daily rate of mortality decreased in the subsequent days.

The obtained results concerning the efficacy of CO_2 gas against the tested insects are in agreement with those obtained by Zakladnoi (1976) who reported that pure CO_2 was more rapidly toxic than pure nitrogen to the adults of *Sitophilus granairus, S. oryzae, Rhizopertha domanica* and *T. castaneum* at all three

experimental temperatures (20, 25 and 35°C). Locatelli and Daolio (1991) investigated the effectiveness of CO2 at different temperature (20, 25, 30 and 40°C) against *Sitophilus oryzae and Rhizopertha dominica* and found that few eggs of *R. dominica* less than 1% could survive at 20°C for 48h., while 100% mortality was recorded within 24 h. at 30°C and within 9h. at 40°C. Total larval mortality was recorded within 30, 24, 24, 12 and 9 h. at 20, 25, 30, 35 and 40°C, respectively. Pupae were less sensitive to CO2 than the other stages. Total adult mortality occurred within 24, 12, 12 and 6h. at 20, 25, 30 and 35°C, respectively. *S. oryzae* was slightly more sensitive to CO2 than the other tested species. Total mortality of eggs was recorded within 36, 24 and 12h. at the same temperature. At 30°C, 100% mortality of pupae was recorded within 24h. and of adults within 6h. These results greatly confirm our results.

Ofuya and Reichmuth (1993) studied the sensitivity of adults, eggs, larvae and pupae of *C. maculatus and Aconthoscelides obtectus* in cowpea to pure CO2 gas at 25 and 32°C. and the 70 ± 5% R.H. and found that irrespective of temperature all adults of both bruchids were killed within 1 day of exposure to pure CO2 atmosphere. All eggs were killed in 5 days at 25°C and in 3 days at 32°C. All larvae and pupae were also killed after 4 and 6 days of exposure at 32 and 25°C resp.

Also, Leong and Ho (1994) studied the response of *Liposcelies bostrychophila* and *L.entomophila* (psocoptera) to carbon dioxide and found that eggs were the most tolerant stage. The authors added that increase of exposure period to gas resulted in an increased mortality. Suss and Locatelli (1991) mentioned that carbon dioxide under high temperature was toxic than at low temperature. If the temperature is below the optimum for insect development, its metabolism is slower and thus less oxygen is required, the authors added that increase in temperature resulted in higher mortality and could be useful in reducing the treatment period. Some food stuffs could be subjected to changes in organoleptic quality and the use of increased temperature for reducing treatment time will be restricted to some products only.

Sunita *et al.* (2002) and Mahla *et al.* (2003) studied the effect of carbon dioxde at different

concentrations (40, 50, 60, 70, 80, 90 and 98%) and exposure periods (1, 3, 7, 15, 30 and 45 days) on the mortality of *Trogoderma granarium* and wheat grain damage.

The mean grub mortality after 45 days at natural exposure period was 7.6%. A maximum of 70.83% grub mortality was observed at 98% CO2 concentration which decreased to 69.8, 67.7, 66.3, 63.6, 56.8 and 46.1% with the reduction of carbon dioxide doses to 90, 80, 70, 60, 50 and 40%, respectively. Grub mortality was lowest (10.86%) after one day exposure followed by 3 (29.4%), 7(60.18%), 15 (81.06%), 30 (96.67%) and 45 (100%) day exposure. Carbon dioxide at 70% recorded 100% grub mortality after a 30 days exposure.

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فعالية غاز ثانى أكسيد الكربون على بعض حشرات المخازن على درجات حرارة مختلفة

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أجريت دراسات معملية على تأثير غاز ثانى أكسيد الكربون على الأطوار الكاملة والغير كاملة لأربعة أنوع من حشرات المخازن وهى خنفساء اللوبيا وخنفساء الدقيق الصدئية وفراشة الحبوب وثاقبة الحبوب الصغرى وذلك على أربعة درجات حرارة مختلفة وهى ٢٠، ٢٥، ٣٠، ٣٥ درجة مئوية. أوضحت النتائج أن ثانى أكسيد الكربون كان أكثر فعالية على درجات الحرارة العالية عنها على درجات الحرارة المنخفضة وكانت فراشة الحبوب أكثر الأنواع حساسية للغاز مقارنة بالحشرات الأخرى. كانت الأطوار الكاملة لكل الأنواع تحت الدراسة أكثر الأطوار حساسية بينما كان طور العذراء أكثر الأطوار تحملا للغاز على جميع درجات الحرارة المستخدمة إزدادت نسبة الموت كلما زادت فترة التعريض للغاز وكلما زادت درجة الحرارة تقل فترة التعريض اللازمة للوصول إلى نسبة موت كاملة.