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Control of the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) using some insecticide alternative safe methods

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Abstract:

This work aimed to evaluate the efficacy of certain safe control methods against the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Adults of *S. oryzae* were exposed to CO_2 or N_2 gas differ in relative humidity content at two degrees of temperature. Mortality of insect was influenced significantly by the level of relative humidity in the inert gas ambient. The highest percentages of insect mortality were recorded for lowest and the highest level of humidity at both degrees of temperature. At the moderate levels of humidity (50 and 60%) the insect mortality was slightly reduced than that of the lowest and the highest levels of humidity at both degrees of temperature, especially for the shortest exposure periods. It was showed also that a complete mortality was occurred for the insects exposed to the CO_2 at the lowest (35%) and the highest (80%) levels of humidity. Four doses of ECO_2-Fume gas (30, 35, 40 and 50 g\m^3) were tested against the different developmental stages and adults of *S. oryzae* in wheat grains piles under natural conditions. All tested doses induced a considerable control for all stages of the rice weevil, efficacy was increased as the dose increased, so that, a dose of 50 g\m^3 induced 100% mortality of all insect stages after 3 days of treatment.

Introduction

The rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a very serious pest of stored grain. This species infests kernels of various cereals causing excessive losses in quality and quantity during grain storage, many practices were used, one of them was the use of insecticides, which considered the most commonly utilized method by many countries (Harein and Davis, 1992; Bell, 1993 and Arthur, 1994). In these countries, the indiscriminate and improper use of insecticides to control the proliferation of stored grain insects, has resulted in several problems.

It is therefore imperative to develop alternative methods that are economically feasible and ecologically oriented to control stored grain insects and fungi. Hermetic storage is considered one of these alternative methods (Villers *et al.,* 2010 and Boardman *et al.,* 2011). The use of hermetic storage for the preservation of food stuffs is as old as it is new and modern. Hermetic storage of grain was practiced in ancient times in underground pits in the dry, subtropical regions of the Middle East, North Africa and India, etc. Modified atmosphere is a technique involves changes the composition of the normal atmospheric constituents of O_2, N_2 and CO_2 in a sealed store to create an atmosphere lethal to insects at an adequate length of time. It appears that high concentrations of CO_2 in combination with low humidity could prove lethal to insects by causing rapid loss of water through the spiracles. This possibility was also suggested by the results of earlier investigations (Pearman and Jay, 1970).

ECO_2-Fume fumigant gas is a liquefied mixture of 2% wt. PH_3 (2.6% vol.) with carbon dioxide (CO_2). This mixture has been patented by Boc Gases of Australia. This mixture of PH_3 with CO_2 ensures it in non-flammable in all proportions with air. This eliminates all safety concerns with dispensing rates or dilution rates. Traditional solid formulations can generate PH_3 concentration above the lower flammability limit for PH_3 thereby creating a hazard. ECO_2-Fume is considered one of the most alternatives of methyl bromide (MB), that will not harm the ozone layer.

The present work was undertaken to investigate the following points: 1. Effect of relative humidity levels on the efficacy of CO_2 and N_2 gases against *S. oryzae* adults under laboratory conditions. 2. Efficacy of ECO_2-Fume gas against all stages of *S. oryzae* under field conditions.

Materials and methods

1. Test insect:

One of the most dangerous insect pests to cereal grains *(S. oryzae*) was chosen for this study. The cultures were started by large batches of adults initially collected form the infested stored products in warehouses at Zagazig region, Sharqyia Governorate , the collected insects were mass reared for several generations using the following technique:

1.1. Rearing technique of stock cultures:

1.1.1. Rearing medium:

Wheat grains variety Sakha 93 was used as a rearing host. Fresh wheat grains were firstly examined to insure it in a good case and free of toxic residues then it was tightly packaged in plastic bags and kept at -13^{oc}, in a deep freezer for at least 48 h to kill any infestation that might be present. The kernels were conditioned with respect to the moisture content to be about 14% before starting the rearing or any experiment.

1.1.2. Rearing of adult stage developmental stages of *Sitophilus oryzae* for gas exposure:

Batches of 200 adults each of 1 - 2 weeks old were sifted out from the cultures and introduced into 1 kg glass jars half filled with fresh medium (conditioned wheat grains + dry yeast). After two days of egg deposition the adults were removed and transferred to other new prepared jars and the medium were returned to the jars. A quantity of medium with 0 – 2 day old eggs was thus maintained in the same jars of culture at the same condition of rearing until emergence of adults. Batches of 30 adults each (1– 2 weeks old) were collected from the cultures using a fine brush and glass tubes. These batches were transferred into wire gauze cages contained quantities of medium and used for testing.

2. Gas experiments:

2.1. Vessels of insect exposure to gases:

2.1.1. Cages of insect gas exposure (exposure vials):

Ready made plastic-wire gauze cages (3x6 cm) used for water household apparatus were used for insect exposure to gases.

2.1.2. Plastic bags (exposure chamber):

The known urine collection bags (call also drainage bags in field of medicine) were used as gas exposure chambers for the experiments of gases bioassay against the tested insects. These bags are rectangular in a shape (18 x 23 cm) and 2000 ml capacity. Each bag has two tubes (hoses) at the both long ends. One of the two tubes is long (inlet tube) at the front of the bag and the other tube (outlet tube) is very short and located at the other end of the bag. Each tube has at its free end a built -in valve with cover which could be tightly closed.

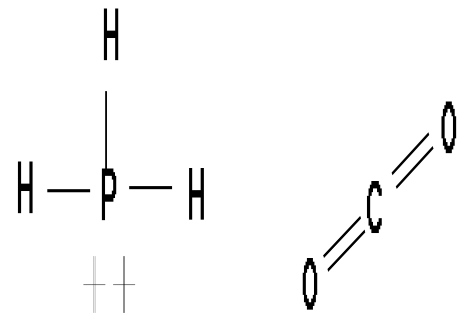
2.2. Gas exposure procedures:

The cages of the adults which reared and prepared as mentioned before were put (three replicates for each experiment) inside the plastic bags (exposure chambers) through a lateral cut wool were wet thoroughly by the tap water to give the respective level of relative humidity when introduced inside the gas exposure chamber. Piece of 1 g cotton wool to give about 60% RH. and a piece of 3 g to give about 80% RH.). Small hygrometer was introduced inside each gas exposure chamber to indicate the level of humidity. Silica gel bags of 20.2 g (total weight) were introduced into the exposure chamber to obtain the low level of relative humidity (35% RH.). The bags were tightly sealed using a hand pressing sealer. The air inside the bag could get out by gently pressing on the bag and then the bag was connected to gas cylinder (CO_2 or N_2) through a rubber hose and the long tube of the bag. The gas could purge very slowly inside the bag for about 20 -30 seconds to ensure completely exchanging the air inside the bag with the tested gas without any pressure inside the bag. Valves of the treated bags were tightly closed, and the bags were kept in the rearing room at the tested temperature, (20 or 30±2°C) for different periods of gas exposure, i.e., 7, 14 and 21 days. 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 adult stage. The percentages mortality was calculated, corrected and recorded. The data obtained were statistically analyzed using the analysis of variana F-test.

3. ECO_2-Fume gas experiment:

3.1. ECO_2-Fume formulation:

- Phosphine (PH_3): 2% (by weight) 2.6% by volume.
- Carbon Dioxide (CO_2): 98%.
- Molecular formula: PH_3 and CO_2
- Structural formula:



Ready gas cylinder manufacture by Cytec Company (Canada) was used for this experiment.

3.2. Fumigation procedures by ECO_2-Fume:

This experiment was conducted on wheat grains stored in jute bags at Sindiyun Shona, Qalyubiya Governorate. Three piles of 240 Jute bags each bag was filled with 100 kg of wheat grains for each bag. From the stock cultures maintained in the rearing room, cloth bags (10x16 cm) each contained 50 g wheat kernels infested with one of the different stages of *S. oryzae*; eggs, larvae, pupae and adults (30 individuals for each bag in case of adult) were prepared. The total numbers of bags for each concentration of ECO_2-Fume gas were 48 bags; 12 bags for each direction (North, south, middle and west). The prepared bags were introduced into the pile and distributed on the four direction as mentioned before. The pile was covered exactly and tightly with a plastic sheet 14x20 m. After sealing the place of fumigation, whose of the gas cylinder was introduced inside the pile and the gas cylinder was put on platform balance to calculate the required dose. Four doses (concentrations) of ECO_2-Fume (30, 35, 40 and 50 g/m^3) were used. After 3 days of exposure to ECO_2-Fume gas the piles were aerated, and the cloth bags of insects were inspected directly for adult mortality. Bags of the other insect stages were kept in the rearing room until adult emergence and the mortality percentages were calculated and corrected for each stage. Similar numbers of cloth bags of insect stages were distributed in another wheat pile using the same procedures without ECO_2-Fume gas to be used as a control. The concentration of phosphine gas in the treated ECO_2-Fume pile was monitored by a portable Silo**.** Chek MARKII manufactured by the CANARY COMPANY Pty Ltd, AUSTRALIA. This device detects high fumigation phosphine levels greater than 1 ppm up to 2000 ppm. The automatic sampling model (which used in this study) had a sample tube, which connect to the gas-sampling lines coming from the pile under fumigation and built-in pump and battery. After connecting of the sample tube with the gas-sampling lines, the key switch down turned to on. A period up to 3 minutes was elapsed to allow the PH_3 sensor to record the final reading of PH_3 concentration. The data obtain were statistically analyzed using the analysis of variance F-test.

Results and discussion

1. Effect of relative humidity on the efficiency of CO_2 and N_2 gas against *Sitophilus oryzae* adults:

The purpose of this experiment was to evaluate the relationship between the ambient relative humidity and mortality of *S. oryzae* adults exposed to CO_2 or N_2 pure gases.

1.1. Carbon dioxide gas:

Data in Table (1) show generally that efficacy of CO_2 gas against adults of *S. oryzae* was influenced significantly by the exposure period to the gas. Mortality of adults increased as the exposure period increased recording averages of 72.75, 94 and 99.75% for the exposure periods of 7, 14 and 21 days, respectively, at 30 ºC. This trend was also recorded at 20 ºC averaging of 62.5, 90.75 and 98.00%, respectively. However, the mortality percentages of insects were increased at the higher temperature (30 ºC) comparing with the lower one (20 ºC) but the difference between them was insignificant.

Mortality of insects was influenced significantly by the level of relative humidity in the inert gas ambient. The highest percentages of insect mortality were recorded for the lowest and the highest level of humidity at both degrees of temperature. At the moderate levels of humidity (50 and 60%), the insect mortality was slightly reduced than that of the lowest and the highest levels of humidity at both degrees of temperature, especially for the shortest periods (Table, 1).

It was showed also that a complete mortality was occurred for the insects exposed to the CO_2 at the lowest (35%) and the highest (80%) levels of humidity whatever the tested temperature after the exposure period of 21 days. Averages of mortality % of insects were approximately the same for the lowest level and the highest level of humidity at both degrees of temperature.

Table (1): Effect of relative humidity levels on the efficacy of pure CO_2 gas against Sitophilus *oryzae* adults at 30 and 20± 2 ºC.

Temperature (T)	Exposure periods (in days) (E)	% mortality of adults Relative humidity levels %(R.) 35	50	60	80	means	General means of% of adult mortality at different levels of Temp.
30 ºC	7	85	48	69	89	72.75	88.83
	14	100	97	79	100	94	
	21	100	100	99	100	99.75	
	Means	95	81.66	82.33	96.33		
20 ºC	7	70	34	67	79	62.5	83.75
	14	100	82	89	92	90.75	
	21	100	100	92	100	98	
	Means	69	72	82.66	90.33		
General means of % mortality of adult at different levels of RH.		82.0	76.83	82.49	93.33		

$LSD_{0.05}$ for temperature (T)=N. S
$LSD_{0.05}$ for exposure period: (E)= 2.069
$LSD_{0.05}$ for relative humidity (R)=1.915
$LSD_{0.05}$ for (TxE)=2.927%
$LSD_{0.05}$ for (ExR)=3.612%
$LSD_{0.05}$ for (TxR)= 2.708%
$LSD_{0.05}$ for (TxE x R)= 5.108%

1.2. Nitrogen gas:

The results presented in Table (2) show generally that efficiency of N_2 gas against *S. oryzae* adults at all the tested conditions was less than that of CO_2 gas. However, similar trends were found for the results of the tested factors (temperature, relative humidity and exposure period) on adult mortality of *S. oryzae*. Mortality percentages of insects was significantly increased at the higher temperature (82.47%) than that at the lower one (77.5%). Relative humidity had affected significantly the mortality of insects, high levels of insect mortality were recorded at the lowest level of humidity (35%), the mortality of insects was decreased at 50% relative humidity at both temperatures.

Mortality of insects increased again as the relative humidity was increased to 60 and 80%, recording the highest percentages of insect mortality 85.89 and 88.11 % at the two degrees of temperature 20 and 30^{oc}., respectively. As mentioned before in case of CO_2, the mortality of insects increased gradually as the exposure period to the gas was increased. The highest percentages of insect mortality (91.08 and 87.75 %) were recorded for 21 days exposure period at both degree of temperature (30 and 20 ºC), respectively. The lowest percentages of insect mortality were recorded for the shortest exposure period (7 days). The interaction effect between exposure period and relative humidity on the insect mortality was also significant. The highest percentages of insect mortality (99 and 96 %) were recorded for the longest exposure period (21 days) and the highest level of relative humidity (80%) at both temperature 30 and 20 ºC., respectively.

Table (2): Effect of relative humidity levels on the efficacy of pure N_2 gas against *Sitophilus oryzae* adults at 30 and 20$\pm$ 2 ºC.

Temperature (T)	Exposure periods (in days) (E)	% Mortality of adults					General means of% of adult mortality at different levels of Temp.
		Relative humidity levels %(R)					
		35	50	60	80	Means	
30 ºC	7	74	70	79	81	76.08	82.47
	14	79	76	82	84	80.25	
	21	90	84	91	99	91.08	
	Means	81.11	76.66	84.0	88.11		
20 ºC	7	68	63	71	76	69.5	77.5
	14	73	73	76	79	75.25	
	21	86	81	88	96	87.75	
	Means	75.66	72.33	78.33	83.66		
General means of % mortality of adult at different levels of R.H.		78.39	74.49	81.17	85.89	7	

$LSD_{0.05}$ level for temperature (T)=3.726%.
$LSD_{0.05}$ level for exposure period: (E)=1.545%.
$LSD_{0.05}$ level for relative humidity (R)=1.892%.
$LSD_{0.05}$ level for (EXR)=3.009%.

Very little is known about the combined effect of the inert gases (CO_2 and N_2) and ambient relative humidity. However, this point could be discussed as follows: Insect normally keep their spiracles closed, opening them just enough to satisfy their oxygen (O_2) requirements (Hazelhoff, 1927 and Wigglesworth, 1935). However, the frequency of opening and closing is influenced by several factors, including the concentration of carbon dioxide (CO_2) and O_2 in the environment. Mellanby (1934) found that a 2% concentration of CO_2 is enough to produce sustained opening of the spiracles in some insects. The same effect can be achieved if the O_2 concentration is reduced below 1%. There is considerable evidence that a large part of the water loss from insects takes place through the respiratory surfaces. Mellanby (1934) found that when the spiracles of *Xenopsylla cheopis* (Rothschild) (Siphonaptera: Pulicidae) adult and larval *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Tineola bisselliella* (Hummel)(Lepidoptera: Tineidae) are kept open with high concentrations of CO_2 or low concentrations of O_2, the rate of water loss was two to seven times higher than under normal concentrations of these gases. *X. cheopis* larvae, however, which lack a spiracular closing mechanism, lose water at about the same rate under all these conditions. Bursell (1957) reported that *Glossina morsitans* Westwood (Diptera: Glossinidae) in air at 0% r.h. lose water at the rate of 0.12 mg/h when its spiracles are functioning normally, but when the spiracles are blocked with paraffin wax it loses water at the rate of 0.09 mg/h. It appears then that high concentrations of CO_2 in combination with low humidity could prove lethal to insects by causing rapid loss of water through the spiracles. Dry air having a low O_2 concentration should have the same effect. This situation presents the possibility of using altered concentrations of atmospheric gases in combination with low humidity to control insects infesting stored products. This possibility was also suggested by the results of earlier investigations by two of the authors (Pearman and Jay, 1970). They found that the mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) exposed to altered concentrations of atmospheric gases was greater at low than at high humidity. The high mortality levels of insects at the highest level of relative humidity may be attributed to the combine effect of high relative humidity and the high concentration of CO_2 or N_2. The results concerning the effect of the high relative humidity level on the percentage mortality of the tested insects agree with those recorded by (Navarro and Calderon, 1974). The authors reported that pupae of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) *exposed* to high concentration of CO_2 gas (89%) at a high relative humidity level (95%) were completely died after 5 days of exposure. In this case insect mortality resulting from the treatments cannot be attributed to loss of water which is the causative factor at the lower relative humidity. We suggest that carbon dioxide itself acts as a fumigant at high concentrations causing a lethal effect on insects.

2. Efficacy of ECO_2- Fume gas against *Sitophilus oryzae* under practical conditions:

Different concentrations of ECO_2-Fume gas was evaluated against the different stages of *S. oryzae* under practical conditions in wheat piles stored at Sindiyun, Qalubiyah Governorate under plastic sheet. Data presented in Table (3) showed the mortality percentages of the different developmental stages and adults of *S. oryzae* exposed to gas concentrations for three days. Insect mortality values increased as the concentration of the gas increased. Mortality values were calculated after three days of exposure to gas. A complete mortality (100%) was recorded for (eggs, larvae, pupae and adults) at the concentration of 50% g\m^3. Mortality % of the abovementioned stages (eggs, larvae, pupae and adults) were 23, 39, 26 and 98% respectively, at the concentration of 30 g\m^3. Thus, the results indicated that higher concentrations induced higher percentages of mortality. Adults of *S. oryzae* was the most susceptible stage to ECO_2-Fume gas recording the highest percentages of mortality, while pupae and eggs were the most tolerant, (Table,3)

Table (3): Mortality percentages of developmental stages and adults of *Sitophilus oryzae* exposed to ECO_2-Fume gas used in wheat pills for three days under plastic sheet.

Insect stages	**Gas tested concentrations (g/m$^{3)}$**			
	30	35	40	50
Eggs	23	35	67	100
Larvae	39	56	80	100
Pupae	26	43	72	100
Adults	98	99	100	100
Means of mortality percentages after exposed to different concentrations of gas.	46.37	58.06	79.75	100

$LSD_{0.05}$ level for insect stages (IS) = 8.13 %.
$LSD_{0.05}$ level for gas concentrations (GS) = 3.08%.
$LSD_{0.05}$ level for interactions (ISxGS) = 7.12%.

As for the changes of gas concentrations during the three days immediately after treatment; results of Table (4) show generally that concentrations of the gas were very high in the initial time (at the beginning of treatment) then decreased gradually as the period post- treatment was prolonged. Concentrations of the gas at the initial time and during the three consecutive days post-treatment were the tested dose depended. The highest concentration of the gas at any time was recorded for the highest tested dose (50 g\m^3) and the lowest one was recorded for the least dose (30 g\m^3) (Table, 3). The rate of gas concentration decrease may be attributed to the degree of sealing and the level of gas sorption by the treated grains. As shown from the results of Table (3) that percentages of gas concentration reduction were increased as the time post-treatment was elapsed. ECO_2-Fume fumigant gas is non-flammable mixture of phosphine and carbon dioxide that enables highly effective fumigation in a wide variety of sealed storage applications. It is dispensed externally to stores or structures using simple techniques which avoid the applicator's exposure and enhance worker's safety. The obtained results are in harmony with the findings of other investigators on the efficacy of combinations of phosphine plus carbon dioxide against some stored product insects El-Lakwah *et al.* (1992a) studied the efficacy of mixtures of phosphine and carbon dioxide against the various stages of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Adults of *C. maculatus* proved to be the most susceptible stage. The time of exposure was a more critical factor than the concentration of phosphine. El-Lakwah *et al.* (1992b) investigated the efficacy of phosphine and carbon dioxide mixtures against the various stages of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). These mixtures were effective on all stages of the insect. Adults of *R. dominica* proved to be the most susceptible stage at 30ºC. El-Lakwah *et al*. (1992c) investigated the efficacy of phosphine and carbon dioxide mixtures against the various stages of *S. oryzae*. These mixtures were effective on all stages of the insect. Adults of *S. oryzae* proved to be the most susceptible stage at the 30 ^{o}C. El-Lakwah *et al*. (1989) investigated the efficacy of phosphine with that of mixtures of the gas plus carbon dioxide against diapause larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). It was found that for longer exposure periods of 48 and 72 hours, the addition of CO_2 to phosphine induced significantly higher larval mortality than that by PH_3 alone. In conclusion, ECO_2-Fume gas is considered one of the most promising fumigants for controlling *S. oryzae* in stored grains causing a complete mortality of the insects after 3 days of exposure to 50 g\m^3 of stored grains in good sealing atmosphere.

Table (4): Changes in the concentrations of ECO_2-Fume gas during three consecutive days immediately after initial gas dosing in wheat pile under the plastic sheet.

Tested doses (g/m^3)	Gas concentrations (in ppm)				
	Initial time	1st day	2nd day	3rd day	Average Concentrations
30	844.8	484.8 (42.6%) *	244.2 (71.0 %)	217.8 (74.2%)	447.9 (46.9%)
35	1011.6	441.0 (56.4%)	223.8 (77.8%)	221.4 (78.1%)	474.5 (53.1%)
40	1020	391.8 (61.6 %)	253.6 (75.1%)	174.2 (82.9%)	459.9 (54.9%)
50	1104	683.4 (38.1 %)	435.6 (60.5%)	206.4 (81.3%)	607.35 (44.9%)

*** Numbers between brackets represent % reduction of gas concentrations.**

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