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Reddy Shekar V
Department of Environmental Science & Technology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

B Anil Kumar
Department of Environmental Science & Technology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

A Padmasri
Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad. Telangana, India

M Shanti
AICRP on Forage Crops and Utilization, PJTSAU, ARI, Rajendranagar, Hyderabad, Telangana, India

Correspondence
Reddy Shekar V
Department of Environmental Science & Technology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Mortality of *Sitophilus oryzae* in modified atmosphere with elevated levels of CO_2

Reddy Shekar V, B Anil Kumar, A Padmasri and M Shanti

Abstract
The effect of modified atmosphere with elevated levels of CO_2 against *Sitophilus oryzae* was studied by directly exposing the *S. oryzae* adults to eight concentrations of CO_2 *viz*., 10, 20, 30, 40, 50, 60, 70 and 80 per cent with five exposure periods of 1, 2, 3, 4 and 5 hours to study the adult mortality. The results indicated that 80, 70 and 60 per cent CO_2 concentrations caused complete mortality of adults at two, six and seven days after treatment, respectively after exposing to five hours directly. At 40 and 50 per cent CO_2 concentrations, though some of the adults survived even after seven days, but they did not lay eggs and thus 40 per cent and above CO_2 concentrations were found to be fatal for the development of *S. oryzae*. And damage of *S. oryzae* by CO_2 was confirmed with scanning electron microscope.

Keywords: Mortality of *Sitophilus oryzae*, modified atmosphere, carbon dioxide, scanning electron microscope

1. Introduction
Sitophilus oryzae L. is known to cause extensive loss to quality and quantity of stored grains besides reducing viability of raw seeds. An adult rice weevil, *Sitophilus oryzae* (Linnaeus) lays upto 450 eggs singly in holes chewed in cereal grains and each egg hatches into a white, legless larva, which eats the grain from inside. The rice weevil has four orange-brown areas on the wing and is about 3 mm long with a characteristic rostrum (snout) protruding from its head. It has biting mouth parts at the front of the rostrum and two club like antennae. The rice weevil is winged and may occasionally fly. The infestation starts in the field. The female weevil makes a small hole on the seed, deposits an egg and covers it with a gelatinous fluid. The apodus grub feeds inside the grain, pupates there itself and emerges through a hole made on the seed (Vasanthraj David and Kumara Swami, 1975) and damage is multiplied by several folds under storage.

There are increasing restrictions on the use of pesticides and the number of chemical compounds officially registered for pest control in durable food products. Moreover, the use of methyl bromide for the fumigation of food commodities and facilities must be phased out in accordance with the Montreal protocol due to its effect on the ozone layer (UNEP, 2006). Modified atmospheres (MA) or controlled atmospheres (CA) offer an alternative to the use of conventional residue‐producing chemical fumigants for controlling insect pests attacking stored grain, oilseeds, processed commodities and some packaged foods. These atmospheres also prevent fungal growth and maintain product quality.

Hypercarbia generally reduces the rate of respiration. Oxygen consumption by *E. cautella* pupae was significantly reduced by hypercarbia (Navarro, 1975) [8]. As elevated CO_2 reduces O_2 consumption, it appears that the net effect of elevated CO_2 on the insect respiratory metabolism is similar to that of reduced O_2. A high CO_2 concentration will arrest the heart activity of many insects (Jones, 1974) [5] while a low CO_2 level (5%) stimulates it. As soon as the insect is in contact with pure CO_2, the heart beat stops. Also CO_2 acts directly on events in the neuromuscular transmission sequence (Hoyle, 1960). However, in some insect species, if CO_2 initially causes the valves to open by local action on the muscle, when it reaches the central nervous system it causes a reduction in the tonic discharge to the closer muscle, which may allow the valve to open further. In certain insects a 30-min to one hour exposure to a high CO_2 concentration reduces egg production and hatchability (Aliniazee and Lindgren 1970., Barrer and Jay 1980) [2]. Daily, repeated 2-hr exposures of adult *Tribolium castaneum* to CO_2 before maturation suppressed oocyte development in the ovarioles (Press, 1976) [10].

2. Materials and methods
The present investigation on "Mortality of *Sitophilus oryzae* in modified atmosphere with elevated levels of CO_2" was conducted in the laboratory at Seed Research and Technology

Centre, (SRTC), PJTSAU, Rajendranagar, Hyderabad, Telangana during 2017-2018.

2.1 Effect of CO_2 concentrations on adult mortality of *S. oryzae* (L.)

To study the effect of modified atmosphere with elevated levels of CO_2, Ten freshly emerged adults were transferred to air tight plastic containers of 250 grams capacity separately and directly exposed to different concentrations *viz*., 10, 20, 30, 40, 50, 60, 70 and 80 per cent with five different exposure periods *viz*., 1, 2, 3, 4 and 5 hours by replicating each treatment thrice.

The required concentration of CO_2 was released into the container with a pressure of 2 kg cm^{-2} from CO_2 cylinder. Before releasing the CO_2 into airtight container, the air present in the air tight container was flushed out by opening the outlet present at the top of the container and then it was closed with rubber cork and then the desired concentration of CO_2 was released into the airtight containers through the inlet located at the bottom of the containers by injecting the needle of CO_2 cylinder. After releasing the CO_2, the concentration of CO_2 was checked by using CO_2/O_2 analyzer (PBI 2006, Denmark). For determination of CO_2, the analyzer was calibrated with atmospheric air (20.9 % and 0.03% CO_2), then the needle of the analyzer was introduced into the top outlet tube of the air tight container and the measuring button of the CO_2 / O_2 analyzer was pressed. The concentration of CO_2 and O_2 present in the air tight containers was displayed on screen within 10 seconds which helped in determining the concentration of CO_2 present in the containers and then inlet and outlet tubes were closed at one stroke using rubber corks to prevent escape of CO_2 from the container.

After releasing the desired concentration into the containers, they were made air tight by plugging them with rubber corks and sealing with rubber tape. Control was maintained by following the same procedure adopted for the CO_2 studies in plastic containers under laboratory conditions without exposing the insect to CO_2.

After exposure to various CO_2 concentrations and time periods, the adults whichever survived were placed in plastic jar containing 100 grams disinfested healthy maize seed. The mortality was observed daily and per cent adult mortality was calculated by using the following formula.

$$\text{Adult mortality (per cent)} = \frac{\text{Number of adults dead}}{\text{Total number of adults released}} \text{ X } 100$$

2.2 Effect of CO_2 on *S. oryzae* adults as seen under Scanning Electron Microscopy (SEM)

The two different CO_2 concentrations *viz*., 40 and 80 per cent used for the mortality of *S. oryzae* along with untreated control were studied under scanning electron microscope for their effect on the spiracle and other parts of *S. oryzae* adults. Samples were fixed in 2.5 per cent glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4°C and post fixed in 2 per cent aqueous osmium tetroxide for four hours and dehydrated in series of graded alcohols and dried to critical point drying (CPD) with CPD unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape and thin layer of gold coat over the samples was done by using an automated sputter coater (Model - JEOL JFC-1600) for three minutes and scanned under scanning electron microscopy (SEM Model- JOEL-JSM 5600) at required magnifications as per the standard procedures (John and Lonnie, 1998) [4] at RUSKA Lab, College of Veterinary Science, PV Narsimha Rao Telangana State Veterinary University (PVNRTSVU), Rajendrangar, Hyderabad, India.

2.3 Statistical analysis

The data was subjected to angular transformations wherever necessary and analysed by adopting Completely Randomized Design (CRD) and Factorial Completely Randomized Design (FCRD) as suggested by Panse and Sukhatme (1978) [9].

3. Results and Discussion

3.1 Adult mortality of *S. oryzae* exposed to elevated levels of CO_2 after one hour of exposure period

The adult mortality of *S. oryzae* exposed to different concentrations of CO_2 after one hour of exposure period indicated that low concentrations of CO_2 *i.e.*, 10 per cent did not show any effect on adult mortality after one day of treatment and even after seven days of treatment (Table 1). At higher concentrations of 20, 30 and 40 per cent CO_2, low mortality (3.67, 5.67 and 8.67 per cent, respectively) was observed, after one day of treatment and it increased to 54.67, 63.67 and 67.00 per cent, respectively after seven days of treatment. Among all the concentrations, 80 per cent concentration recorded highest mortality of 20.33 per cent followed by 70 per cent CO_2 (15.33 per cent) at one day after treatment. Among all the concentrations, 80 per cent CO_2 was proved to be significantly superior to other treatments as 50 per cent mortality was observed at two days after treatment and all the CO_2 exposed adults died by seventh day after treatment. At 70 per cent CO_2 concentration, 54.00 per cent mortality was recorded by third day and it increased to 87.33 per cent after seven days of treatment. There was no adult mortality in control (0.00 per cent). The mean adult mortality of *S. oryzae* observed in different concentrations varied from 0.00 to 67.57 per cent.

3.2 Adult mortality of *S. oryzae* exposed to elevated levels of CO_2 after two hours of exposure period

The results (Table 2) showed that low concentrations of 10, 20, 30 and 40 per cent CO_2 caused less than 20 per cent mortality of adults (9.00-19.00 per cent) after one day of treatment and by seventh day it varied from 37.00 to 68.67 per cent. The mean mortality of adults recorded in all the above four concentrations of CO_2 ranged from 20.19 to 45.52 per cent. The next three higher concentrations of CO_2 *viz*.,50, 60 and 70 per cent concentrations recorded 23.67 to 32.33 per cent mortality after one day of treatment and it increased to 72.00 per cent to 88.67 per cent by seventh day. Among all the concentrations, the highest concentration of 80 per cent CO_2 recorded 54.00 per cent mortality after one day of treatment and cent per cent mortality was recorded after seven days of treatment. The mean adult mortality was also found to be significantly the highest at 80 per cent CO_2 concentration (73.43 per cent) followed by 70 per cent CO_2 (60.71 per cent) and 60 per cent CO_2 (56.90 per cent) which were significantly different from each other.

3.3 Adult mortality of *S. oryzae* exposed to elevated levels of CO_2 after three hours of exposure period

Exposure of *S. oryzae* adults to different concentrations of CO_2 upto three hours of exposure (Table 3) indicated that low concentrations of CO_2 (10, 20, 30 and 40 per cent) recorded 22.00 to 36.67 per cent adult mortality after one day of treatment and it increased to 57.33 to 70.33 per cent after

seven days of treatment. The mean adult mortality of 38.38 to 54.38 per cent was recorded at 10 to 40 per cent CO_2 concentrations. The next higher concentrations of 50 and 60 per cent CO_2, recorded 43.67 and 45.67 per cent mortality, respectively after one day of treatment and 77.33 per cent to 85.67 per cent mortality after seven days of treatment. The higher concentrations of CO_2 *i,e.,*70 and 80 per cent recorded 52.00 and 62.33 per cent mortality after one day of treatment and 100 per cent mortality after seven and six days of treatment, respectively. The higher CO_2 concentrations of 50 to 80 per cent showed 60.38 to 80.33 per cent mean adult mortality and differed significantly from each other.

3.4 Adult mortality of *S. oryzae* exposed to elevated levels of CO_2 after four hours of exposure period

The adult rice weevils exposed to longer exposure periods of four hours showed a significant effect on adult mortality (Table 4). The adult mortality recorded with lower concentrations of 20 and 10 per cent CO_2 was found to be more than fifty per cent after fourth and fifth day of treatment and it reached to 73.67 and 66.00 per cent mortality by seventh day after treatment, respectively. The 50 per cent CO_2 concentration showed 48.67 per cent mortality of adults at one day after treatment and it further increased to 89.00 per cent at seven days after treatment. The next higher concentration of 60 percent recorded 58.67 per cent mortality by first day and 100.00 per cent mortality by seventh day. The adult mortality recorded with higher CO_2 concentrations (80 and 70 per cent) after four hours exposure resulted in cent per cent mortality of adults after five and six days of treatment, respectively. However, the mean mortality of adults obtained with 80 per cent CO_2 concentration (88.71 per cent) was significantly superior over all CO_2 treatments.

3.5 Adult mortality of *S. oryzae* exposed to elevated levels of CO_2 after five hours of exposure period

The results (Table 5) revealed that low concentrations of 10, 20, 30 and 40 per cent CO_2 caused 32.67 per cent to 47.00 per cent adult mortality after one day of treatment and by seventh day it ranged from 65.33 percent to 85.00 percent. The mean mortality of adults recorded in all the above four concentrations of CO_2 ranged from 48.90 per cent to 64.95 per cent. The adult mortality recorded at 80, 70 and 60 per cent CO_2 after five hours exposure period was cent per cent at second, sixth and seventh day, respectively. The mean mortality of adults obtained with 80 per cent CO_2 concentration (95.81 per cent) was significantly superior over the rest of the treatments taken into consideration. The interaction effect of concentrations and exposure periods also showed significant variation. Among all the interactions, exposure of adult insects to 80 per cent CO_2 concentration for five hours resulted in 95.81 per cent mortality.

The overall findings obtained from adult mortality studies of *S. oryzae*, when exposed to various concentrations and exposure periods of CO_2 (Table 6) indicated that the concentrations of CO_2 as well as exposure periods had significant influence on adult mortality and increasing the exposure period from one hour to five hours drastically reduced the time required to cause the mortality of adults.

The results are in agreement with the findings of Ofuya and Reichmuth (2002) who concluded that the mortality of *C. maculatus* to CO_2 was significantly influenced by CO_2 concentration and exposure period. Spratt *et al.* (1985) [11] subjected several developmental stages of laboratory strains of *T. granarium* to 60 per cent CO_2 and they observed that the mortality increased with the increase in exposure period. Mannad *et al.* (1999) [7] and Bera *et al*. (2004) stated that modified atmosphere system involving CO_2 concentration ranging from 20 to 80 per cent in paddy effectively controlled rice weevil and lesser grain borer. Krishnamurthy *et al*. (1993) [6] used 80 per cent CO_2 to get 100 per cent mortality of *T. castaneum* and *S. oryzae* adults.

The present findings revealed that exposure of *S. oryzae* adults to 80 per cent CO_2 for 5 hours as the best treatment for the control of adult weevils as this treatment resulted in cent per cent adult mortality within two days after treatment and it can be recommended for effective management of the *Sitophilus oryzae*.

Table 1: Mortality (per cent) of *Sitophilus oryzae* (L.) adults after one hour exposure to different concentrations of CO_2

CO_2 concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00(4.06)
20%	3.67 (10.53)	5.33 (13.34)	15.00 (22.78)	24.00 (29.32)	27.00 (31.29)	33.67 (35.46)	54.67 (47.68)	23.33(28.88)
30%	5.67 (13.76)	14.33 (22.21)	22.00 (27.95)	54.00 (47.30)	59.33 (50.38)	60.33 (50.96)	63.67 (52.94)	39.90(39.18)
40%	8.67 (17.00)	17.33 (24.55)	34.00 (35.66)	55.00 (47.87)	61.00 (51.36)	63.67 (52.94)	67.00 (54.95)	43.81(41.44)
50%	12.33 (20.54)	22.00 (27.95)	40.33 (39.41)	59.33 (50.38)	62.00 (51.95)	65.67 (54.13)	69.00 (56.18)	47.24(43.42)
60%	13.67 (21.64)	25.67 (30.43)	42.33 (40.59)	65.33 (53.93)	67.33 (55.15)	80.33 (63.68)	85.33 (67.49)	54.29(47.46)
70%	15.33 (23.04)	28.67 (32.35)	54.00 (47.30)	70.33 (57.00)	73.67 (59.14)	82.33 (65.18)	87.33 (69.21)	58.81(50.08)
80%	20.33 (26.78)	50.33 (45.19)	62.67 (52.34)	74.00 (59.35)	78.67 (62.52)	87.00 (69.00)	100.00 (85.95)	67.57(55.29)
Control	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00(4.06)
SEm±	1.31	0.81	0.82	0.48	0.71	0.80	0.66	0.20
CD (P=0.05)	3.36	2.41	2.43	1.44	2.13	2.38	1.95	0.58
CV (%)	12.47	6.21	4.65	2.13	3.01	3.13	2.31	0.97

Figures in the parentheses are angular transformed values

Table 2: Mortality (per cent) of *Sitophilus oryzae* (L.) adults after two hours exposure to different concentrations of CO_2

CO_2 Concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	9.00 (17.33)	10.67 (19.06)	13.67 (21.65)	18.67 (25.57)	22.00 (27.95)	30.33 (33.42)	37.00 (37.46)	20.19(26.70)
20%	12.00 (20.20)	17.00 (24.31)	20.67 (27.03)	28.33 (32.14)	28.67 (32.36)	37.00 (37.46)	57.00 (49.03)	28.67(32.37)
30%	15.33 (23.05)	19.00 (25.81)	23.67 (29.10)	54.67 (47.68)	60.33 (50.96)	62.33 (52.15)	67.00 (54.95)	43.19(41.09)
40%	19.00 (25.82)	20.33 (26.80)	25.67 (30.44)	56.33 (48.64)	62.00 (51.95)	66.67 (54.75)	68.67 (55.98)	45.52(42.43)
50%	23.67 (29.10)	25.33 (30.22)	27.33 (31.50)	59.33 (50.39)	65.67 (54.13)	67.33 (55.16)	72.00 (58.07)	48.67(44.24)
60%	28.67 (32.36)	34.00 (35.66)	36.67 (37.26)	66.00 (54.33)	67.67 (55.35)	80.33 (63.68)	85.00 (67.21)	56.90(48.97)
70%	32.33 (34.65)	35.33 (36.47)	37.00 (37.46)	72.33 (58.28)	75.33 (60.22)	84.00 (66.51)	88.67 (70.44)	60.71(51.19)
80%	54.00(47.30)	57.33 (49.22)	62.33 (52.15)	75.33 (60.22)	77.33 (61.59)	87.67 (69.46)	100.0(85.95)	73.43(58.97)
Control	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00(4.06)
SEm±	0.91	0.65	0.84	0.73	0.70	0.89	0.91	0.24
CD(P=0.05)	2.70	1.93	2.49	2.16	2.09	2.65	2.70	0.73
CV (%)	6.06	4.03	4.82	2.96	2.74	3.18	2.93	1.09

Figures in the parentheses are angular transformed values

Table 3: Mortality (per cent) of *Sitophilus oryzae* (L.) adults after three hours exposure to different concentrations of CO_2

CO_2 Concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	22.00 (27.95)	26.67 (31.07)	32.33 (34.65)	37.33 (37.66)	45.67 (42.51)	47.33 (43.47)	57.33 (49.22)	38.38 (38.28)
20%	25.33 (30.22)	34.00 (35.66)	37.00 (37.46)	45.33 (42.32)	47.00 (43.28)	59.00 (50.19)	64.00 (53.13)	34.52 (41.86)
30%	33.67 (35.46)	35.33 (36.47)	42.00 (40.39)	48.67 (44.24)	54.00 (47.30)	60.00 (50.77)	67.00 (54.95)	48.67 (44.24)
40%	36.67 (37.26)	40.67 (39.62)	50.33 (45.19)	55.33 (48.06)	61.00 (51.36)	66.33 (54.54)	70.33 (57.00)	54.38 (47.51)
50%	43.67 (41.36)	47.00 (43.28)	54.00 (47.30)	62.00 (51.95)	65.33 (53.93)	73.33 (58.93)	77.33 (61.59)	60.38 (51.00)
60%	45.67 (42.51)	50.33 (45.19)	55.33 (48.06)	63.67 (52.94)	69.00 (56.17)	84.00 (66.44)	85.67 (67.76)	64.81 (53.62)
70%	52.00 (46.15)	54.00 (47.30)	63.33 (52.74)	74.33 (59.58)	79.00 (62.74)	87.33 (69.22)	100.00 (85.95)	72.86 (58.61)
80%	62.33 (52.15)	65.33 (53.93)	68.67 (55.97)	78.67 (62.52)	87.33 (69.21)	100.00(85.95)	100.00 (85.95)	80.33 (63.67)
Control	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)
SEm±	0.74	0.57	0.85	0.78	0.62	0.78	0.62	0.39
CD(P=0.05)	2.22	1.68	2.53	2.32	1.84	2.32	1.86	1.15
CV (%)	3.67	2.62	3.62	3.02	2.23	2.51	1.87	1.50

Figures in the parentheses are angular transformed values

Table 4: Mortality (per cent) of *Sitophilus oryzae* (L.) adults after four hours exposure to different concentrations of CO_2

CO_2 Concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	32.00 (34.44)	38.67 (38.44)	43.67 (41.35)	45.67 (42.47)	53.33 (46.91)	63.33 (52.74)	66.00 (54.33)	48.95 (44.40)
20%	38.67 (38.44)	42.00 (40.39)	47.33 (43.47)	53.67 (47.10)	56.33 (48.64)	68.33 (55.76)	73.67 (59.14)	54.29 (47.46)
30%	40.00 (39.23)	47.00 (43.28)	55.67 (48.25)	55.67 (48.25)	61.67 (51.76)	73.67 (59.14)	84.67 (66.95)	59.76 (50.63)
40%	47.33 (43.47)	50.67 (45.38)	63.67 (52.94)	64.33 (53.34)	67.00 (54.95)	77.00 (61.39)	85.33 (67.49)	65.05 (53.76)
50%	48.67 (44.24)	57.67 (49.41)	67.33 (55.15)	67.33 (55.15)	68.67 (55.99)	85.00 (67.21)	89.00 (70.71)	69.10 (56.23)
60%	58.67 (50.00)	61.33 (51.56)	75.00 (60.00)	75.33 (60.22)	79.00 (62.74)	89.00 (70.71)	100.00 (85.95)	76.90 (61.28)
70%	62.33 (52.15)	65.33 (53.93)	84.00 (66.44)	85.33 (67.49)	88.67 (70.39)	100.00 (85.95)	100.00 (85.95)	83.67 (66.16)
80%	70.33 (57.04)	73.33 (58.93)	85.67 (67.76)	91.67 (73.40)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	88.71 (70.39)
Control	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)
SEm±	0.87	0.73	0.67	0.90	0.92	0.82	0.57	0.36
CD(P=0.05)	2.58	2.18	1.98	2.67	2.74	2.43	1.68	1.07
CV (%)	3.73	2.96	2.34	3.12	2.98	2.35	1.52	1.23

Figures in the parentheses are angular transformed values

Table 5: Mortality (per cent) of *Sitophilus oryzae* (L.) adults after five hours exposure to different concentrations of CO_2

CO_2 Concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	32.67 (34.85)	38.33 (38.25)	43.33 (41.16)	45.67 (42.51)	53.33 (46.91)	63.67 (52.94)	65.33 (53.93)	48.90 (44.37)
20%	38.67 (38.44)	42.33 (40.59)	54.00 (47.30)	54.00 (47.30)	57.33 (49.22)	68.67 (55.97)	73.67 (59.14)	55.52 (48.17)
30%	40.67 (39.62)	46.67 (43.09)	55.67 (48.25)	55.67 (48.25)	62.00 (51.95)	73.67 (59.14)	84.00 (66.44)	59.76 (50.63)
40%	47.00 (43.28)	50.67 (45.38)	63.67 (52.94)	64.33 (53.34)	67.33 (55.15)	76.67 (61.15)	85.00 (67.21)	64.95 (53.70)
50%	49.00 (44.43)	57.33 (49.22)	66.67 (54.75)	68.00 (55.56)	69.33 (56.42)	85.67 (67.76)	89.33 (71.05)	69.33 (56.38)
60%	58.67 (50.00)	62.67 (52.34)	75.67 (60.45)	76.33 (60.90)	78.67 (62.51)	89.00 (70.67)	100.00 (85.95)	77.29 (61.54)
70%	62.00 (51.95)	65.33 (53.93)	84.33 (66.71)	85.67 (67.76	88.67 (70.39)	100.00 (85.95)	100.00 (85.95)	83.71 (66.20)
80%	70.67 (57.25)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	95.81 (78.21)
Control	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)
SEm±	0.84	0.65	0.67	0.48	0.98	0.69	0.67	0.35
CD(P=0.05)	2.50	1.95	1.99	1.43	2.92	2.04	1.98	1.04
CV (%)	3.61	2.48	2.26	1.61	3.17	1.96	1.79	1.17

Figures in the parentheses are angular transformed values

Table 6. Effect of different concentrations and exposure periods of CO_2 on mean adult mortality of *Sitophilus oryzae* (L.)

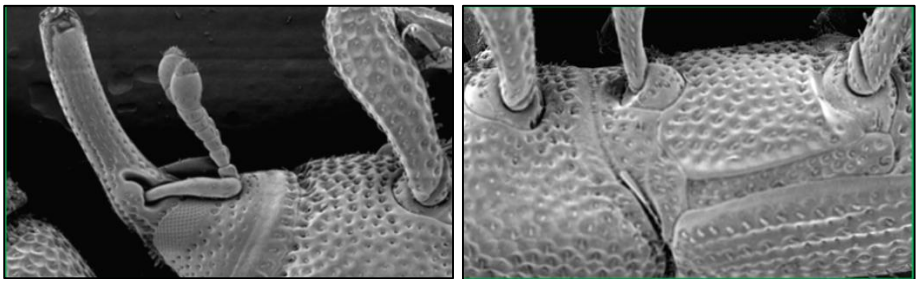
CO_2 Concentrations	Per cent adult mortality					
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	Mean
10%	0.00 (4.06)	20.19 (26.70)	38.38 (38.28)	48.95 (44.40)	48.90 (44.37)	31.29 (31.56)
20%	23.33 (28.88)	28.67 (32.37)	44.52 (41.86)	54.29 (47.46)	55.52 (48.17)	41.27 (39.75)
30%	39.90 (39.18)	43.19 (41.09)	48.67 (44.24)	59.76 (50.63)	59.76 (50.63)	50.26 (45.15)
40%	43.81 (41.44)	45.52 (42.43)	54.38 (47.51)	65.05 (53.76)	64.95 (53.70)	54.74 (47.77)
50%	47.24 (43.42)	48.67 (44.24)	60.38 (51.00)	69.10 (56.23)	69.33 (56.38)	58.94 (50.25)
60%	54.29 (47.46)	56.90 (48.97)	64.81 (53.62)	76.90 (61.28)	77.29 (61.54)	66.04 (54.57)
70%	58.81 (50.08)	60.71 (51.19)	72.86 (58.61)	83.67 (66.16)	83.71 (66.20)	71.95 (58.45)
80%	67.57 (55.29)	73.43 (58.97)	80.33 (63.67)	88.71 (70.39)	95.81 (78.21)	81.17 (65.31)
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
Mean	37.22 (34.87)	41.92 (38.89)	51.59 (44.76)	60.71 (50.48)	61.70 (51.47)	
	SE(m)±			CD (P=0.05)		
Concentrations (F1)	0.14			0.40		
Exposure period (F2)	0.11			0.30		
Interaction (F1 X F2)	0.32			0.89		
CV (%)	1.24					

Figures in the parentheses are angular transformed values

3.6 Effect of CO_2 fumigation on *Sitophilus oryzae* as seen under Scanning Electron Microscope (SEM)

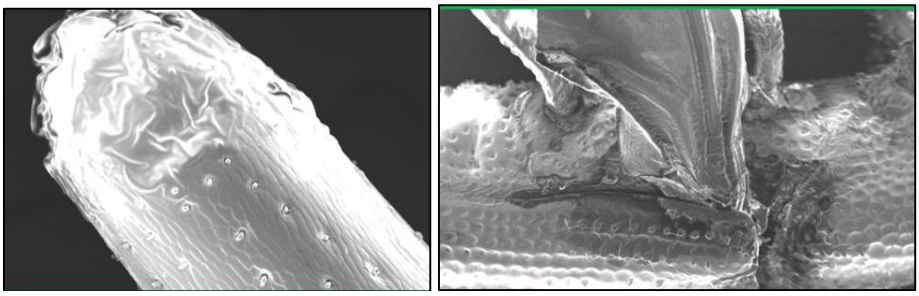
The scanning electron microscope (SEM) images of adult insect exposed to CO_2 fumigation (Pressure 2 kg cm^{-2}) clearly showed the damage of the integument (cuticle) (Plate 2) and rostrum (Plate 3) over the normal integument (cuticle) and rostrum in untreated check (Plate 1). CO_2 initially causes the spiracle valves to open by local action on the muscle, when it reaches the central nervous system it causes a reduction in the tonic discharge to the closer muscle which may allow the valve to open further, as soon as the insect is in contact with pure CO_2, the heartbeat stops (Jones, 1974) [5]. As CO_2 enters with high pressure (2 kg cm^{-2}), expands first and then rapidly equilibrates to atmospheric pressure thereby causing severe damage to the insect body with loss of integument (Plate 2 and 3).

A. Rostrum (Snout) of test insect B. Ventral view of test insect



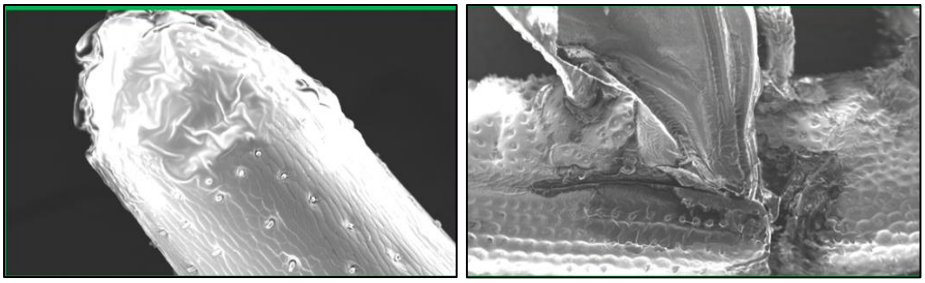
A. Rostrum (Snout) of test insect B. Ventral view of test insect

Plate 1: SEM Images of untreated control test insect



A. Rostrum (Snout) of test insect B. Ventral view of test insect

Plate 2: SEM Images of test insect at 40 per cent CO_2



A. Rostrum (Snout) of test insect B. Ventral view of test insect

Plate 3: SEM Images of test insect at 80 per cent CO_2

References

1. Aliniazee MT, Lindgren DL. Egg hatch of *Tribolium confusum* and *Tribolium castaneum* in different carbon dioxide and nitrogen atmospheres. Ann. Entomol. Soc. Am. 1970; 63:10-12.
2. Barrer PM, Jay EG. Laboratory observations on the ability of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) to locate, and to oviposit in response to a source of grain odour. J Stored Prod. Res. 1980; 16:1-7.
3. Hoyle G. The action of carbon dioxide gas on an insect spiracular muscle. J Insect Physiot. 1960; 4:63-79.
4. John JB, Lonnie DR. In: Electron Microscopy principles and techniques of biologists. 2nd Ed. Jones and Bartlett, publishers, Sudbury, Massachusetts. 1998, 19-24, 54-55 63-67.
5. Jones JC. Factors affecting heart rates in insects. See Ref. 1974; 19(5):1 19-67.
6. Krishnamurthy TS, Muralidharan N, Krishna Kumari MK. Disinfesting food commodities in small storages using carbon dioxide rich atmospheres. Int. Pest Control. 1993; 35:153-156.
7. Mannad D, Jayas DS, White NDG, Muir WE. Mortality of *Cryptolestes ferrugenius* (Stephens) exposed to changing CO_2 concentrations. Journal of Stored Products Research. 1999; 35:385-395.
8. Navarro S. Studies on the effect of alterations in pressure and compositions of atmospheric gases on the tropical warehouse moth, *Ephestia cautella* (Walker), as a model for stored products insects. *Ph.D. Thesis*. Hebrew University of Jerusalem, Israel, 1975.
9. Panse VG, Sukhatme PV. Statistical methods for Agricultural workers. ICAR New Delhi, 1978.
10. Press JW, Flaherty BR, Arbogast RT. Oocyte maturation in *Tribolium castaneum* after repetitive sublethal carbon dioxide exposures. Ann. Entomol. Soc. Am. 1976; 66:480-481.
11. Spratt EC. The effect of high concentration of CO_2 in air on *Trogoderma granarium* Everts. J Stored. Prod. Res. 1985; 21:41-46.
12. UNEP (United Nations Environment Programme). Report of the Methyl Bromide Technical Options committee (MBTOC), 2006.
13. Vasanthraj David B, Kumara Swamy T. Elements of economic entomology. 4th Edition, Madras. Popular Book Depot. 1975, 279-280.