Int.J.Curr.Microbiol.App.Sci (2018) *7*(8): 4064-4075

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.708.422

Effect of Modified Atmosphere with Elevated Levels of CO_2 on *Sitophilus oryzae* (Linnaeus)

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Keywords

Mortality of *Sitophilus oryzae*, Modified atmosphere, Carbon dioxide, Scanning electron microscope

Article Info

Accepted:
22 July 2018
Available Online:
10 August 2018

A B S T R A C T

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.

Introduction

In India, maize is the third most important food crop after rice and wheat, contributing nearly 9 per cent in the national food basket. According to the Ministry of Agriculture & Farmers welfare, Government of India, Statistics, 2015-16, the area, production and productivity of maize is 8.80 m ha, 22.56 mt and 2563 kg ha^1, respectively. The maize is cultivated throughout the year in all states of the country for various purposes including

grain, fodder, green cobs, sweet corn, baby corn, popcorn etc. The predominant maize growing states that contribute more than 80 per cent of the total maize production are Karnataka, Madhya Pradesh, Maharashtra, Uttar Pradesh Telangana, Rajasthan and Bihar.

Most of the maize grain harvested is stored on the farm, where post-harvest pest management practices are inadequate (Dubale**,** 2011) leading to huge amounts of maize seed losses due to pests of stored grain. Among the several insects attacking maize grain during storage, *Sitophilus zeamais* (Motsch) and *Sitophilus oryzae* (L.) are major pests. *Sitophilus zeamais* (Motsch) causes substantial losses to stored corn, amounting to 18.30 per cent (Adams, 1976), while a high damage of 92.40 to 98.30 per cent was reported by Bitran *et al.,* (1978) in different parts of the world except India.

On the other hand, *S. oryzae* causes enormous losses upto 100 per cent in stored maize in India and other countries (Irabagon, 1959 and Singh *et al.,* 1974). This evidently indicates the importance of *S. oryzae* in the storage of maize seed.

In the new state like Telangana, maize seeds are often traditionally stored in jute bags. This leads to significant increase of moisture during rainy seasons, thereby creating conducive conditions for weevil infestation (Hossain, *et al.,* 2007 and Zunjare *et al.,* 2014). Infested seed fetches lower market price due to reduced weight. Seed viability of the damaged grain is drastically reduced and affects subsequent planting (Tefera, 2012).

Wide use of insecticides for the control of stored grain insect pests is of global concern with respect to environmental hazards, insecticide resistance development, chemical residues in food, side effects on non-target organisms and the associated high costs (Cherry *et al.,* 2005). Keeping in view of environment safety study was conducted to develop alternate control strategies. Modified atmosphere treatment is a safe and environmentally friendly way to control stored grain pests. Recently, the worldwide ban of the fumigant insecticide methyl bromide, under the international agreement of the Montreal Protocol has motivated researchers to search various alternatives to replace methyl bromide (Fields and White, 2002). The use of CO_2 has several advantages, there is no accumulation of toxic residues after the treatment in the final product and is considered as the safest traditional fumigant. Treatment with CO_2 is residue free and approved by Environmental Protection Agency (EPA), USA. CO_2 treated grains are also accepted in the organic market (Bera *et al.,* 2008). The objective of the present work is to demonstrate the effect of elevated levels CO_2 on *Sitophilus oryzae* so as to prevent insect pest's development during the storage of maize seed.

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.

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 500 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.

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

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^oC 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) at RUSKA Lab, College of Veterinary Science, PV Narsimha Rao Telangana State Veterinary University (PVNRTSVU), Rajendrangar, Hyderabad, India.

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).

Results and Discussion

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 (3.33, 5.00 and 8.33 per cent, respectively) was observed, after one day of treatment and it increased to 55.00, 63.33 and 66.67 per cent, respectively after seven days of treatment. Among all the concentrations 80 per cent concentration recorded highest mortality of 18.33 per cent however, it was on par with 70 per cent CO_2 which resulted in 15.00 per cent mortality 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 after two days after treatment and all the CO_2 exposed adults died by seventh day after treatment. At 70 per cent CO_2 concentration, 53.33 per cent mortality was recorded by third day and it increased to 86.67 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 66.90 per cent.

Adult mortality of *S. oryzae* exposed to elevated levels of CO2 after two hours of exposure period

The results (Table 2) showed that low concentrations of 10,20and 30 and 40 per cent CO_2 caused less than 20 per cent mortality of adults (8.33-18.33 per cent) after one day of treatment and by seventh day it varied from 36.67 to 68.33 per cent. The mean mortality of adults recorded in all the above four concentrations of CO_2 ranged from 19.76 to 44.76 per cent. The next three higher concentrations of CO_2 *viz.*,50, 60 and 70 per cent concentrations recorded 23.33 to 31.67 per cent mortality after one day of treatment and it increased to 71.67 per cent to 88.33 per cent by seventh day. Among all the concentrations, the highest concentration of 80 per cent CO_2 recorded 56.33 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 (72.85 per cent) followed by 70 per cent CO_2 (60.24 per cent) and 60 per cent CO_2 (56.19 per cent) which were significantly different from each other.

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 up to three hours of exposure (Table 3) indicated that low concentrations of CO_2 (10, 20, 30 and 40 per cent) recorded 21.67 to 36.67 per cent adult mortality after one day of treatment and it increased to 56.67 to 70.00 per cent after seven days of treatment. The mean adult mortality of 37.62 to 54.05 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 43.33 and 45.00 per cent mortality, respectively after one day of treatment and 76.67 per cent to 85.00 per cent mortality after seven days of treatment. The higher concentrations of CO_2 *i,e.*,70 and 80 per cent recorded 51.67 and 61.67 per cent mortality after one day of treatment and 100 per cent mortality after seven days of treatment.

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%	3.33 (9.97)	5.00 (12.92)	15.00 (22.79)	23.33 (28.86)	26.67 (31.07)	33.33 (35.26)	55.00 (47.87)	23.07 (28.71)
30%	5.00 (12.92)	13.33 (21.34)	21.67 (27.71)	53.33 (46.91)	58.33 (49.80)	60.00 (50.77)	63.33 (52.74)	39.28 (38.81)
40%	8.33 (16.60)	16.67 (24.05)	33.33 (35.25)	55.00 (47.87)	60.00 (50.77)	63.33 (52.75)	66.67 (54.75)	43.33 (41.17)
50%	11.67 (19.99)	21.67 (27.71)	40.00 (39.22)	58.00 (49.80)	61.67 (51.76)	65.00 (53.73)	68.33 (55.77)	46.90 (43.22)
60%	13.33 (21.34)	25.00 (30.00)	41.67 (40.20)	65.00 (53.73)	66.67 (54.75)	80.00 (63.44)	85.00 (67.21)	53.81 (47.19)
70%	15.00 (22.79)	28.33 (32.14)	53.33 (46.91)	70.00 (56.79)	73.33 (58.93)	81.67 (64.70)	86.67 (68.66)	58.34 (49.70)
80%	18.33 (25.31)	50.00 (54.00)	61.67 (51.76)	73.33 (58.93)	78.33 (62.90)	86.67 (68.67)	100.00 (85.95)	66.90 (54.88)
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±	1.41	83.00	0.94	0.69	0.85	0.80	0.76	0.23
CD (P=0.05)	4.19	2.46	2.81	2.05	2.53	2.37	2.25	0.67
CV (%)	16.06	6.40	5.42	3.07	3.62	3.12	2.68	1.13

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%	8.33 (16.60)	10.00 (18.44)	13.33 (21.35)	18.33 (25.30)	21.67 (27.71)	30.00 (33.21)	36.67 (37.26)	19.76 (26.39)
20%	11.67 (19.86)	16.67 (24.05)	20.00 (26.57)	28.33 (32.14)	26.67 (31.07)	36.67 (37.26)	56.67 (48.84)	28.10 (32.00)
30%	15.00 (22.77)	18.33 (25.37)	23.33 (28.86)	55.00 (47.87)	60.00 (50.77)	61.67 (51.76)	66.67 (54.75)	43.57 (41.30)
40%	18.33 (25.31)	20.00 (26.57)	25.00 (30.00)	56.67 (48.84)	61.67 (51.76)	66.7 (54.75)	68.33 (55.77)	44.76 (41.99)
50%	23.33 (28.86)	25.00 (30.00)	26.67 (31.07)	58.33 (49.81)	65.00 (53.73)	66.67 (54.75)	71.67 (57.86)	47.87 (43.77)
60%	28.33 (32.15)	33.33 (35.25)	35.00 (36.24)	65.00 (53.73)	66.67 (54.75)	80.00 (63.44)	85.00 (67.21)	56.19 (48.56)
70%	31.67 (34.24)	35.00 (36.27)	36.67 (37.26)	71.67 (57.86)	75.00 (60.00)	83.33 (65.95)	88.33 (70.12)	60.24 (50.91)
80%	53.33 (46.94)	56.67 (48.84)	61.67 (51.76)	75.00 (60.00)	76.67 (61.15)	86.67 (68.66)	100.00 (85.95)	72.85 (58.60)
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±	1.13	0.76	1.03	0.80	0.80	0.93	0.90	0.26
CD(P=0.05)	3.36	2.25	3.06	2.37	2.38	2.75	2.66	0.76
CV (%)	7.63	4.74	6.01	3.28	3.16	3.33	2.90	1.15

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%	21.67 (27.71)	26.67 (31.07)	31.67 (34.23)	36.67 (37.26)	45.00 (42.13)	46.67 (43.09)	56.67 (48.84)	37.62 (37.83)
20%	25.00 (30.00)	33.33 (35.25)	36.67 (37.26)	45.00 (42.13)	46.67 (43.09)	58.33 (49.80)	63.33 (52.74)	44.05 (41.58)
30%	33.33 (35.26)	35.00 (36.27)	41.67 (40.20)	48.33 (44.04)	53.33 (46.91)	60.00 (50.77)	66.67 (54.75)	48.34 (44.05)
40%	36.67 (37.26)	40.00 (39.23)	50.00 (45.00)	5.00 (47.87)	60.00 (50.77)	66.67 (54.75)	70.00 (56.77)	54.05 (47.32)
50%	43.33 (41.16)	46.67 (43.09)	53.33 (46.91)	61.67 (51.76)	65.00 (53.73)	73.33 (58.93)	76.67 (61.15)	60.24 (50.91)
60%	45.00 (42.13)	50.00 (45.00)	55.00 (47.87)	63.33 (52.74)	68.33 (55.77)	83.33 (65.95)	85.00 (67.21)	64.29 (53.30)
70%	51.67 (45.96)	53.33 (46.91)	63.33 (52.75)	73.33 (58.93)	78.33 (62.29)	86.67 (68.66)	100.00 (85.95)	72.38 (58.30)
80%	61.67 (51.76)	65.00 (53.73)	68.33 (55.77)	78.33 (62.29)	86.67 (68.66)	100.00 (5.5)	100.00 (85.95)	80.00 (63.43)
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±	0.83	0.67	0.98	0.84	0.84	0.93	0.69	0.40
CD (P=0.05)	2.46	1.98	2.91	2.48	2.48	2.75	2.04	1.19
CV (%)	4.09	3.11	4.19	3.25	3.05	3.00	2.07	1.56

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%	31.67 (43.23)	38.333 (38.25)	43.33 (41.16)	45.00 (42.13)	63.00 (46.91)	63.33 (52.74)	65.00 (53.73)	48.57 (44.18)
20%	38.33 (38.25)	41.67 (40.20)	53.33 (46.91)	46.67 (43.09)	70.00 (48.84)	68.33 (55.77)	83.00 (58.93)	54.05 (47.32)
30%	40.00 (39.23)	46.67 (43.09)	55.00 (47.87)	51.67 (45.96)	61.67 (51.76)	73.33 (58.93)	83.33 (65.95)	58.81 (50.07)
40%	46.67 (43.09)	50.00 (45.00)	63.33 (52.74)	63.33 (52.75)	66.67 (54.75)	76.67 (61.15)	91.64 (67.21)	64.53 (53.45)
50%	48.33 (44.04)	58.33 (49.81)	66.67 (54.74)	66.67 (54.75)	68.33 (55.82)	85.00 (67.22)	88.33 (70.16)	68.81 (56.05)
60%	58.33 (49.80)	61.67 (51.76)	75.00 (60.00)	73.33 (5.93)	78.33 (62.29)	88.33 (70.16)	100.00 (85.95)	76.19 (60.79)
70%	61.67 (51.76)	65.00 (53.73)	83.33 (65.95)	85.00 (67.22)	88.33 (70.12)	100.00 (85.95)	85.95 (85.85)	83.33 (65.91)
80%	70.00 (56.84)	73.33 (5893)	85.00 (67.21)	91.67 (73.40)	100.00 (85.95)	100.00 (85.95)	100.00 (5.95)	88.57 (70.26)
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±	1.00	0.81	0.78	0.96	1.14	0.85	0.73	0.39
CD (P=0.05)	2.98	2.40	2.31	2.86	3.38	2.53	2.18	1.16
CV (%)	4.32	3.27	2.75	3.40	3.69	2.46	1.98	1.35

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

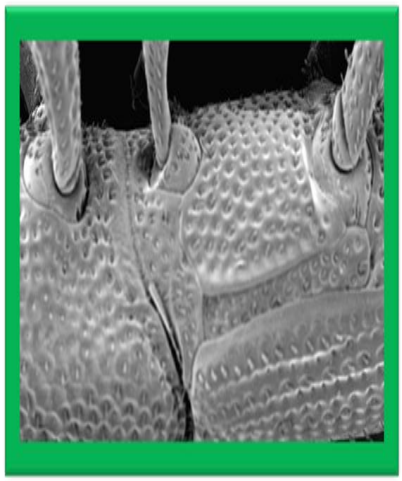
CO_2 concentrations (%)	Per cent adult mortality							
	Days after treatment (DAT)							
	1	2	3	4	5	6	7	Mean
10%	30.00 (34.23)	38.33 (38.25)	43.33 (41.06)	45.00 (42.13)	63.00 (46.91)	63.33 (52.74)	65.00 (53.73)	48.57 (44.18)
20%	50.00 (38.25)	41.67 (40.20)	53.33 (46.91)	53.33 (46.91)	70.00 (48.84)	68.33 (55.77)	73.33 (58.93)	54.05 (47.32)
30%	50.00 (39.23)	46.67 (43.09)	55.00 (47.87)	51.67 (45.96)	61.67 (51.76)	73.33 (58.93)	83.33 (65.95)	58.81 (50.07)
40%	46.67 (43.09)	50.00 (45.00)	63.33 (52.74)	63.33 (52.74)	66.67 (54.75)	76.67 (61.15)	85.00 (67.21)	64.53 (53.45)
50%	48.33 (44.04)	58.33 (49.81)	66.67 (54.75)	66.67 (54.75)	68.33 (55.82)	85.00 (67.21)	88.33 (70.16)	68.81 (56.05)
60%	58.33 (49.80)	61.67 (51.76)	75.00 (60.00)	73.33 (58.93)	78.33 (62.29)	88.33 (70.11)	100.00 (85.96)	78.57 (62.48)
70%	61.67 (51.76)	65.00 (53.73)	83.33 (65.95)	85.00 (67.21)	88.33 (70.16)	100.00 (85.95)	100.00 (85.96)	83.43 (65.99)
80%	70.00 (56.84)	100.00 (85.95)	100.00 (85.95)	100..00 (85.95)	100.00 (85.96)	100.00 (85.95)	100.00 (85.96)	92.38 (73.99)
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±	1.00	0.72	0.79	0.74	1.14	0.85	0.73	0.63
CD (P=0.05)	2.98	2.15	2.31	2.21	3.38	2.34	2.18	1.87
CV (%)	4.32	2.74	2.64	2.53	3.69	2.46	1.98	2.14

Figures in the parentheses are angular transformed values

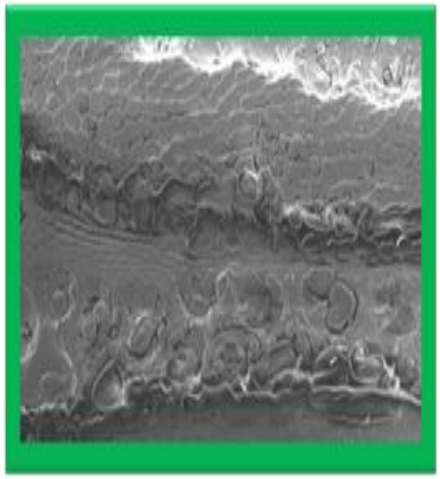
Table.6 Effect of different concentrations and exposure periods of CO_2 on mean adult mortality of *Sitophilus oryzae* (L.)

CO_2 Concentrations	Per cent adult mortality					
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	Mean
10%	0.00 (4.05)	19.76 (26.39)	37.62 (37.83)	48.57 (44.18)	48.57 (44.18)	30.91 (31.33)
20%	23.07 (28.71)	28.10 (32.01)	44.05 (41.58)	54.05 (47.32)	54.05 (47.32)	40.66 (39.39)
30%	39.29 (38.81)	43.57 (41..31)	48.34 (44.05)	58.81 (50.07)	58.81 (50.07)	49.76 (44.86)
40%	43.33 (41.17)	44.76 (41.99)	54.05 (47.32)	64.53 (53.45)	64.53 (53.45)	54.24 (47.48)
50%	46.90 (43.22)	47.86 (43.77)	60.24 (50.91)	68.81 (56.53)	68.81 (56.05)	58.52 (50.00)
60%	53.81 (47.19)	56.19 (48.56)	64.29 (53.30)	76.19 (60.79)	18.57 (62.48)	65.81 (54.46)
70%	58.34 (49.70)	60.24 (50.91)	72.38 (58.30)	83.33 (65.91)	83.43 (92.38)	71.54 (58.18)
80%	66.90 (54.88)	72.85 (58.60)	80.00 (63.43)	88.57 (70.26)	92.38 (73.99)	80.14 (64.23)
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	36.85 (34.65)	41.48 (38.62)	51.22 (44.53)	60.32 (50.23)	61.02 (50.84)	
	SE(m)±			CD (P=0.05)		
Concentrations (F1)	0.18			0.51		
Exposure period (F2)	0.14			0.38		
Interaction (F1 X F2)	0.41			1.14		
CV (%)	1.61					

Figures in the parentheses are angular transformed values



A untreated control test insect



B. Lateral view of test insect exposed to 40 per cent CO_2 concentration

C. Lateral view of test insect exposed to 80 per cent CO_2 concentration

Plate.1 SEM Images of *Sitophilus oryzae* exposed to elevaated levels of CO_2

The higher concentrations of CO_2 *viz*., 70 and 80 per cent were found to be significantly superior to other treatments and recorded cent per cent mortality after seven and six days of treatment, respectively. The higher CO_2 concentrations of 50 to 80 per cent showed 60.24 to 80.00 per cent mean adult mortality and differed significantly from each other.

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

The *S.oryzae* adults subjected to longer exposure periods of four hours (Table 4) showed 48.33 per cent mortality of adults even at 50 per cent CO_2 concentrations at one day after treatment and it further increased to 88.33 per cent at seven days after treatment.

The next higher concentration of 60 per cent recorded 58.33 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 (88.57 per cent) was significantly superior over all CO_2 treatments.

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 30.00 per cent to 46.67 per cent adult mortality after one day of treatment and by seventh day it ranged from 65.00 per cent to 85.00 per cent. The mean mortality of adults recorded in all the above four concentrations of CO_2 ranged between 48.57 per cent and 64.53 per cent.

The adult mortality recorded at 80, 70 and 60 per cent CO_2 after five hours exposure resulted in cent per cent mortality at second, sixth and seventh day, respectively. The mean mortality of adults obtained with 80 per cent CO_2 concentration (92.38 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 92.38 per cent mortality. Exposure to low concentration (10 per cent) for one hour resulted in significantly lowest adult mortality (zero per cent).

The overall findings obtained from adult mortality studies of *S. oryzae,* when exposed to various concentrations and exposure periods of CO_2 (Table 4.18) 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 (1993) 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) subjected several developmental stages of laboratory strains of *T. granarium* to 60 per cent CO_2 and they observed mortality increased with the increase in exposure period. Mannad *et al.,* (1999) 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) used 80 per cent CO_2 to get 100 per cent mortality of *T. castaneum* and *S. oryzae* adults. Zhou *et al.,* (2000) found that elevated CO_2 reduced the O_2 consumption of *Platynota staltana*. They found that O_2 consumption rate was decreased by 62 per cent at 20 per cent CO_2 and by 73 per cent at 79 per cent CO_2. Empirical mortality data showed that levels of CO_2 toxicity to insects are generally above 20 per cent (Banks and Annis, 1990; Carpenter and Potter, 1994; Mitcham *et al.,* 1997; Zhou *et al.,* 2001). Carbon dioxide can initially have a narcotic effect leading to knock down (Edwards and Batten, 1973). Most insects are more easily killed with higher CO_2 concentrations (Jay, 1984).

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). 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).

The high mortality of *S. oryzae* adults obtained with high CO_2 concentrations and prolonged exposure periods could be attributed to the following effects. Elevated CO_2 affects the respiration of insects by reducing the oxidative phosporylation and inhibits the respiratory enzymes such as succinate dehydrogenase (Edwards, 1968) and malic enzyme (Fleurat-Lessard, 1990). Reduced oxidative phosporylation leads to reduced ATP generation. Carbon dioxide poisoning inhibits O_2 utilization by specific enzymes, such as succinic dehydrogenase, or causes a weak oxidative metabolism resulting in accumulation of toxic products (Bell, 1984) such as lactate, pyruvate, and succinic acid. Zhou *et al.,* (2001) suggested that elevated CO_2 could increase the permeability of membranes. Therefore, the failure of

membrane function under hypercarbia could result from both energy insufficiency and increased membrane permeability. It is more likely that the decreased energy supply under metabolic arrest cannot meet the need of maintaining a more permeable membrane due to elevated CO_2.

Carbon dioxide has also been shown to increase intercellular Ca^{+2} ion concentration by decreasing pH (Lea and Ashley, 1978). According to Hochachka (1986), a high concentration of Ca^{+2} in the cytosol can cause the cell and mitochondrial membranes to become more permeable leading to cell damage or death. Very important effect of raised concentrations of CO_2 is prolonged opening of the spiracles, which leads to desiccation and mortality (Bursell, 1974). 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). Daily, repeated 2-hr exposures of adult *Tribolium castaneum* to CO_2 before maturation suppressed oocyte development in the ovarioles (Press, 1976). The present findings confirmed that exposure of *S. oryzae* adults to 80 per cent CO_2 for 5 hours was considered as the best treatments for 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* in maize.

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How to cite this article:

Padmasri, A., B. Anil Kumar, C. Srinivas, K. Vijaya lakshmi, T. Pradeep, J. Aruna kumara and Sheker, V. 2018. Effect of Modified Atmosphere with Elevated Levels of CO_2 on *Sitophilus oryzae* (Linnaeus). *Int.J.Curr.Microbiol.App.Sci*. 7(08): 4064-4075.
doi: https://doi.org/10.20546/ijcmas.2018.708.422