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Management of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) under modified atmospheric condition on stored sorghum

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ABSTRACT

Management of Sitophilus oryzae (L.) under modified atmospheric (MA) condition on stored sorghum containing various concentrations of carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2) was studied. The tested MA was N_2: O_2: CO_2- 80: 00: 20 MA_1, 80: 05: 15 MA_2, 80: 10: 10 MA_3, 80: 15: 5 MA_4, and Untreated MA_5. Adult emergence and mass loss (%) was recorded after exposure periods of 45, 90, 135, 180, 225, 270 and 315 days. Fifteen and 20 per cent CO_2 concentrations exposed up to 315 days resulted in the cent per cent mortality of insects. Further, there was no adult emergence thereby mass loss (%) was nil and also there was no loss in germination of grains.

Key words: Carbon dioxide, Modified atmosphere, *Sitophilus oryzae*

INTRODUCTION

It is well established fact that lot of efforts should be put for the production of “every single grain” but this is of no use if the produced grains are not saved, which recalls the proverb “a grain saved is a grain produced”. This adage depends mainly on how best we protect the quality of grains during storage. Loss of grains stored as seed and the future food of our country is to the tune of 7-8 per cent accounting to major share of economic loss worth Rs. 600-700 crores. Scientists are equally putting their efforts and attempting to find ways and means to reduce losses in storage due to store pests.

There are increasing restrictions on the use of pesticides and on 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 is being phased out in accordance with the Montreal Protocol due to its effect on the ozone layer [25]. The development of alternative treatments for pest control is an increasing demand from the food industry and has been promoted by governments through legislation and the funding of research projects. Alternatives should meet consumer demands for the reduced use or elimination of pesticides while, at the same time, maintaining a high degree of control efficacy [24].

Hermetic storage of grain was practiced in ancient times in underground pits in the dry, subtropical regions of the Middle East and other dry regions of the world, such as Africa and India. Underground pits for grain storage were used in Egypt during 1940s. Very old but active hermetic storages were reported to be in operation in India [17] and in Yemen, Somalia, Sudan, and Egypt [21].

Hermetic storage for generating a dynamic modified atmosphere has been demonstrated extensively in Israel and parts of Asia and Africa, and provided a means of safe storage in locations where electricity or access to gases or

permanent storage structures is limited [22]. Toxicity responses of insects to controlled or modified atmospheres are similar to those with chemical fumigants. Modified atmosphere provide a way to eliminate insects from stored commodities without polluting the atmosphere and are safer than traditional fumigants. No harmful residues remain after the treatment of the commodity with Nitrogen (N_2) or Carbon dioxide (CO_2). CO_2 is now used in several countries for the treatment of stored products, particularly grain in bulk, to control insect pest [20]. The attraction of CO_2 in modified atmosphere (MA) treatment lies on availability, relative convenience and safety of application and the facts that it does not leave toxic residue has received the U.S. Food and Drug Administration (FDA) approval for its use as a fumigant [19].

Controlled atmospheres have been used for disinfesting raw or semi-processed food products, such as cereal grains and dried fruits, while still in storage. Treatments based on reduced oxygen (O_2) and high CO_2 or N_2 contents are technically suitable alternatives for arthropod pest control in durable commodities [16, 1, 22 and 24]. Atmospheres rich in CO_2, those with over 40% in air, are faster at controlling pests than those with high contents of N_2 [22]. Data on the effects of different types of CO_2 treatments and dosages on key pests are available for many species and stages of stored-product pests under particular sets of conditions [5, 29, and 3]. CO_2 has received considerable attention for the disinfection of stored foodstuffs, particularly durable products [4, 2, and 6]. The toxicity of CO_2 to insects is known to vary among species, developmental stages and age groups.

Rice weevil is economically important storage pest on sorghum and other cereals in tropical and sub-tropical regions of the world. Rice weevil infestation alone resulted in sorghum grain loss of 61.3 per cent over a period of five months [26]. Further, the presence of storage pest in grain reduces the value of sorghum for milling and bread quality [9]. It is reported that major loss of food grains in storage is contributed by two internal feeder's *viz*., rice weevil, *Sitophilus oryzae* Linn. and lesser grain borer, *Rhyzopertha dominica* Fab. Survey conducted by Food and Agriculture Organization revealed that *R. dominica* is the major pest of wheat, rice and millets in India [11]. Therefore, this study was designed to quantify the dosage of gas combination for *S. oryzae* for safe storage of Sorghum seeds.

MATERIALS AND METHODS

2.1 *Insect culture and its maintenance*

S. oryzae insect were collected from the old stocks. Sorghum seeds were kept in an oven for one hour at 50^o C for disinfection and 250 gram of sorghum seeds in 20 bottles were taken separately and 50 newly emerged adults were released in bottles. The containers were covered with muslin cloth and maintained at room temperature of 27 ± 3^{o}C throughout the period of study. After about 35 to 40 days, adults that started emerging from the culture were utilized for the maintenance of subcultures. Sub culturing of these insects was done at 20 days intervals so that a continuous supply of insects for experiments was ensured. For getting uniform aged adults, 250g of disinfected sorghum seeds were exposed to 20 pairs of insects and allowed to lay eggs for 10 days. All the adult insects were removed after 10 days and uniform aged adults were harvested after 25 days. Various sub cultures were maintained for conducting experimental studies on modified atmosphere against these pests. Details of the treatments and gas concentrations are as mentioned below.

Treatments	Gas combination (%) N_2: O_2: CO_2
MA_1	80: 00: 20
MA_2	80: 05: 15
MA_3	80: 10: 10
MA_4	80: 15: 5
MA_5	Atmospheric gas

2.2. Laboratory experiments

Modified atmospheres (MAs) were investigated in the Laboratory. Experiments aimed to study the effect of O_2 and CO_2 gases at different concentrations on the development of *S. oryzae* for different exposure periods *viz*., 45, 90,135, 180, 225, 270 and 315 days. The newly-hatched adults were required for the MA experiments were obtained from the stock culture as described earlier. After exposure to MAs, treated packets were maintained under laboratory conditions of 27 ± 3^{o}C throughout the period of study. All the tests with MAs were repeated four times, and four similar replicates of every treatment were left untreated for control purpose.

2.3. *Procedure to use MAP instrument*

Ten pairs each of rice weevils and lesser grain borers were released in to it and this cloth bag was put in a polyethylene cover (700 gauge) in such a way that the cloth avoids loss of insects while creating vacuum before

filling the gas in the polyethylene covers. The seeds containing rice weevil and lesser grain borers were filled with required gas as in each treatment using the modified atmosphere filling equipment,

The connections of CO_2, O_2 and N_2 gas cylinder to the mixing chamber were checked and the pressure of the gases was adjusted so that the alarming red light in gas mixing chamber is switched off. Later the required gas concentrations were adjusted as follows.

a) Adjusted the top dial in the mixing chamber to the required CO_2 gas concentration and the value of the X (mentioned below the upper dial) is noted then adjusted the bottom dial by calculating the value. (N2)/X, where: N2 = Nitrogen X = Value below the upper dial

b) The gas concentrations have been checked by using check mate gas analyzer. Through the gas sampling port the gases are allowed to pass through needle and the obtained gas concentration from the gas mixing chamber is checked.

c) Required gas concentration is achieved by slightly changing the dialler and later the sampling port was closed and vacated the buffer tank which is meant for collection and supply of gas in combination

d) Buffer tank was vacated to avoid deviation in the required gas concentration

In Packing Unit the heat of sealing was adjusted to 2.0 to 2.5 for proper sealing. The packing material (poly ethylene chloride, 700 gauges) kept in a packing unit where, it first creates vacuum so that the old gas if may removed from the packing material so that it fills the required gas concentration from buffer tank and seal it immediatly.

Proper care was to be taken to avoid loss of seed and escape of insects from poly ethylene bag. Once this process was over the respective were kept under ambient condition in the laboratory after proper labelling.

1.4. Adult count

Observations on live and dead adult insect count were taken after 45 days of storage. Prior to count the bags were kept in deep freezer for 2-3 minutes to make insects inactive. Then by sieving the seeds were sieved and insects were separated for recording observation.

2.5. Weight loss in sorghum seeds

Weight loss of sorghum seeds was recorded by using following formula

$W = W1 - W0$

Where: W= Weight loss W1=Initial weight W0= Final weight

2.6. Per cent weight loss

Seed weight loss was computed by the following formula as suggested by [18].

$$\text{Per cent weight loss} = \frac{\text{O.W.} - \text{F.W.}}{\text{O.W.}} \times 100$$

Where;

O.W. = Original weight of seeds on dry weight basis
F.W. = Final weight of seeds on dry weight basis

2.7 Statistical analysis

Weight loss, adult emergence and Change in carbon dioxide gas concentration (%) level were analyzed using one way ANOVA. Significant differences between treatments were determined. Analyses were performed with the original data.

Table 1 Weight loss of sorghum grains by *S. oryzae* when exposed to different modified atmospheres (MAs) combined with different exposure periods

Treatments	Gas combination $N_2:O_2:CO_2$	Number of adults released	Weight loss at different days after exposure													
			`45 days		90 days		135 days`		180 days		225 days		270days		315 days	
			(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
MA_1	**80: 00: 20**	20.0	0.00 (1.00)*	0.00 (0.01)**	0.00 (1.00)*	0.00 (0.01)**	0.00 (1.00)*	0.00 (0.01)**	0.00 (1.00)*	0.00 (0.01)**	0.00 1.00)*	0.00 (0.01)**	0.00 (1.00)*	0.00 (0.01)**	0.00 (1.00)*	0.00 (0.01)**
MA_2	**80: 05: 15**	20.0	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)	0.00 (1.00)	0.00 (0.01)
MA_3	**80: 10: 10**	20.0	20.25 (4.64)	8.4 (16.84)	26.50 (5.24)	10.5 (18.89)	30.5 (5.61)	12.3 (20.52)	29.5 (5.61)	11.7 (19.99)	27.75 (5.30)	11.3 (19.63)	30.50 (5.61)	12.3 (20.52)	30.50 (5.61)	12.00 (20.02)
MA_4	**80: 15 : 05**	20.0	30.00 (5.54)	11.9 (20.17)	34.25 (5.94)	13.8 (21.80)	41.45 (6.50)	16.7 (24.11)	40.05 (6.50)	16.3 (23.80)	40.50 (6.44)	16.2 (23.72)	41.25 (6.50)	16.7 (24.11)	41.48 (6.61)	16.04 (24.12)
UTC	**control**	20.0	34.25 (5.93)	13.3 (21.37)	39.75 (6.38)	15.8 (23.41)	49.0 (7.07)	19.9 (26.48)	65.05 (8.17)	26.3 (30.84)	72.00 (8.59)	28.6 (32.32)	78.25 (8.90)	31.0 (33.82)	82.50 (9.14)	33.2 (35.17)
S. Em±			**0.05**	**0.22**	**0.04**	**0.16**	**0.03**	**0.11**	**0.03**	**0.22**	**0.03**	**0.09**	**0.04**	**0.17**	**0.29**	**0.11**
CD (p=0.01)			**0.23**	**0.93**	**0.16**	**0.67**	**0.13**	**0.47**	**0.12**	**0.73**	**0.15**	**0.41**	**0.20**	**0.71**	**0.12**	**0.49**

** Figures in the parentheses are $\sqrt{x+1}$ transformed values*
*** Figures in the parentheses are arc sine transformed values*

Table 2 Adult emergence of exposed to different modified atmospheres (MAs) combined with different exposure periods: *S. Oryzae*

Treatments	Gas combination $N_2:O_2:CO_2$	Number of adults released	Total adults [Live and dead] (no / 250 g of seeds)						
			45 days	90 days	135 days	180 days	225 days	270 days	315 days
MA_1	**80: 00: 20**	20.0	20.00 (4.58)*	20.00 (4.58)*	20.00 (4.58)*	20.00 (4.58)*	20.00 (4.58)*	20.00 (4.58)*	20.00 (4.58)*
MA_2	**80: 05: 15**	20.0	20.00 (4.58)	20.00 (4.58)	20.00 (4.58)	20.00 (4.58)	20.00 (4.58)	20.00 (4.58)	20.00 (4.58)
MA_3	**80: 10: 10**	20.0	329.75 (18.19)	458.25 (21.43)	339.5 (18.45)	354.00 (18.84)	356.5 (18.91)	356.75 (18.91)	347.25 (18.66)
MA_4	**80: 15 : 05**	20.0	344.5 (18.59)	480 (21.93)	383.25 (19.60)	382.5 (19.58)	348.25 (18.69)	348.5 (18.69)	346.00 (18.63)
UTC	**control**	20.0	388.75 (19.74)	560.75 (23.70)	561.25 (23.25)	567.5 (23.84)	546.25 (23.39)	577 (24.04)	516 (22.74)
S. Em±			**0.10**	**0.08**	**0.11**	**0.08**	**0.07**	**0.12**	**0.07**
CD (p=0.01)			**0.51**	**0.33**	**0.52**	**0.32**	**0.32**	**0.53**	**0.33**

** Figures in the parentheses are $\sqrt{x+1}$ transformed values*
*** Figures in the parentheses are arc sine transformed values*

Table 3 Change in CO_2 and O_2 gas concentration (%) level exposed to different modified atmospheres (MAs) combined with different exposure periods: *S. Oryzae*

Treatments	Gas combination $N_2:O_2:CO_2$	Number of adults released	45 days		90 days		135 days		180 days		225 days		270 days		315 days	
			CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
MA_1	**80: 00: 20**	20.0	20.00 (26.55)*	0.00 (0.01)*	20.00 (26.55)*	0.00 (0.01)*	20.00 (26.55*	0.00 (0.01)*	20.00 (26.55)*	0.00 (0.01)*	20.00 (26.55*	0.00 (0.01)*	20.00 (26.55)*	0.00 (0.01)*	20.00 (26.55*	0.00 (0.01)*
MA_2	**80: 05: 15**	20.0	17.00 (24.34)	3.00 (9.95)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)
MA_3	**80: 10: 10**	20.0	15.68 (23.31)	4.33 (11.99)	16.33 (23.82)	3.68 (11.04)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.57)
MA_4	**80: 15 : 05**	20.0	11.00 (19.47)	9.00 (17.45)	15.23 (22.96)	4.78 (12.61)	17.25 (24.81)	2.75 (9.53)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)	20.00 (26.55)	0.00 (0.01)
UTC	**control**	20.0	0.50 (4.05)	21.88 (27.87)	0.63 (4.47)	21.63 (27.70)	0.63 (4.41)	21.63 (27.70)	0.63 (4.47)	21.50 (27.61)	0.63 (4.47)	21.50 (27.61)	0.63 (4.47)	21.50 (27.61)	0.63 (4.47)	21.50 (27.61)
S. Em±			**0.15**	**0.21**	**0.20**	**0.15**	**0.21**	**0.13**	**0.18**	**0.06**	**0.18**	**0.06**	**0.18**	**0.06**	**0.18**	**0.06**
CD(p=0.01)			**0.63**	**0.84**	**0.84**	**0.66**	**0.89**	**0.56**	**0.78**	**0.26**	**0.78**	**0.26**	**0.78**	**0.26**	**0.78**	**0.26**

** Figures in the parentheses are $\sqrt{x+1}$ transformed value*

RESULTS

3.1 Weight loss (g)

There was no weight loss in 20(%) and 15(%) CO_2 treatment due to the death of all insects affected by CO_2 concentration at different days of exposure. Weight losses recorded during different experimental periods were 20.25, 26.50, 30.5, 29.5, 27.75, 30.50 and 30.50 g for 10(%) CO_2 at 45, 90, 135, 180, 225, 270 and 315 days respectively. In 5(%) CO_2 30.00, 34.25, 41.45, 40.05, 40.50, 41.25 and 41.48 g at 45, 90, 135, 180, 225, 270 and 315 days respectively. Highest weight loss was noticed in untreated control (T_5) 34.25, 39.75, 49.0, 65.05, 72.00, 78.25 and 82.50 g at 45, 90, 135, 180, 225, 270 and 315 days respectively. Among all the treatments weight loss of sorghum increased with decreasing CO_2 concentration where as weight loss was decreased after 180 days after exposure because of increased carbon dioxide concentration as compared to the weight loss in untreated control (Table 1).

3.2 Weight loss (%)

Similar to above observation the per cent weight loss of seeds followed similar trend a weight loss increased with increased level of CO_2 (Table 1).

3.3 Adult emergence

Adult emergence in treatments *viz*., 20 and 15(%) CO_2 was nil throughout the period of observation followed by the treatment 10 (%) CO_2 (329.75, 458.25, 339.5, 354.00, 356.5, 356.75 and 347.25 adults were noticed at 45, 90 135, 180, 225, 270 and 315 days respectively) and 5 (%) CO_2 (344.5, 480.00, 383.25, 382.5, 348.25 and 346.00 adults were noticed at 45, 90 135, 180, 225, 270 and 315 days respectively). However maximum adult population was observed in untreated control with (388.75, 560.75, 561.25, 567.5, 546.25, 577, and 516 adults at 45, 90 135, 180, 225, 270 and 315 days respectively) (Table 2).

3.4 Change in carbon dioxide gas concentration (%)

Observation on change in gas level within polyethylene cover was recorded by check mate gas analyser. Prior to opening of polyethylene cover. There was no change in CO_2 concentration in 20 (%) remained same throughout the period while in 15 (%) CO_2 after 45 days it was increased from15 to 17.00 per cent at 90 days after exposure CO_2 level increases to 20 per cent in all exposure periods. 10 (%) CO_2 concentration at 45 days increased to 15.68 and 16.33 at 90 days, at 135 days after exposure CO_2 increases to 20 per cent. In 5 (%) CO_2 at 45 days CO_2 increased to 11.00, 15.23-90 days, 17.25 (%)-135 days, at 180 days after exposure CO_2 reaches to 20 per cent in all exposure periods. In case of untreated control treatment CO_2 concentration was 0.63 per cent in all exposure period (Table 3).

3.4 Change in oxygen gas concentration (%)

Similar to CO_2 observation O_2 concentration was checked prior to the opening of polyethylene bags.

There was no change in O_2 concentration in 20 (%) it remains same throughout the period of observation where as in 15 (%) CO_2 at 45 days O_2 was decreased from 5 to 3.00 per cent, at 90 days after exposure O_2 level decreased to zero per cent in all exposure periods. 10 (%) CO_2 at 45 days O_2 decreased to 4.33and 3.68 at 90 days, at 135 days after exposure O_2 decreased to zero per cent. In 5(%) CO_2 at 45 days O_2 decreased to 9.00, 4.78(%)-90 days, 2.75(%)-135 days, at 180 days after exposure O_2 decreased to zero per cent in all exposure periods. In case of untreated control O_2 concentration was 21.55 per cent in all exposure period (Table 3).

DISCUSSION

Several researchers have applied modified or controlled atmospheres as a means of controlling the stored product pests [19]. Applied MAs where cent per cent mortality was achieved within a week; even in quite moderate conditions of temperature (29 to 37^o C) with low O_2 percentage (5 to 8 %). At lower O_2 and higher CO_2 concentrations metabolism level of insects become too low, combined with accumulation of toxic end products, which is a cause for stress in the insects eventually leading to death [13 and 23]. Modified atmospheres are used to protect commodities throughout their storage life using low oxygen levels [12] to prevent population growth.

The present study applied MAs containing N_2:O_2:CO_2 of 80:00:20(MA_1), 80:05:15(MA_2), 80:10:10(MA_3), 80:15:05(MA_4) and untreated control against adults of *S. Oryzae.* [12] Applied N_2 based Modified Atmosphere storage with elevated CO_2 (10–20 %) at 20^o and 25°C with 75 per cent and 85 per cent RH at each temperature. When CO_2 was increased to 10 per cent or 20 per cent, reducing O_2 to five per cent it eliminated emergence of *Sitophilus granarius* (L.) at 20°C, but few individuals emerged at 25˚C. Decreased respiration can be used as a measurement of reduced metabolism. Respiration decreased by 50 per cent of the normal rate in larval *Phormia regina* (Meigen) in two per cent O_2 and in *Calliphora vomitoria* (Linnaeus) larvae at one per cent O2. In

Rhyzopertha dominica immature stages and eggs, respiration rate decreased proportional to the amount of O_2 in the atmosphere as reported by [15].

These results are also comparable with the results of [28] who indicated that 54 per cent CO_2 for seven days was required for the control of *Cryptolestes ferrugineus* adults at 20^o C, 29 per cent CO_2 for 14 days at 20 to 25 ^{o}C [27]. [2] recommended that between 20 and 29^o C, control of *S. granarius* with 20 per cent CO_2 in air needed at least 22 days, while 40 per cent CO_2 in air required 13 days to kill *C. ferrugineus* adults and 14 days to kill adult of *T. castaneum* are also in agreement with the results of [10] who reported that results of progeny indicated that from the fifth day the number of emerging insects were low at 20, 60 and 80 per cent CO_2. Complete inhibition of the insects was achieved with 30 days of exposure in CO_2 atmospheres. The present study confirmed that high CO_2 concentration with low level O_2 gives cent percent mortality of adults *S. oryzae* with no weight loss, adult emergence and not affected on the germination of sorghum after 45 days of exposure.

The results on germination corroboration with the results of [7] where the storage of wheat seeds in CO_2 rich atmosphere irrespective of concentrations and periods, showed no adverse effect on germinability, vigour and no change in dehydrogenase enzyme activity as well as molondialdehyde contents. Paddy seeds can be stored safely at least up to 12 months without reduction in seed viability under modified atmospheric storage up to 80 per cent CO_2 as reported by [8].

In conclusion, In the long term storage studies four tested MAs containing 20%, 15%, 10% and 5% CO_2 varied in their lethal effects against adults of the *S. oryzae*. No adult emergence was seen up to 315 days after exposure at 20 and 15 per cent CO_2 concentrations. At lower O_2 and higher CO_2 concentrations metabolism level of insects become too low, combined with accumulation of toxic end products, which may be cause of stress for the insects eventually leading to death. These findings give a hint that the seeds can be stored at 20 per cent for a period of 315 days without any impact on germination as well as weight loss on sorghum seeds.

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