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Effect of modified atmosphere with elevated levels of CO₂ on *Sitophilus oryzae* (L.) in stored maize

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Abstract

The present study was aimed to investigate the effect of modified atmosphere with elevated levels of CO_2 against rice weevil, *Sitophilus oryzae* in maize. Seeds were artificially infested with *S. oryzae* and exposed to different concentrations of CO_2 (20, 40, 60 and 80 percent) and packed in air tight containers. Eighty percent CO_2 Concentration checked the seed damage (0.67 percent) and weight loss (0.37 percent) below permissible limits (one percent) by restricting the adult emergence (4.33) of rice weevil upto six months of storage followed by sixty percent CO_2 concentration. Storage of maize seeds in the CO_2 rich atmosphere (60 and 80 percent) also maintained seed quality (viability of seed) without any detrimental effect on germination (maintained above 90 percent), seedling vigour and moisture content (12 percent) upto six months of storage.

Keywords: Maize seeds, modified atmosphere storage, carbon dioxide, rice weevil, quality of seeds

1. Introduction

Maize, *Zea mays* (L.) is one of most versatile emerging crop having wider adoptability under varied agro climatic conditions. Globally, maize is known as queen of cereals as it has the highest genetic yield potential. It is cultivated on nearly 185 m ha in about 160 countries having wide diversity of soil, climate, biodiversity and management practices thus contributing to 36 percent (782 Mt) in the global grain production. In India, maize is the third most important food crop after rice and wheat, contributing to nearly nine percent in the national food basket. 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.

Maize is attacked by a number of insect pests in different parts of the world during storage. The most important insect pests that cause damage to maize are lepidopterous stalk borers and coleopterous insects in the field and storage, respectively^[1]. The coleopterous insects attacking maize seeds during storage are rice weevil, *Sitophilus spp* (Linnaeus); lesser grain borer, *Rhizopertha dominica* (Fabricius) and red flour beetle, *Tribolium castaneum* (Herbest). Among these coleopterous insect pests, *Sitophilus spp* (Linn) is the most destructive insect pest and gained economic importance of the stored raw cereal grains in the world ^[2]. Among *Sitophilus spp*, the *Sitophilus oryzae* has been reported as an important insect pest of storage causing considerable loss ^[3, 4].

The growing concerns about pesticide residues has resulted in research on modified atmosphere with high levels of carbon dioxide thus leading to identification of an alternative for chemical fumigants like methyl bromide and phosphine. The use of this technique has been in practice in developed countries as early as in 1975. Earlier, Bailey and Banks ^[5] reviewed the effect of CO_2 atmosphere on stored product insects. Modified atmosphere ^[6] is a method to eliminate insects from stored commodities without polluting the atmosphere and is considered as the safer traditional fumigants. The use of CO_2 has several advantages, There is no accumulation of toxic residues after the CO_2 treatment in the final product and is considered as the safest traditional fumigant. Although, research has been carried out on modified atmosphere with CO_2 on various storage pests including beetle pests and moths ^[7-9], the information regarding its role in controlling *S. oryzae* in maize seeds is very meagre. Hence, the present study was undertaken to evaluate the efficacy of various elevated levels of CO_2 in controlling rice weevil in stored maize at different storage intervals and seed quality attributes.

2. Materials and Methods

The present study was carried out at the Seed Entomology Lab, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India during 2017-18.

2.1 Effect of modified atmosphere with elevated CO₂ as a seed protectant against *S. oryzae* and seed quality of maize during storage

To study the effect of modified atmosphere with elevated CO₂, forty five air tight containers were filled with 500 g of disinfected maize seed and ten pairs of freshly emerged S. oryzae adults were released into the containers twenty five days prior to treatment with CO₂ to ensure a uniform level of infestation. Then CO₂ was released at four concentrations viz., 20, 40, 60 and 80 percent with three replications of each treatment. The required concentration of CO₂ was released into the container with a pressure of 2 kg cm⁻² from CO_2 cylinder. Before releasing the CO₂ 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 CO2 was released into the airtight containers through the inlet located at the bottom of the containers by injecting the needle of CO₂ cylinder. After releasing the CO₂, the concentration of CO2 was checked by using CO2/O2 analyzer (PBI Dansensor, PBI 2006, Denmark). For determination of CO₂, the analyzer was calibrated with atmospheric air (20.9% and 0.03% CO₂), then the needle of the analyser 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 will be displayed on screen within 10 seconds which helps in determining the concentration of CO₂ present in the containers then inlet and outlet tubes were closed at one stroke using rubber corks to prevent escape of CO₂ 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 seed to CO_2 .

The air tight containers containing the disinfested seed exposed to different concentrations of CO_2 were observed at bimonthly intervals upto six months of storage. After completion of each exposure period, seal of the container was opened and observations on adult emergence, seed damage percent, weight loss percent, germination percent, seedling vigour and moisture content were recorded.

Adult emergence was recorded by counting the number of live and dead insects emerged out from 500 g sample of each replication of the treatment.

The seed damage percent was calculated by taking a random sample of 400 seeds and counting the number of seeds with bored holes caused by *S. oryzae* and converted to percentage. Number of damaged seeds.

The weight loss percentage was recorded by count and weight method using the formula.

$$\mathbf{W} (\%) = \frac{(Wu X Nd) - (Wd X Nu)}{Wu X (Nd+Nu)} X 100$$

W = Weight loss (%) Wu = Weight of undamaged seeds Nd = No. of damaged seeds Wd = Weight of damaged seeds

Nu = Number of undamaged seeds

Seed germination was measured using standard paper towel method as per the ISTA rules ^[10]. Germinated seeds were counted on the seventh day and ten germinated seedlings were selected from each replication of the treatment for calculating the seedling vigour index. The shoot and root length of each of the ten seedlings were measured in centimeters and total length of the seedling was calculated. Seedling vigour index was calculated by multiplying germination percentage with seedling length as suggested by Abdul Baki and Anderson ^[11].

Seedling vigour index (SVI) = Seed germination percentage x Seedling length (cm)

The Moisture content of the seed in each replication of every treatment at bimonthly intervals was recorded by using Dickyjohn moisture meter.

2.2 Statistical analysis

The data was subjected to square root and angular transformations values wherever necessary and analysed by adopting factorial completely randomized design (FCRD) as suggested by Panse and Sukhatme ^[12].

3. Results and Discussion

3.1 Effect of modified atmosphere (MA) with elevated CO₂ as a seed protectant against *S. oryzae*

3.1.1 Adult emergence:

As observed from the results (Table 1 and Fig. 1), it was evident that all concentrations above 20 percent CO₂ were lethal to the test insect and resulted in complete mortality and did not record any adult emergence under artificial infestation during two months of study where as in control which was subjected to artificial infestation, number of adults emerged were 7.33 after two months and it was further increased to 11.33 and 28.33 adults after four and six months of storage, respectively. Among concentrations, the lowest concentration of 20 percent CO₂ was least effective and recorded 0.00, 7.67, 14.33 mean adults after two, four and six months of storage, respectively. All the CO₂ concentrations protected the seed from infestation by S. oryzae upto two months of treatment but after four and six months of storage, adult emergence was observed. Seeds exposed to 80 percent CO₂ are found to be effective with the lowest adult emergence even after four (2.33) and six (4.33) months of storage, followed by seeds exposed to 60 percent CO_2 concentration at four (5.00) and six (8.67) months of storage, respectively.

The interaction effect between the treatments and exposure period with respect to adult emergence showed that no adult emergence (zero) was recorded in all the CO_2 treatments at two months of treatment imposition.

3.1.2 Seed damage percentage caused by the infestation of *S. oryzae*:

From the data (Table 1 and Fig. 2), it is evident that after two months of CO_2 treatment, zero percent seed damage was observed in 20, 40, 60 and 80 percent CO_2 whereas 0.33

percent of seed damage was recorded in untreated control. After four months of the CO_2 treatment, lowest seed damage (0.33 percent) was recorded in 80 percent CO_2 which was found to be significantly superior to remaining treatments, while untreated control recorded the highest seed damage of 10.33 percent. After six months of CO_2 treatment, significantly lowest percent (0.67) seed damage was observed in 80 percent CO_2 as compared to remaining CO_2 concentrations, while highest seed damage percent was seen in untreated control (14.67 percent).

The results from mean percent seed damage showed 20 percent (2.44 percent) and 40 percent CO_2 concentration (2.22 percent) are on par among the treatments. Significantly least seed damage of 0.33 percent was recorded at 80 percent CO_2 . The untreated control recorded the highest mean seed damage of 8.44 percent. Results on exposure period revealed that mean seed damage showed increased infestation with increase in exposure periods. The mean seed damage of 0.07, 3.80 and 5.20 percent were recorded after two, four and six months of treatment, respectively.

From the results, it is evident that CO_2 concentration of all the treatments (20, 40, 60, and 80 percent) was fatal to *Sitophilus oryzae* in the initial stage (upto 2 months) but 20 and 40 percent CO_2 could not protect the seed during prolonged storage of four months and above period. Eight percent CO_2 concentration (0.67 percent) could protect the seed upto six months of storage within permissible limit of seed damage (1 percent).

3.1.3 Weight loss percentage caused by the infestation of *S. oryzae*

The data (Table 1 and Fig. 3) shows that the weight loss upto two months of storage in 20, 40, 60 and 80 percent CO_2 was zero percent, but 1.09 percent of weight loss was found in untreated control. After four months of treatment, the least weight loss percent was observed at 80 percent CO_2 (0.21 percent) which was superior over remaining treatments, while 60 percent CO_2 concentration (0.75 percent) was on par with 40 percent CO_2 concentration (0.86 percent) and these two CO_2 concentrations are significantly superior over 20 percent CO_2 concentration (1.44 percent), whereas highest weight loss (2.19 percent) was found in untreated control.

Least weight loss (0.37 percent) was found in 80 percent CO_2 concentration after six months of storage followed by 60 percent CO_2 concentration (0.82 percent). Forty percent CO_2 concentration (1.51 percent) was on par with 20 percent CO_2 concentration (1.67 percent), but highest weight loss (3.96 percent) was recorded in untreated control.

The results on mean weight loss showed differences among the treatments. The highest mean weight loss percent of 2.41 was recorded in untreated control. While, lowest mean weight loss percent was recorded in 80 percent CO_2 concentration (0.20 percent) and it was significantly superior over remaining treatments.

The interaction effect between the treatments and exposure period with respect to weight loss showed that no weight loss (zero percent) was recorded in all the CO_2 treatments after two months of treatment imposition.

Table 1: Effect of carbon dioxide (CO ₂) treatment on adult emergence of Sitophilus oryzae (L.), seed damage and weight loss of maize during
different months of storage.

CO ₂ Concentrations	Adult emergence				Seed damage (%)				Weight loss (%)			
	2MAT	4MAT	6MAT	Mean	2MAT	4MAT	6MAT	Mean	2MAT	4MAT	6MAT	Mean
20% CO ₂	0.00	7.67	14.33	7.33	0.00	3.33	4.00	2.44	0.00	1.44	1.67	1.15
	(0.71)	(2.86)	(3.85)	(2.47)	(4.05)	(10.50)	(11.54)	(8.70)	(4.05)	(6.90)	(7.42)	(6.36)
40% CO ₂	0.00	5.67	11.33	5.67	0.00	3.00	3.67	2.22	0.00	0.86	1.51	0.79
	(0.71)	(2.48)	(3.44)	(2.21)	(4.05)	(9.97)	(11.02)	(8.35)	(4.05)	(5.53)	(6.95)	(5.51)
60% CO ₂	0.00	5.00	8.67	4.56	0.00	2.00	3.00	1.67	0.00	0.75	0.82	0.52
	(0.71)	(2.35)	(3.03)	(2.03)	(4.05)	(8.13)	(9.88)	(7.36)	(4.05)	(4.88)	(4.05)	(4.33)
80% CO ₂	0.00	2.33	4.33	2.22	0.00	0.33	0.67	0.33	0.00	0.21	0.37	0.20
	(0.71)	(1.68)	(2.20)	(1.52)	(4.05)	(4.61)	(5.18)	(4.62)	(4.05)	(4.05)	(4.05)	(4.05)
Control	7.33	11.33	28.33	15.67	0.33	10.33	14.67	8.44	1.09	2.19	3.96	2.41
Collitor	(2.80)	(3.44)	(5.37)	(3.87)	(4.62)	(18.75)	(22.51)	(15.29)	(4.50)	(7.28)	(8.67)	(6.82)
Mean	1.47	6.40	13.40		0.07	3.80	5.20		0.22	1.12	1.70	
	(1.13)	(2.56)	(3.58)		(4.17)	(10.39)	(12.03)		(4.14)	(5.73)	(6.37)	
	SE(m)±		CD (P=0.05)		SE(m)±		CD (P=0.05)		SE(m)±		CD (P=0.05)	
Concentrations (F1)	0.03		0.08		0.24		0.70		0.06		0.18	
Months after treatment (F2)	0.02		0.06		0.19		0.54		0.05		0.14	
Interaction (F1*F2)	0.05		0.14		0.42		1.21		0.11		0.32	
CV (%)	3.55				8.18				3.49			

Figures in parentheses are transformed values MAT- Months after treatment

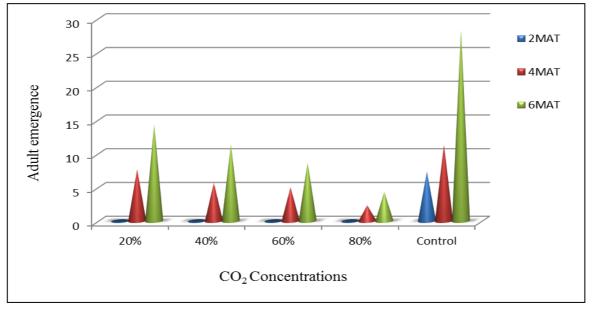


Fig 1: Effect of elevated levels of CO2 on adult emergence of S. oryzae

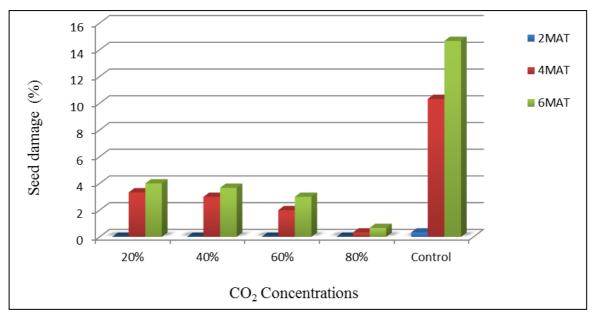


Fig 2: Effect of elevated levels of CO2 on seed damage caused by S. oryzae

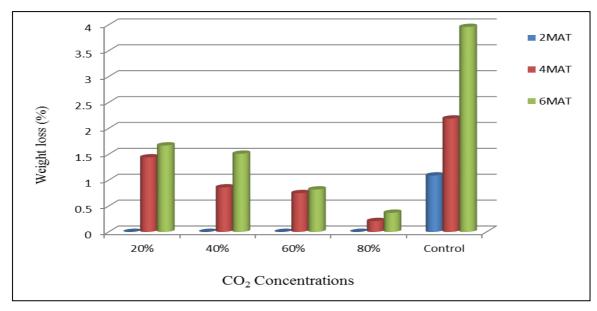


Fig 3: Effect of elevated levels of CO2 on weight loss caused by S. oryzae

3.2 Effect of modified atmosphere (MA) with elevated CO₂ on viability and vigour of seed **3.2.1** Germination

The CO₂ did not show any effect on seed germination but in turn helped in the control of the insects thus indirectly preventing loss of germination. The study revealed that the germination percentage of maize seeds exposed to different concentrations of CO₂ had no significant variation among the concentrations during two months of exposure (Table 2 and Fig. 4). Highest percent germination (98.00) was recorded in 80 percent CO₂ followed by 60 percent CO₂ (97.33) and 40

percent CO_2 (97.33) treatments. Lowest germination of 95.67 percent was recorded in untreated control. Similar trend was observed at four months of storage, among the treatments 80 percent CO_2 showed high germination (96.00 percent), followed by 60 percent CO_2 concentration

(95.33 percent), while germination percentage observed in untreated control was 93.00 percent.

The germination percentage recorded after six months of treatment imposition was found to vary from 81.33 to 95.67 percent in all the CO₂ concentrations as compared to 78.33 percent germination recorded in control. The treatments 20 percent (81.33%) and 40 percent (88.67%) CO₂ concentrations recorded below IMSCS i.e., 90 percent germination, while the treatment 80 percent CO₂ showed highest percent of germination (95.67%) followed by the treatment of 60 percent CO₂ (93.00%).

3.2.2 Seedling vigor index

Seedling vigour index (Table 2 and Fig. 5) showed variation among the treatments during different storage intervals, as CO_2 concentration increased the seedling vigour also increased but decreased as storage duration was increased.

Significantly highest seedling vigour index was recorded at 80 percent (3323) followed by 60 percent (3090) while, the lowest seedling vigour index was recorded in control (2846) at two months after treatment. Similar trend was observed after four months after treatment. Highest seedling vigour

index was recorded at 80 percent (3165), while lowest (2683) was recorded in untreated control. At six months after treatment, highest seedling vigour index was recorded in 80 percent CO_2 concentration (2732) and was on par with 60 percent CO_2 concentration (2682).

Highest seedling vigor index was recorded at 80 percent concentration of CO_2 which showed decreasing trend from two to six months of exposure period (3323 to 2732), while the lowest seedling vigor index was recorded in untreated control which ranged from 2846 to 1886 during two months to six months of seed storage which could be due to increase in storage period and natural ageing process. The mean seedling vigor index of 3049, 2949 and 2422 was recorded at two, four and six months of storage, respectively.

3.2.3 Moisture content

The moisture content (Table 2 and Fig. 6) of maize seeds exposed to different concentrations of CO_2 did not show any significant variation among the treatments upto two months of treatment, while highest moisture content of 11.63 percent was recorded in untreated control which was found to be significantly superior to all other treatments. Four months after treatment imposition, moisture content varied from 11.37 to 11.73 percent in different concentrations of CO_2 , while in control 11.80 percent was recorded. Even after six months of treatment imposition, similar trend was observed where the moisture content of CO_2 exposed maize seeds varied from 11.87 to 12.13 percent as against 12.20 percent moisture content recorded in untreated control.

The mean moisture content (Table 2 and Fig. 6) has showed slight variation among the treatments. The Mean moisture content of 11.47, 11.64 and 12.05 percent was recorded at two, four and six months of storage, respectively. Maximum moisture content of 12.20 percent was recorded after six months of storage in untreated control. Overall, the moisture content of maize seed in the present investigation did not show much variation even after six months of treatment, in spite of being stored under hermetic storage.

Table 2: Effect of carbon dioxide (CO₂) treatment on germination, seed vigour index and moisture content of maize during different months of storage.

CO ₂ Concentrations		Germina	tion (%)			SV	/ I		Moisture content (%)			
	2MAT	4MAT	6MAT	Mean	2MAT	4MAT	6MAT	Mean	2MAT	4MAT	6MAT	Mean
20% CO ₂	96.67	95.00	81.33	91.00	2933	2813	2131	2625	11.40	11.57	12.00	11.66
	(79.60)	(77.12)	(64.40)	(73.70)					(19.73)	(19.88)	(20.27)	(19.96)
40% CO ₂	97.33	95.00	88.67	93.70	3055	2996	2678	2910	11.33	11.37	11.87	11.52
	(80.92)	(77.25)	(70.35)	(76.20)					(19.70)	(19.70)	(20.15)	(19.84)
60% CO ₂	97.33	95.33	93.00	95.20	3090	3088	2682	2953	11.47	11.73	12.03	11.74
	(81.21)	(77.54)	(74.68)	(77.80)					(19.79)	(20.03)	(20.30)	(20.04)
80% CO ₂	98.00	96.00	95.67	96.60	3323	3165	2732	3073	11.50	11.73	12.13	11.79
	(82.05)	(78.52)	(78.00)	(79.50)					(19.82)	(20.03)	(20.38)	(20.08)
Control	95.67	93.00	78.33	89.00	2846	2683	1886	2472	11.63	11.80	12.20	11.88
	(78.00)	(74.68)	(62.26)	(71.60)					(19.94)	(20.09)	(20.44)	(20.16)
Mean	97.00	94.80	87.47		3049	2949	2422		11.47	11.64	12.05	
	(80.36)	(76.92)	(70.05)						(19.79)	(19.95)	(20.31)	
	SE(m)±	CD (P=0.05)		SE(m)±		CD (P=0.05)		SE(m)±		CD (P=0.05)	
Concentrations (F1)	0.68		1.95		24.98		72.14		0.07		0.20	
Months after treatment (F2)	0.52		1.51		19.35		55.88		0.05		0.15	
Interaction (F1*F2)	1.17		3.38		43.26		124.94		0.12		0.34	
CV (%)	2.67				2.67				1.01			

Figures in parentheses are transformed values MAT- Months after treatment

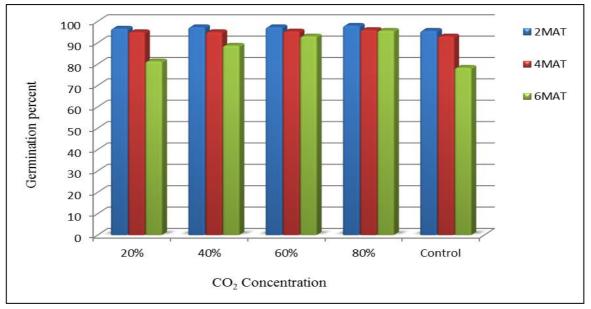


Fig 4: Effect of elevated levels of CO2 on germination percentage of maize seeds

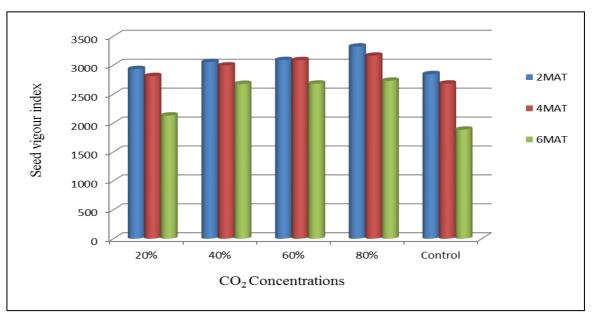


Fig 5: Effect of elevated levels of CO2 on seedling vigour index of maize seeds

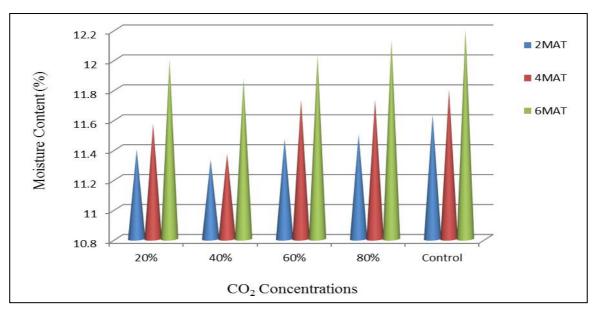


Fig 6: Effect of elevated levels of CO2 on moisture content of maize seeds

The results (Table 1 and 2) indicated that lowest adult emergence was noticed in 80 percent of CO₂ upto six months of maize storage. 20 percent CO₂ concentration protected the seed upto two months, while 3.33 percent seed damage and 1.67 percent weight loss was recorded after four months and six months of treatment, respectively. But 80 percent CO₂ concentration is able to maintain germination (95.67 percent) above IMSCS ^[13] (90 percent) and moisture content (12.13 percent) marginally higher than IMSCS ^[13] (12.00 percent) good seed vigour by restricting the seed damage (0.67 percent) and weight loss (0.37 percent) below permissible limit (1 percent) upto six months of maize storage.

Bera ^[14] reported that complete control of seed damage by the lesser grain borer, R. dominica and rice moth, Corcyra cephalonica was achieved when the seed was subjected to 20 percent CO₂ concentration within two months of storage period. The increase in CO₂ concentration caused decrease in the adult emergence in many insects as reported by Divya^[15]., Jayashree [16]. Yadav and Mahla [17] estimated the grain damage caused by Trogoderma granarium to wheat grains treated with different concentrations of CO₂ and found that the lowest infestation of grains (0.4 and 0.6 percent) was noticed in grains exposed to 90 and 98 percent CO2 concentrations as compared to 4 and 2 percent infestation noticed with 40 and 50 percent CO₂ concentrations, respectively after 45 days of exposure period. Shehata [18] observed lowest infestation by C. maculatus when cowpea seeds were treated with 80 percent CO₂. Sharaf ^[19] also observed that weight loss of faba bean seeds by C. chinensis decreased with the increasing concentrations of CO2 and exposure periods. Shehata ^[18] reported that cowpea seeds treated with gases containing 80 percent CO₂ showed the lowest weight loss by C. maculatus.

From our studies, it can be inferred that the adult emergence, percent seed damage and percent weight loss decreased with the increasing concentrations of CO_2 . Similarly, for prolonged protection of maize seeds from insect damage, 60 and 80 percent concentrations of CO_2 are most preferred, while 20 and 40 percent concentration of CO_2 is sufficient for two or three months' protection.

Low germination percent observed in control in our studies could be due to insect infestation which might have damaged seed embryo. Jayas and Jeyamkondan [20] concluded that modified atmosphere do not cause any detrimental effect on the functional characteristics of seeds and help in maintaining seed germination and viability. Lowest germination recorded at 20 and 40 percent CO₂ concentrations could be due to the infestation of maize seeds by S. oryzae at 6 months after treatment. Rathi ^[21] reported that CO₂ exposed red gram seeds had less insect infestation. less mould attack and retained high germination percent when compared to untreated seeds. Bera ^[14] opined that storage in the CO₂ rich atmosphere irrespective of concentrations and the exposure period showed no adverse effect on germinability of wheat seed. Shehata [18] stated that the germination percent of cowpea seeds stored up to six months under controlled atmosphere was higher than the untreated seeds.

In the present study, seed vigour index of the maize seedlings have shown decreasing pattern from two to six months after treatment because of increased insect infestation and natural ageing. The highest decrease in seed vigour index in 20 percent CO_2 and 40 percent CO_2 concentrations could be attributed to the insect damage noticed at 20 percent CO_2 and 40 percent CO_2 treatments which might have damaged the embryo of the seed, thereby affecting the germination percent and vigour of seedlings. Bera ^[14] reported decrease in vigour with the increasing storage period and attributed decrease in germination after 6 months of treatment to natural ageing process rather than exposure of seeds to CO_2 .

Results on different exposure periods revealed that mean moisture percent goes on increasing with increase in exposure period. Increase in moisture content resulted due to increased insect damage and also adversely affected the germination of maize seed which dropped below IMSCS ^[13] (<90%) in untreated control (78.33%), 20 percent (81.33%) and 40 percent CO₂ (88.67%) concentrations. CO₂ rich atmosphere i.e., 80 percent CO₂ concentration maintained high germination of maize seeds (95.67%) upto six months of storage, followed by 60 percent CO₂ concentration (93.00%). The present investigations are in conformity with the findings of Bera ^[22] reported that under modified atmosphere (upto 80 percent CO₂) paddy seed with 11 percent moisture content can be stored safely atleast upto 12 months without much reduction in seed viability.

Modified atmosphere with higher concentrations of CO_2 was effective in preventing the adult emergence of *S. oryzae* and subsequent seed damage and weight loss without affecting seed germination, seedling vigour and moisture content upto six months of storage. Finally it is evident that the modified atmosphere with higher CO_2 content (>60%) was found to be ideal and eco-friendly approach for the management of *S. oryzae* in stored maize instead of conventional harmful fumigants and poisonous insecticidal sprays. Hence, these findings are important for the recommendation of safe, residue free and long-term storage of maize without any qualitative and quantitative losses.

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