International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

IJBSM November 2022, 13(11):1176-1185 Print ISSN 0976-3988 Online ISSN 0976-4038 Article AR2835

Research Article Stress Management

DOI: HTTPS://DOI.ORG/10.23910/1.2022.2835

Management of Pulse Beetle in Green Gram Seed Using Modified Atmosphere

K. Kanaka Durga[1], T. Sowmya[2], Kamala Venkateshwaran[3], K. Keshavulu[4] and Vidyasagar[5]

[1]Dept. of Genetics and Plant Breeding, Agricultural College, Sircilla, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana (500 030), India

[2]Dept. of Seed Science and Technology, Telangana State Seed and Organic Certification Authority, Warangal, Telangana (506 001), India

[3]Dept. of Economic Botany, ICAR –National Bureau of Plant Genetic Resources, Rajendranagar, Hyderabad, Telengana (500 030), India

[4]Dept. of Seed Science and Technology, Telangana State Seed and Organic Certification Authority, HACA Bhavan, Hyderabad, Telangana (500 082), India

[5]Dept. of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad, Telangana (500 030), India

Corresponding kanakakilaru@gmail.com

0000-0002-6506-0026

ABSTRACT

The experiment was conducted at Seed Research and Technology Center, PJTSAU, Rajendranagar, Hyderabad, Telangana State, India during June, 2019 under laboratory conditions using modified atmosphere with elevated levels of CO_2 in two varieties of green gram namely LGG-460 and LGG-407. The study indicated that biological parameters of the test insect and the seed quality parameters were highly influenced by different levels of CO_2 and storage intervals and varied between the two accessions of green gram. Emergence of adults decreased with increased concentrations of CO_2. Among the two accessions, LGG-460 had least adult emergence with comparatively less insect damage than LGG-407. Higher concentrations of CO_2 (45%) significantly reduced the per cent insect infestation and weight loss of both the accessions. The efficacy of different concentrations of CO_2 on seed infestation and population build up revealed that exposing the bruchid infested green gram seed to 45% and higher concentrations of CO_2 not only checked seed infestation but also checked the progeny production of the pest even after prolonged periods of storage up to 2 MAS. The green gram seeds stored in CO_2 rich atmosphere also maintained seed quality with high germination percentage and seedling vigor up to 2 MAS. Two months storage period was effective for control of insect infestation and weight loss. Among the 2 accessions, seedling vigour I and II were found to be high in LGG 460 even at 6 months storage interval.

KEYWORDS: CO_2 levels, Green gram, modified atmosphere, pulse beetle

***Citation* (VANCOUVER):** Durga et al., Management of Pulse Beetle in Green Gram Seed Using Modified Atmosphere. *International Journal of Bio-resource and Stress Management,* 2022; 13(11), 1176-1185. HTTPS://DOI.ORG/10.23910/1.2022.2835.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 18th January 2022 RECEIVED in revised form on 12th October 2022 ACCEPTED in final form on 30th October 2022 PUBLISHED on 18th November 2022

1. INTRODUCTION

Stored grain pests cause huge weight loss to the seed and the intensity of damage depends upon temperature, humidity and moisture content of the seed (Marks and Stroshine, 1995) at the time of storage period. Losses of 5-10%, and up to 40% in developing countries, caused by insects in stored products have been reported World wide (Shaaya et al., 1997, Weaver and Petroff, 2005). The losses caused by storage pests are more in case of pulses compared to cereals. In green gram, pulse beetle *Callasobruchus chinensis* L. is the most destructive stored grain pest (Singh and Jambunadham, 1990). Gujar and Yadav (1978) reported losses of about 55–60% in seed weight and 45.5–66.3% in protein content in green gram due to bruchids and the infested seeds become unfit for human consumption.

Control of insects in stored grain relies largely on fumigation or synthetic insecticides which provide adequate control against the *C. maculatus* on pulses. Yesteryear chemicals/ insecticides used to control stored insects leave objectionable residues in treated commodity and generally are hazardous to handle and apply (Bekele et al., 1996). Indiscriminate use of insecticides has led to the development of insecticide resistant strains of stored product insects as well as insecticide residue problems in food grains (Anonymous, 1996). Cancellation of registration of almost all fumigants including methyl bromide and aluminium phosphide in many developed countries due to carcinogenic effect on human beings has resulted in increased reliance on alternative eco-friendly pest management strategies such as modified atmosphere storage, use of botanical treatments, inert dusts and novel insecticide molecules that have relatively low mammalian toxicity (Kalpna et al., 2022).

Modified atmosphere provides a way to eliminate insects from stored commodities without polluting the atmosphere and are safer than traditional fumigants (Banks and Annis, 1990, Cheng et al., 2012, Mehmood et al., 2018, Cao et al., 2019). CO_2 is now being used in several countries for the treatment of stored products, particularly grain in bulk, to control insect pests (Aliniazee and Lindgren, 1970, Wang et al., 2009). Modified atmosphere with elevated CO_2 levels is gaining popularity on a large scale in the recent past due to easy accessibility and availability, relative convenience and safety in application (Bera et al., 2008) and non-residual toxicity. Farmers and warehouse managers are interested in using hermetic storage for pest control in stored products (Njoroge et al., 2016, 2017) as modified atmosphere provide a highly effective non-chemical control measure for stored-product pests (Rasool et al., 2017). Several studies have been conducted to study the period for insect mortality under controlled atmospheres with elevated CO_2 (Soderstrom et al., 1990, Ofuya and Reichmuth, 2002, Gunasekaran and Rajendran, 2005).

Though studies on CO_2 rich modified atmosphere on different storage pests like *Caryedon serratus*, *Callosobruchus maculatus*, *Sitotroga cerealella* and other storage pests (Radhika et al., 2014, Divya et al. 2016) and beetle pests and moths (Riudavets et al., 2009) were conducted, the research data on specific period of CO_2 exposure with different concentrations of CO_2 to kill the adult pulse beetles under airtight conditions, their survival rate, fecundity and progeny production was scanty. Therefore, keeping in view the advantages of modified atmosphere as a storage technique, the present study was initiated in green gram for management of pulse beetle during storage.

2. MATERIALS AND METHODS

The experiment was conducted at Seed Research and Technology Center, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana state, India during June, 2019 under laboratory conditions using modified atmosphere with elevated levels of CO_2 in two varieties of green gram namely LGG-460 and LGG-407. Air tight plastic containers of five hundred grams capacity consisting of an inlet, an outlet and an air tight lid were filled with 250 g of disinfested green gram seeds and ten pairs of *C. chinensis* adults were released into the container 25 days prior to treatment with CO_2 to ensure uniform level of infestation. After 25 days, the weight of the green gram seeds was taken and CO_2 was released at four different concentrations viz., 15%, 30%, 45% and 60% with three replications of each treatment.

The seed was observed for adult emergence (Number), per cent insect damage, per cent weight loss, seed moisture content, per cent germination (Anonymous, 1999), seedling characters and seedling vigour indices I and II (Abdul-Baki and Anderson, 1973) at 2, 4, and 6 months after treatment, by using destructive sampling method. Untreated control was also maintained under normal conditions without exposing the seed to CO_2 concentration. For recording data on mean adult emergence, F_1 progeny emerged from each treatment at 60 days after release (DAR) were counted and adult beetles were discarded daily and the process was continued till they completely cease to emerge. Per cent insect damage and per cent weight loss were calculated using the following formulae:

Per cent insect infestation×(Number of seeds with bored holes at 60 DAR/ Total number of seeds observed)×100

Per cent weight loss =((Initial weight of the sample − final weight of the sample at 60 DAR)/Initial weight of the sample)×100

3. RESULTS AND DISCUSSION

3.1. Effect of CO_2 on biological parameters of the insects at different storage intervals

3.1.1. Adult emergence

Biological parameters of the test insect and the seed quality parameters were highly influenced by different levels of CO_2 and storage intervals and varied among the two accessions of green gram (LGG-407 and LGG-460) (Table 1).

The adult emergence was reported in 2 kinds i.e., dead insects and live insects. After 2 months of storage interval, the population of dead and live insects was observed to be more in the accession of LGG-407 (98.3) than LGG-460 (93.0). The population was decreased with increasing CO_2 concentration. In LGG-407, the dead and live insects were noticed as (131.0, 7.7), (109, 4.0), (83.0, 4.0), (21.0, 7.7) and (147.3, 14.3) at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively. In case of LGG-460, the emergence of dead and live insects was noticed as (111.0, 6.7), (96.0, 1.3), (86.0, 2.3), (39.0, 0.0) and (132.0, 18.0) at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively. After 4 months of storage, the population of dead and live insects was recorded as (217.6, 14.6), (136.0, 9.3), (116.6, 10.6), (112.0, 10.3) and (221.3, 42.6) in LGG-407 and in LGG-460, it was noticed as (156.6, 8.3), (103.0, 7.6), (88.0, 8.3), (69.3, 2.3) and (225.6, 26.6) at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively. After 6 months of storage, the number of adults were found to be high than the previous storage levels. The dead and live beetles observed in LGG-407 was (252.0, 14.3), (138.6, 11.6), (143.6, 20.0), (133.0, 15.3) and (453.0, 51.6) and in case of LGG-460 it was found as (322.6, 28.6), (273.3, 13.0), (176.6, 10.0), (114.6, 9.3) and (454.0, 45.6) at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively.

The overall present findings on the efficacy of different concentrations of CO_2 on seed damage and population build up revealed that exposing the bruchid infested green gram seed to 45% and 60% CO_2 not only checked seed damage but also checked the progeny production as compared to 15% and 30% CO_2 levels. From the above results, it was observed that the emergence of adults was decreased with increased concentrations of CO_2 and increased with increasing the storage period. Among the two accessions, LGG-460 had least adult emergence than LGG-407 and this could be attributed to inherent resistance mechanism of that particular variety against pulse beetle.

Table 1: Effect of modified atmosphere with elevated levels of CO_2 on adult emergence in two green gram accessions at different storage intervals

Treatments	Adult emergence (Number)											
	2 Months						4 Months					
	Dead			Live			Dead			Live		
	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean
15% CO_2	131.0 (11.4)	111.0 (10.5)	121.0 (11.0)	7.7 (2.8)	6.7 (2.6)	7.2 (2.7)	217.6 (14.7)	156.6 (12.5)	187.1 (13.7)	14.6 (3.8)	8.3 (2.9)	11.5 (3.4)
30% CO_2	109.0 (10.4)	96.0 (9.8)	102.5 (10.1)	4.0 (2.1)	1.3 (1.2)	2.7 (1.6)	136.0 (11.7)	103.0 (10.2)	120.0 (10.9)	9.3 (3.1)	7.6 (2.8)	8.5 (3.0)
45% CO_2	83.0 (9.2)	86.0 (9.3)	84.5 (9.2)	4.0 (2.1)	2.3 (1.5)	3.2 (1.8)	116.6 (10.8)	88.0 (9.4)	101.2 (10.1)	10.6 (3.3)	8.3 (2.9)	9.5 (3.1)
60% CO_2	21.0 (4.6)	39.0 (6.03)	30 (5.3)	7.7 (2.8)	0.0 (0.7)	4.0 (1.8)	112.0 (10.6)	69.3 (8.3)	91.0 (9.5)	10.3 (3.3)	2.3 (1.6)	6.3 (2.4)
Control	147.3 (12.2)	132.0 (11.5)	139.7 (11.8)	14.3 (3.8)	18.0 (4.3)	16.2 (4.0)	221.3 (14.9)	225.6 (15.0)	223.5 (15.0)	42.6 (6.6)	26.6 (5.2)	35.0 (5.9)
General mean	98.3 (9.6)	93.0 (9.4)	95.6 (9.5)	7.6 (2.7)	5.7 (2.1)	6.7 (2.4)	161.0 (12.6)	129.0 (11.1)	145.0 (11.8)	17.5 (4.02)	10.7 (3.1)	14.2 (3.6)
	SEm±	SEd	CD*	SEm±	SEd	CD*	SEm±	SEd	CD*	SEm±	SEd	CD*
Variety	0.2	0.2	0.5	0.1	0.2	0.4	0.09	0.1	0.3	0.1	0.2	0.4
Conc.	0.3	0.4	0.8	0.2	0.3	0.7	0.1	0.2	0.4	0.2	0.3	0.6
Interaction	0.4	0.6	1.2	0.3	0.5	1.0	0.2	0.3	0.6	0.3	0.4	0.9
*p=0.05			CV			CV			CV			CV
			7.2			23.8			3.0			15.1

Table 1: Continue...

Treatments	Adult emergence (Number)					
	6 Months					
	Dead			Live		
	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean
15% CO_2	252.0 (15.9)	322.6 (18.0)	287.3 (16.9)	14.3 (3.8)	28.6 (5.4)	21.5 (4.6)
30% CO_2	138.6 (11.7)	273.3 (16.5)	206.0 (14.1)	11.6 (3.4)	13.0 (3.7)	12.3 (3.5)
45% CO_2	143.6 (12.0)	176.6 (13.3)	160.2 (12.7)	20.0 (4.5)	10.0 (3.2)	15.0 (3.9)
60% CO_2	133.0 (11.6)	114.6 (10.7)	123.8 (11.1)	15.3 (3.9)	9.3 (3.1)	12.3 (3.5)
Control	453.0 (21.3)	454.0 (21.3)	453.5 (21.3)	51.6 (7.2)	45.6 (6.8)	48.6 (7.0)
General mean	224.0 (14.5)	268.2 (16.0)	246.2 (15.2)	22.6 (4.6)	21.3 (4.4)	21.9 (4.5)
	SEm±	SEd	CD*	SEm±	SEd	CD*
Variety	0.1	0.2	0.4	0.1	0.2	0.4
Conc.	0.2	0.3	0.8	0.2	0.3	0.7
Interaction	0.2	0.5	1.1	0.3	0.4	0.9
*p=0.05			CV			CV
			4.2			12.2

At higher CO_2 and lower O_2 concentrations metabolic activity of insects become too low which when combined with accumulation of toxic end products leads to death of the insects (Donahaye and Navarro, 2000). Bera et al. (2007) stated that CO_2 treatment ranging from 20% - 80% were equally effective in controlling insect population build-up of rice weevil and lesser grain borer even after 12 months of storage in rice. Caril et al. (2010) reported that the number of emerging insects was low at 20%, 60% and 80% CO_2 from the 5th day and complete inhibition was achieved within 30 days of exposure in CO_2 atmosphere. Decrease in adult emergence in many insects with increase in CO_2 concentration was reported in horsegram and sorghum (Divya et al., 2016).

3.1.2. Insect damage (%)

Insect damage followed descending trend from the lower concentrations to higher concentrations of CO_2 viz., 15%, 30%, 45% and 60% even after 6 months of treatment on both accessions of green gram i.e., LGG-407 and LGG-460 (Table 2). After two months of storage the control (without CO_2 exposure into the seeds) exhibited more insect infestation and was noticed to be 6.88% and 7.39% in LGG-407 and LGG-460, respectively compared to treated seeds. In LGG-407 the insect damage was recorded as 5.49%, 4.87%, 3.22% and 2.59% in 15%, 30%, 45% and 60% levels of CO_2, respectively. While, in case of LGG-460, the damage was recorded as 4.33%, 2.87%, 2.63% and 1.43% in 15%, 30%, 45% and 60% levels of CO_2, respectively. After 4 months of storage period increased insect damage was noticed as compared to the earlier storage periods. The per cent insect damage was found to be 6.74%, 4.37%, 3.80%, 2.12% and 12.33% in LGG-407 and in LGG-460 it was 3.36%, 3.45%, 2.52%, 2.03% and 14.44% at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively. After 6 months of storage period the damage was highly increased than in previous storage periods. In LGG-407, the damage noticed at 15%, 30%, 45% and 60% levels of CO_2 and control was 11.01%, 7.71%, 5.57%, 2.92% and 15.67%, respectively and in LGG-460 the damage was 13.35%, 8.24%, 6.14%, 3.05% and 20.93%, respectively. From the above results it can be seen that the CO_2 concentration of 45% and above was fatal to *C. chinensis,* while 15% and 30% CO_2 though controlled the pest in the initial stages could not protect the seed during prolonged storage of six months and above period. The effect of CO_2 on pulse beetle was decreased by increasing the duration of storage period. Among the two accessions LGG-460 showed comparatively less insect damage than LGG-407. Rathi et al. (2000) reported that higher % of CO_2 concentration decreased the infestation %. Lowest infestation of wheat grains (0.4 and 0.6%) by *Trogoderma granarium* was noticed in grains exposed to 90% and 98% CO_2 concentration after 45 days of exposure period (Yadav and Mahla, 2002).

3.1.3. Weight loss (%)

After two months of storage the % weight loss recorded in LGG-407 was 1.53%, 1.51%, 0.71%, 0.41% and 2.37% and the accession LGG-460 showed 1.45%, 1.04%, 0.65%, 0.33% and 1.65% at respected levels of CO_2 viz., 15%, 30%, 45% and 60% and control condition. After 4 months of storage, the % weight loss was observed as 4.36%, 2.61%, 1.47%, 1.08% and 5.31% in LGG-407 and LGG-460 had 4.65%, 2.79%, 1.71%, 1.05% and 7.72% at respected

Table 2: Effect of modified atmosphere with elevated levels of CO_2 on insect damage (%) in two green gram accessions at different storage intervals

Treatments	Insect damage (%)**								
	2 Months			4 Months			6 Months		
	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean
15% CO_2	5.49 (13.53)	4.33 (12.0)	4.91 (12.77)	6.74 (15.03)	3.36 (10.55)	5.05 (12.79)	11.01 (19.36)	13.35 (21.42)	12.18 (20.39)
30% CO_2	4.87 (12.74)	2.87 (9.75)	3.87 (11.24)	4.37 (12.04)	3.45 (10.69)	3.90 (11.36)	7.71 (16.10)	8.24 (16.68)	7.97 (16.39)
45% CO_2	3.22 (10.32)	2.63 (9.33)	2.92 (9.83)	3.80 (11.22)	2.52 (9.09)	3.15 (10.16)	5.57 (13.64)	6.14 (14.33)	5.85 (13.99)
60% CO_2	2.59 (9.24)	1.43 (6.79)	2.01 (8.01)	2.12 (8.35)	2.03 (8.13)	2.07 (8.24)	2.92 (9.81)	3.05 (10.04)	2.98 (9.92)
Control	6.88 (15.2)	7.39 (15.7)	7.13 (15.48)	12.33 (20.54)	14.44 (22.33)	13.38 (21.43)	15.67 (23.31)	20.93 (27.22)	18.30 (25.26)
General mean	4.61 (12.2)	3.73 (10.73)	4.17 (11.47)	5.87 (13.43)	5.16 (12.16)	5.51 (12.8)	8.58 (16.44)	10.34 (17.94)	9.45 (17.19)

	SEm±	SEd	CD*	CV	SEm±	SEd	CD	CV	SEm±	SEd	CD	CV
Variety	0.16	0.23	0.49	5.68	0.21	0.29	0.61	6.30	0.18	0.26	0.54	4.15
Conc.	0.26	0.37	0.78		0.33	0.47	0.97		0.29	0.41	0.86	
Interaction	0.37	0.53	1.11		0.41	0.58	1.21		0.466	0.66	1.37	

*p=0.05

** Values in the parentheses are angular transformed values

levels of CO_2 viz., 15%, 30%, 45% and 60% levels of CO_2 and control. After 6 months of storage, the weight loss was observed to be high i.e., 6.53%, 4.03%, 3.23%, 2.96 and 9.47% in LGG-407 and the accession LGG-460 showed 5.87%, 3.68%, 1.83%, 1.25% and 9.97% weight loss at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively (Figure 1). Similar loss in seed weight due to infestation by *Callasobruchus chinensis* with increased concentrations of CO_2 and exposure periods is in conformity with the findings of Raghupathi et al. (2021) and Gujar and Yadav (1978) in green gram.

From the above results, it was noticed that 2 months storage period was effective than 4 and 6 months of storage period for control of the insect infestation and weight loss. Among the different concentrations of CO_2, 45% and 60% levels were highly effective to control weight loss by the infestation of pulse beetle than 15% and 30% levels. These findings are in tune with decreased weight loss with the increasing levels of CO_2 in the atmosphere and exposure periods.

3.2. Effect of CO_2 on seed quality parameters at different storage intervals

3.2.1. Moisture content (%)

Before initiating the experiment, the moisture content was

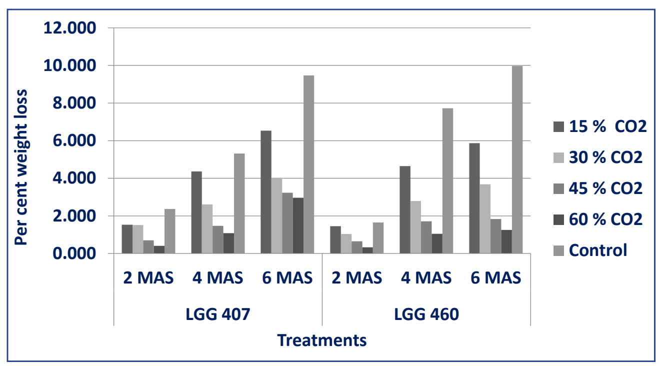

Figure 1: Effect of different concentrations of CO2 on weight loss (%) by C. chinensis in green gram cvs. LGG-460 and LGG-407 at different storage intervals

recorded as 9.1% in LGG-407 and 9.3% in LGG-460. After two months of treatment, highest moisture content was recorded in control conditions (14.70%) followed by 13.30%, 12.20%, 12.10% and 12.20% at 15%, 30%, 45% and 60% levels of CO_2, respectively in LGG-407 and in LGG-460 it was recorded as 8.70%, 8.40%, 8.50%, 7.50% and 9.40% at 15%, 30%, 45% and 60% levels of CO_2 and control, respectively (Figure 2). After 4 months of treatment, the moisture content was slightly increased in

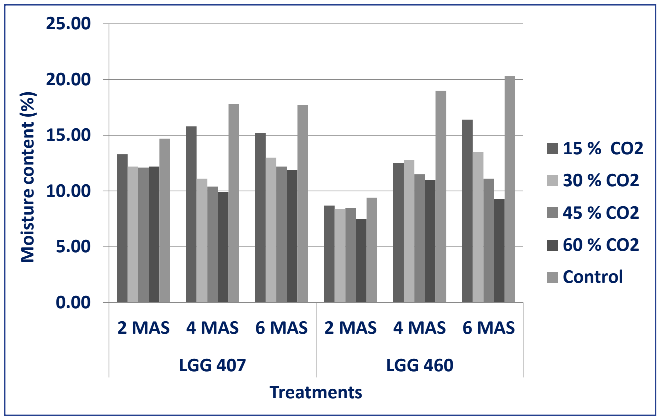

Figure 2: Effect of different concentrations of CO_2 on moisture content (%) in green gram cvs. LGG-460 and LGG-407 at different storage intervals

both the accessions. In case of LGG-407 it was found to be 15.80%, 11.10%, 10.40%, 9.90% and 17.80% and in LGG-460 the moisture content was noticed as 12.50%, 12.80%, 11.50%, 11.00% and 19.00% in the respected levels of 15%, 30%, 45% and 60% of CO_2 and control. After 6 months of treatment, the moisture content was slightly increased than the previous storage period. The observed results were 15.20%, 13.00%, 12.20%, 11.90% and 17.70% in LGG-407 and 16.40%, 13.50%, 11.10%, 9.30% and 20.30% in LGG-460 noticed in the respected levels of 15%, 30%, 45% and 60% of CO_2 and control condition.

From the data, it was evident that the moisture content was directly related to concentrations of CO_2 and storage period. The moisture content was gradually increased with increase in the storage interval. The moisture levels remained below the maximum recommended moisture contents of 12% and 13% for safe storage of mungbean and pigeon pea, respectively (Odogola, 1994). Rathi et al. (2000) reported 50% drop in germination percentage in control showing beneficial effect of modified atmosphere storage on soybean. Bera et al. (2008) reported that paddy seed with 11% moisture content can be stored safely up to 12 months under 80% CO_2 concentration.

3.2.2. Germination (%)

The per cent germination was slightly differed from one concentration to other concentration (Figure 3). The highest germination percentage was observed at two months of storage treatment i.e., 85.0%, 92.2%, 93.3%, 90.7% and 80.5% in LGG-407 and 93.0%, 94.2%, 97.5%, 97.5% and 94.3% in LGG-460 at 15%, 30%, 45% and 60% levels of concentrations of CO_2 and control, respectively and least germination percentage was observed at 6 months storage period i.e., 64.3%, 88.0%, 85.7%, 90.7% and 1.7% was reported in LGG-407 and in LGG-460 it was noticed as 81.7%, 84.7%, 94.7%, 93.3% and 3.0% at 15%, 30%,

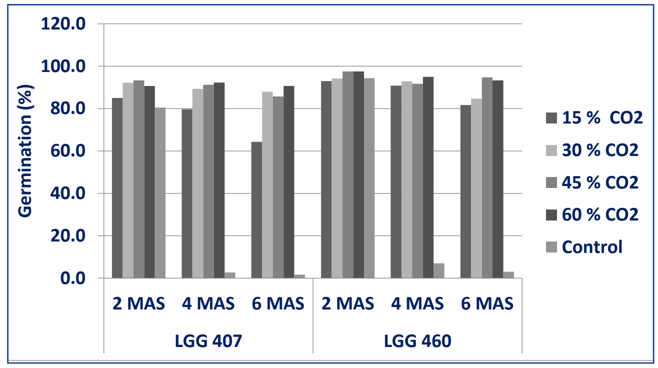

Figure 3: Effect of different concentrations of CO_2 on germination (%) in green gram cvs. LGG-460 and LGG-407 at different storage intervals

45% and 60% levels of concentrations of CO_2 and control, respectively. The reduction in germination percentage in control could be attributed to increased adult emergence consequently leading to increased insect damage and per cent weight loss.

At 4 months of storage, germination percentage was observed to be moderate as compared to 2 months and 6 months storage period. In this period, least germination was observed in control (2.7%, 7.0%) followed by (79.7%, 90.8%) at 15% level, (89.3%, 92.8%) at 30% level and (91.2%, 91.7%) at 45% level and highest germination percentage was recorded at 60% level of CO_2 (92.3%, 95.0%) in LGG-407 and LGG-460, respectively.

White et al. (1990) reported that germination percentage of wheat seed remained unaffected in storage up to 12 weeks when the seed was exposed to 20%–40% CO_2 concentration. White and Jayas (1993) obtained complete control of *Trogoderma castaneum* and *Cryptolestes ferrugenius* which was exposed to 34% CO_2 without significant effect on seed germination. The higher percentage of CO_2 decreased the infestation percentage and lowered per cent fungal count, indicating the antifungal effect of CO_2 and beneficial effect in retaining high germination percentage (Rathi et al., 2000). The cowpea seeds treated with gases mixture exhibited the highest germination percentage up to 6 months of storage as compared to the untreated seeds (Shehata et al., 2009). The pulse beetle infestation in green gram resulted in decreased germination potential and reduction in commercial value of the seed (Booker, 1967).

3.2.3. Seedling characters

Before initiation of the experiment, the root length, shoot length and seedling length was measured as 9.0 cm, 10.5 cm and 19.5 cm in LGG-407 and 8.3 cm, 7.6 cm and 15.9 cm in LGG-460, respectively. In all the seedling parameters, slight variations were observed among the different concentrations and different storage periods in both the accessions.

Longer root length was observed at two months storage period followed by four months and six months storage period viz., (11.30 cm, 7.73 cm), (12.63 cm, 11.15 cm), (11.15 cm, 12.98 cm), (11.35 cm, 12.81 cm) and (11.45 cm, 12.57 cm) at 2 MAS; (9.00 cm, 8.32 cm), (8.97 cm, 9.11 cm), (10.91 cm, 10.27 cm), (10.58 cm, 11.60 cm) and (10.36 cm, 9.68 cm) at 4 MAS; and (7.83 cm, 8.67 cm), (8.90 cm, 8.46 cm), (10.46 cm, 9.26 cm), (8.23 cm, 8.80 cm) and (8.32 cm, 9.73 cm) at 6 MAS; at 15%, 30%, 45% and 60% levels of concentrations of CO_2 and control in LGG-407 and LGG-460, respectively.

Longer shoot length was observed at 2 months storage period followed by 4 months storage period and 6 months storage period viz., (16.43 cm, 17.53 cm), (20.43 cm, 18.66 cm), (19.36 cm, 19.80 cm), (20.73 cm, 20.86 cm) and (21.26 cm, 18.46 cm) at 2 MAS; followed by (14.05 cm, 13.61 cm), (13.90 cm, 14.35 cm), (17.80 cm, 17.26 cm), (20.42 cm, 19.11 cm) and (12.94 cm, 14.74 cm) at 4 MAS; and (11.96 cm, 13.61 cm), (13.37 cm, 14.81 cm), (12.88 cm, 12.95 cm), (13.49 cm, 13.68 cm) and (12.11 cm, 13.42 cm) at 6 MAS; at 15%, 30%, 45% and 60% levels of concentrations of CO_2 and control in LGG-407 and LGG-460, respectively.

The total seedling length was noticed as longer at 2 months storage period followed by 4 months storage period and 6 months storage period viz., (25.36 cm, 25.26 cm), (29.33 cm, 27.13 cm), (29.83 cm, 29.06 cm), (31.00 cm, 30.71cm), (24.66 cm, 26.00 cm) at 2 MAS; followed by (23.26 cm, 25.77 cm), (26.00 cm, 25.96 cm), (28.72 cm, 27.53 cm), (24.84 cm, 26.49 cm), (31.63 cm, 24.14 cm) at 4 MAS; and (21.88 cm, 13.91 cm), (22.88 cm, 23.46 cm), (24.04 cm, 25.93 cm), (28.96 cm, 29.66 cm) and (21.27 cm, 28.23 cm) at 15%, 30%, 45%, 60% concentrations of CO_2 and control in LGG-407 and LGG-460, respectively.

In the present study, both the accessions of green gram showed longer seedlings at 60% concentrations of CO_2 at 2 months storage period and seedling length was observed to be small at lower concentrations of CO_2 viz., 15% and 30% levels in 6 months storage period.

3.2.4. Seedling dry weight (g)

The seedling dry weight recorded before initiation of experiment was 0.23 g in LGG-407 and 0.26 g in LGG-460. After the exposure of CO_2 concentrations, the dry weight was varied in between different concentrations. More seedling dry weight was observed at two months storage period (0.20 and 0.21 g) followed by four months of storage (0.16 g) and six months of storage (0.14 and 0.17 g) in LGG-407 and LGG-460, respectively.

3.2.5. Seedling vigour indices

Significant variation was observed in seedling vigour index-I and seedling vigour index-II. The highest seedling vigour index-I was observed at two months storage interval (2503) followed by 4 months storage period (2108) and 6 months storage (1717) (Table 3). More seedling vigour index-I was observed in the accession LGG-460 (2547, 2325 and1792) as compared to LGG-407 (2460, 1892 and 1641) at 2 MAS, 4 MAS and 6 MAS. The seedling vigour index-I is the product of germination percentage and total seedling length, seedling vigour index-I would be high if the germination percentage and total seedling length were more.

Table 3: Effect of modified atmosphere with elevated levels of CO_2 on seedling vigor index-I in two green gram accessions at different storage intervals

Treatments	Seedling vigor index-I								
	2 Months			4 Months			6 Months		
	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean
15% CO_2	1976	2343	2159	1745	2342	2043	1634	1841	1737
30% CO_2	2572	2446	2509	2324	2293	2309	2111	2178	2144
45% CO_2	2843	2735	2789	2677	2684	2681	2192	2378	2285
60% CO_2	2924	2993	2958	2660	2815	2738	2250	2473	2361
Control	1985	2216	2100	54	1490	772	17	92	55
General mean	2460	2547	2503	1892	2325	2108	1641	1792	1717
	SEm± SEd	CD*	CV	SEm± SEd	CD	CV	SEm± SEd	CD	CV
Variety	45.1 63.8	133.1	6.98	60.4 85.3	178.0	11.08	47.5 67.2	140.2	10.72
Conc.	71.3 100.9	210.4		95.4 134.9	281.5		75.1 106.2	221.6	
Interaction	100.9 142.7	297.6		134.9 190.8	398.1		106.2 150.2	313.4	

*p=0.05

From the data, seedling vigour index-II (Table 4), product of seedling dry weight and germination percentage, showed significant differences among the different concentrations of CO_2 and among the different storage intervals. The highest seedling vigour index-II was recorded at two months of storage period (19.1) followed by 4 months storage period (14.3) and 6 months storage period (11.7). Between the two accessions of green gram, LGG-460 showed more seedling vigour index-II of 19.9, 15.2 and 12.1 at 2, 4 and 6 months storage period, respectively. While, LGG-407 recorded seedling vigour index-II of 18.2, 13.4 and 11.4 at 2 MAS, 4 MAS and 6 MAS, respectively.

From the above data, it was concluded that seedling vigour index-I and seedling vigour index-II were found to be high in LGG-460 at 6 months storage interval as less damage was reported by pulse beetle at 2 MAS than 4 months storage period and 6 months storage period. Similar decrease in seedling vigour with increase in CO_2concentration was in agreement with the finding of Divya et al. (2013) in horse gram genotypes.

Table 4: Effect of modified atmosphere with elevated levels of CO_2 on seedling vigor index-II in two green gram accessions at different storage intervals

Treatments	Seedling vigor index-II								
	2 Months			4 Months			6 Months		
	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean	LGG-407	LGG-460	Mean
15% CO_2	16.7	18.4	17.6	16.2	17.0	16.6	10.2	11.0	10.6
30% CO_2	20.7	20.8	20.8	16.1	19.3	17.7	14.9	15.6	15.3
45% CO_2	19.3	22.5	20.9	16.9	18.3	17.6	16.0	17.1	16.6
60% CO_2	20.7	23.3	22.0	17.7	21.2	19.4	15.7	16.7	16.1
Control	13.6	14.6	14.1	0.2	0.3	0.3	0.03	0.06	0.05
General mean	18.2	19.9	19.1	13.4	15.2	14.3	11.4	12.1	11.7
	SEm± SEd	CD*	CV	SEm± SEd	CD	CV	SEm± SEd	CD	CV
Variety	0.47 0.66	1.39	9.60	0.24 0.34	0.70	6.50	0.38 0.54	1.13	12.67
Conc.	0.74 1.05	2.20		0.38 0.53	1.12		0.60 0.85	1.79	
Interaction	1.05 1.49	3.11		0.53 0.76	1.58		0.85 1.21	2.53	

*p=0.05

4. CONCLUSION

The study on efficacy of different concentrations of CO_2 on seed infestation and population build up in green gram revealed that green gram seed infested with bruchids and exposed to 45% and more concentrations of CO_2 was found effective in minimizing bruchid infestation and their progeny production up to 2 MAS, besides maintaining high germination percentage and seedling vigour. Among the two accessions, LGG-460 had least adult emergence with comparatively less insect damage than LGG-407.

5. REFERENCES

Abdul-Baki, A.A., Anderson, J.D., 1973.Vigor determination in soybean seeds by multiple criteria. Crop Science 13, 630–632.

Aliniazee, M.T., Lindgren, D.L., 1970. Egg hatch of *Tribolium confusum* and *Tribolium castaneum* in different carbon dioxide and nitrogen atmospheres. Annals of Entomology 63(4), 10–12.

Anonymous, 1996. The Food Quality Protection Act. United States of America. Available at https://www.epa.gov/laws-regulations/summary-food-quality-protection-act.

Anonymous, 1999. International rules for seed testing. Seed Science and Technology, Supplement Rules 27, 25–30.

Banks, H.J., Annis, P.C., 1990. Comparative advantages of high CO_2 and low O_2 types of controlled atmospheres for grain storage. In: Calderon, M., Barkai-Golan, R. (Eds.). Food preservation by modified atmospheres, Boca Raton, Florida, CRC Press, 93–122.

Bekele, A.J., Obeng-Ofori, D., Hassanali, A., 1996. Evaluation of *Ocimum suave* (Wild) as a source of repellent, toxicants and protectants in storage against three stored product insect pests. International Journal of Pest Management 42(2), 139–142.

Bera, A., Sinha, S.N., Gaur, A., Srivastava, C., 2007. Effect of carbon dioxide rich atmosphere on storage insects and fungi. Indian Journal of Agricultural Sciences 77(11), 756–761.

Bera, A., Sinha, S.N., Gaur, A., Srivastava, C., 2008. Effect of modified atmosphere storage on seed quality parameters of paddy. Seed Research 36(1), 56–63.

Booker, R.H., 1967. Observation on three bruchids associated with cowpea in Northern Nigeria. Journal of Stored Product Research 3, 1–15.

Cao, Y., Xu, K., Zhu, X., Bai, Y., Yang, W., Li, C., 2019. Role of modified atmosphere in pest control and mechanism of its effect on insects. Frontiers in Physiology 10, 206.

Caril, M.D., Bruna, S., Caciano Pelayo, Z.N., Irineu, L., Adriano, B., 2010. Efficacy of modified atmosphere packaging to control *Sitophilus* sp. in organic maize grain. Brazilian Archives of Biology and Technology 53, 1469–1476.

Cheng, W., Lei, J., Ahn, J.E., Liu, T.X., Zhu-Salzman, K., 2012. Effects of decreased O_2 and elevated CO_2 on survival, development, and gene expression in cowpea bruchids. Journal of Insect Physiology 58, 792–800.

Divya, P., Kanaka Durga, K., Sunil, N., Mandali, R., Kunusoth, K., Udayababu, P., 2016. Modified atmosphere storage technique for the management of pulse beetle, *Callosobruchus chinensis* in horse gram. Legume Research 39(3), 474–478.

Divya, P., Kanaka Durga, K., Udayababu, P., 2013. Studies on the effect of biochemical and physico-chemical characters on bruchid (*Callosobruchus chinensis* L.) resistance in horse gram accessions. Journal of Food Legumes 26(1–2), 70–74.

Donahaye, E.J., Navarro, S., 2000. Comparison of energy reserves among strains of *Tribolium castaneum* selected for resistance to hypoxia and hypercarbia, and the unselected strain. Journal of Stored Products Research 36(3), 223–234.

Gujar, G.T., Yadav, T.D., 1978. Feeding of *Callosobruchus maculatus* Fab (Coleoptera: Bruchidae) reared on different food and temperatures. Journal of Stored Products Research 22(2), 71–75.

Gunasekaran, N., Rajendran, S., 2005. Toxicity of carbon dioxide to drugstore beetle *Stegobium paniceum* and cigarette beetle *Lasioderma serricorne*. Journal of Stored Products Research 41(3), 283–294.

Kalpna, Younis, A.H., Kumar, R., 2022. Management of stored grain pest with special reference to *Callosobruchus maculatus*, a major pest of cowpea: A review. Haylon. 8(1), 1–12. https://doi.org/10.1016/j.heliyon.2021.e08703

Marks, B.P., Stroshine, R.L., 1995. Effects of previous storage history, hybrid and drying method on the storability of maize grain (corn). Journal of Stored Products Research 31(4), 343–354.

Mehmood, K., Husain, M., Aslam, M., Shoaib, M.A., Mehmood, A.A., Shaheen, F.A., 2018. Changes in the nutritional composition of maize flour due to *Tribolium castaneum* infestation and application of carbon dioxide to manage this pest. Environmental Science and Pollution Research 25(19), 18540–18547.

Njoroge, A.W., Affognon, H., Mutungi, C., Rohde, B., Richter, U., Hensel, O., 2016. Frequency and time pattern differences in acoustic signals produced by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zea mais* (Motschulsky) (Coleoptera: Curculionidae) in stored maize. Journal of Stored Products Research 69, 31–40.

Njoroge, A.W., Mankin, R.W., Smith, B.W., Baributsa, D., 2017. Effects of hermetic storage on adult *Sitophilus oryzae* L. (Coleoptera: Curculionidae) acoustic activity patterns and mortality. Journal of Economic Entomology 110(6), 2707–2715.

Odogola, W.R., 1994. Post harvest management and storage of food legumes. Technical Systems for Agriculture. AGROTECUNDP/OPS, Harare, Zimbabwe, 98.

Ofuya, T.I., Reichmuth, C., 2002. Effect of humidity on the susceptibility of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) to two modified atmospheres. Journal of Stored Products Research 38(2), 139–146.

Radhika, P., Mandali, R., Reddy, B.S., 2014. Storage in modified atmosphere: an efficient technique to prevent the infestation of *Caryedon serratus oliver* in stored groundnut. Journal of Entomological Research 38(2), 91–93.

Raghupathi, B., Padmasri, A., Lakshmi Narayanamma, V., Ramya, V., 2021. Effect of modified atmosphere with elevated levels of CO_2 on seed quality parameters of green gram during storage. The Pharma Innovation Journal 10(11), 53–59.

Rasool, K.G., Husain, M., Mehmood, K., Alhamdan, A.M.A., Tufail, M., Aldawood, A.S., 2017. The effectiveness of carbon dioxide and nitrogen on different developmental stages of *Cadra cautella* (Lepidoptera: Pyralidae). Pakistan Journal of Agricultural Sciences 54, 731–736.

Rathi, S.S., Shan, N.G., Zambre, S.S., Kalbande, V.H., Venkatesh, K.V., 2000. Respiration, absorption and germination of seeds stored in controlled atmosphere. Seed Science and Technology 28, 341–348.

Riudavets, J., Castane, C., Alomar, O., Pons, M.J., Gabarra, R., 2009. Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. Journal of Stored Products Research 45(2), 91–96.

Shaaya, E., Kostjukovski, M., Eilberg, J., Sukprakarn, C., 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Products Research 33(1), 7–15.

Shehata, S.A., Hashem, M.Y., Abd El-Gawad, K.F., 2009. Effect of controlled atmosphere on quality of dry cowpeaseeds. In: 4th Conference on Recent Technology in Agriculture. Department of Vegetable Crops and Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

Singh, U., Jambunadhan, R., 1990. Pigeonpea: Post-harvest technology. In: Nene, Y.L., Hall, S.D., Sheila, V.K. (Eds.). The Pigeonpea. ICRISAT, CAB International, Wallingford, UK, 435–455.

Soderstrom, E.L., Brandl, D.G., Mackey, A., 1990. Responses of codling moth (Lepidoptera: Tortricidae) life stages to high carbon dioxide or low oxygen atmospheres. Journal of Economic Entomology 83(2), 472–475.

Wang, F., Jayas, D.S., White, N.D.G., Fields, P., 2009. Combined effect of carbon monoxide mixed with carbon dioxide in air on the mortality of stored-grain insects. Journal of Stored Products Research 45(4), 247–253.

Weaver, D.K., Petroff, A.R., 2005. Pest management for grain storage and fumigation. Pest Control: Grain Storage & Seed Treatment Facilities, Montana Department of Agriculture, 1–84. Available at http://www.docstoc.com/docs/45259080/.

White, N.D.G., Jayas, D.S., 1993. Effectiveness of carbon dioxide in compressed gas or solid formulation for the control of insects and mites in stored wheat and barley. Phyto Protection 74(2), 101–111

White, N.D.G., Jayas, D.S., Sinha, R.N., 1990. Carbon dioxide as a control agent for the rusty grain beetle (Coleopteran: Cucujidae) in stored wheat. Journal of Economic Entomology 83(1), 277–288.

Yadav, S., Mahla, J.C., 2002. Bio efficacy of carbon dioxide concentration and its exposure against khapra beetle in wheat grain. Indian Journal of Entomology 64, 130–137.