

## Research Article

# Optimization of CO<sub>2</sub> Concentration on Mortality of Various Stages of *Callosobruchus maculatus* and Development of Controlled Atmosphere Storage Structure for Black Gram Grains

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Received 27 May 2022; Revised 26 June 2022; Accepted 7 July 2022; Published 20 July 2022

Academic Editor: Jeevan Kumar Reddy Modigunta

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Pulse beetle (*Callosobruchus maculatus*) is a common weevil that is responsible for up to 24% of stored pulse losses. Using black gram grain storage, the efficiency of carbon dioxide gas against all life stages of *Callosobruchus maculatus* insects was tested at various concentrations and exposure times. The trials were carried out in pilot bins with a capacity of 25 kg. At a CO<sub>2</sub> concentration of 50%, complete mortality of the egg stage of *Callosobruchus maculatus* was attained after 72 h of continuous exposure. At a CO<sub>2</sub> concentration of 60% for 48 h, 100% larva mortality was achieved. At the most tolerant stage of pupa, recorded complete mortality is at a CO<sub>2</sub> concentration of 70% for 96 hours of the exposure period. Adult insects are especially vulnerable to the high CO<sub>2</sub> concentration. Adult mortality was achieved at a concentration of 20% with an exposure period of 48 h. The CO<sub>2</sub>-treated black grams were then stored for three months with the optimized CO<sub>2</sub> concentration and exposure period, while physiochemical parameters such as water retention capacity and physiological loss in weight were determined. Grain stored in the silo showing significant 100% mortality of egg was measured after 20-25 days of observation. The use of a controlled atmosphere storage bin increased the mortality of the insect *C. maculatus* at all developmental stages, by means of increasing CO<sub>2</sub> concentration and exposure time. Grain stored in a controlled atmosphere silo showed minimum losses of grain (4.10%) compared to the gunny bag storage (22.56%).

## 1. Introduction

Black gram (*Vigna mungo*) is one of the most important pulses cultivated in India, constituting the protein supple-

ments for a large number of human beings. Due to its fermenting property, it is used in the preparation of various foods [1]. Grain production has been increasing steadily due to the developments in production technology, but high

storage losses in grains occur mainly by the reason of improper postharvest handling. Due to the lack of storage facilities and insufficient money for storage structures, the farmers are forced to sell their produce at low prices. The major insect pest of pulse storage is *Callosobruchus maculatus* (F.). Rahman and Talukder [2] observed the damages by *Callosobruchus maculatus* on the grains of bengal gram, black gram, cowpea, green gram, moth beans, red gram, and rice beans studied under the natural storage conditions in India. Cowpea had maximum damage in terms of weight loss (34.5%) and exit holes (69.2%) observed, followed by moth beans (53.7 and 21.9%) and black grams (50.3 and 19.4%).

Synthetic chemicals were used to control this pest; as a result, some chemical residues may accumulate in the grain, favouring the formation of resistant weevil populations and raising producer costs. Furthermore, the number of other synthetic insecticides has recently reduced, and the possibilities for using those that remain have been severely limited, because of their negative environmental impacts. There are currently just four fumigants that have been registered in India: methyl bromide, aluminium phosphide, and ethylene dichloride-carbon tetrachloride. However, only phosphine and the ethylene dichloride-carbon tetrachloride (EDCT) combo have been permitted for use in treating food grains. However, because of the dangers that these chemicals pose to plants, humans, and the environment, there has been a push to find a biopesticide that uses plant components to control *C. maculatus* [3]. The widespread use of phosphine for storing grains is relatively inexpensive and easy to apply, leaves minimal residues, and can be used in a wide range of storage types and commodities [4]. This insect infests pulses in the field before the mature seeds are harvested. Insects develop quickly in storage, resulting in significant postharvest losses. After 3–5 months of storage, unprotected grains can often become completely infested. Seeds that have been damaged have a lower weight and nutritional content, as well as a lower germination rate [5]. Grains of this grade have no market worth because they are no longer suitable for human consumption and cannot even be utilised as seeds for planting.

Constant monitoring and adjustment of CO<sub>2</sub> and O<sub>2</sub> levels within gas-tight stores or containers are referred to as controlled atmosphere (CA) storage. Fumigation of insects by exposure to elevated levels of CO<sub>2</sub> is more appropriate than exposure to reduced levels of O<sub>2</sub> because the environment does not have to be controlled as precisely. By changing the relative concentrations of oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>), and carbon dioxide (CO<sub>2</sub>), the quality of the grain may be preserved for a longer time than storage under atmospheric air concentrations [6]. Intergranular air composition between the grains gets deteriorated because insects, mites, and microorganisms require oxygen to respire and carbon dioxide could be lethal to living organisms. Therefore, use of CO<sub>2</sub> reduces the cost of modified atmosphere fumigation. Retention and desorption of grains will occur easily without any external chemical reaction during the CO<sub>2</sub> storage, which has no effect on the quality of grains. Carbon dioxide (CO<sub>2</sub>) and nitrogen (N<sub>2</sub>) are used as sustainable alternatives to currently used fumigants for insect con-

trol in stored grains [7]. One of the most essential grain quality parameters is standardised measurement of black gram water retention, which provides direction for predicting cooking retention and is strongly related to yield and quality of by-products [8]. Many factors influence the rate of water retention in seeds, including chemical constitutions, seed size and maturity phase, seed size (fractions), and seed cover structure (permeability). The ability of a substance to absorb water is linked to its qualities, particularly its porosity and pore characteristics [9]. As a result, the current research is aimed at examining the feasibility of assessing the insect mortality and quality parameters of stored grains using the carbon dioxide concentration and exposure time monitoring technique.

## 2. Materials and Methods

**2.1. Raw Materials.** Freshly harvested black gram (VBN6) was procured from the farmers of Dharmapuri district. The grains were kept under ambient conditions, and the moisture content of the grains was determined regularly using the “hot air oven” method at 105°C for 3 hrs [10]. The research was carried out at the Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

**2.2. Insect Culture.** For mass culturing of *Callosobruchus maculatus*, eggs, larvae, pupae, and adults were collected and kept at TNAU’s Food Process Engineering laboratory. Approximately 30 pairs of adult beetles obtained from the Department of Entomology, TNAU, were initially released into plastic containers containing 500 g of disinfested black gram. The mouth of the container was closed with muslin cloth and tied with rubber bands around the container’s neck for ventilation. Plastic containers and glass bottles were used for maintaining the mass culturing of test insects. The containers were kept undisturbed under ambient conditions till the emergence of progeny. 50 adults were kept in ventilated plastic cages on 200 g of black gram to collect newly laid eggs of *C. maculatus*. For carbon dioxide treatments, eggs of known age (1–5 days), larvae (7–18 days), pupae (21–23 days), or adults (24–28 days) were obtained at various times. Figure 1 depicts the life stages of *Callosobruchus maculatus* under the microscope.

**2.3. Lab Scale Metal Bin.** The effect of elevated carbon dioxide on pulses and mortality of different life stages of *Callosobruchus maculatus* was studied. The lab scale metal bin was fabricated in two replicates for the study as shown in Figure 2. The mild steel sheet of 16-gauge thickness was used to fabricate the cylindrical bin with a dimension of height 0.5 m and diameter 0.3 m to contain a grain capacity of 25 kg. The top and bottom portion was welded with a 3 mm thick circular sheet. The inlet and outlet valve of 7 mm MS coupling was welded in the bin. The inlet valve was fitted at 4 mm from the bottom of the bin, and the outlet valve was at the top of the bin. Both loading and unloading of grain were done through the feed hopper. The feed hopper of 75 mm MS coupling with end cap was welded at the top

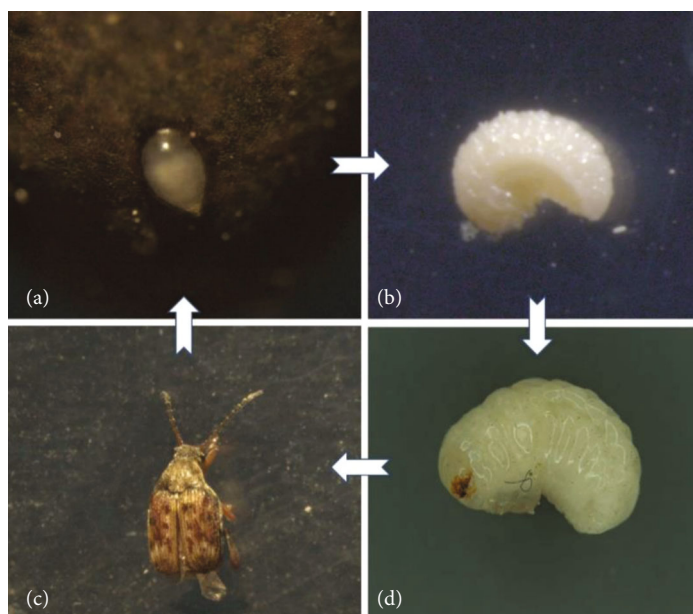


FIGURE 1: A microscopic image of *Callosobruchus maculatus*: (a) egg; (b) larva; (c) pupa; (d) adult insect.



FIGURE 2: A view of pilot bins for CA treatments.

of the bin. Two grain sampling ports of 25 mm size GI couplings with end cap were welded on the bin at a distance of about 150 mm from the top and bottom of the bin. The rubber septum of teflon-coated silicon septum of 20 mm diameter was inserted into the brass check nut. The five septums set were fitted with coupling which was welded outside the bin and positioned vertically at 100 mm distance apart from each

other. Two ball valves were affixed with the bin, one at the top and another at the bottom for purging the gas in and out.

*2.4. Source of Carbon Dioxide.* In this study, carbon dioxide was used in the form of gas. Carbon dioxide gas was purchased from the industrial supplier firm, Covai Air Products Pvt Ltd, Coimbatore. Two carbon dioxide cylinders were

used for the study each with a capacity of 35kg which can hold a volume of  $20\text{m}^3$  of  $\text{CO}_2$ .

**2.5. Estimation of  $\text{CO}_2$  to Be Purged into the Lab Scale Bin.** The volume of  $\text{CO}_2$  to be purged into the bin was determined by properties, and known parameters of grains and bin were used for the calculation, using the below given equations (1) and (2). And the amount of carbon dioxide concentration was checked by a  $\text{CO}_2/\text{O}_2/\text{N}_2$  analyzer (PBI Dansensor):

$$\begin{aligned} \text{Volume of carbon dioxide} &= \text{Volume of total air space} \\ &\times \text{Desired concentration (\%),} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Time required to achieve desired concentration (min)} \\ = \frac{\text{Vol. of carbon dioxide}}{\text{Gas flow rate}}. \end{aligned} \quad (2)$$

Using the time calculation, the carbon dioxide was filled in the bin by allowing the gas for a fixed time to achieve the desired concentration at the constant flow rate of  $10\text{l/min}$ . The required concentration of  $\text{CO}_2$  was released into the container with a pressure of  $2\text{kg/cm}^2$  from the  $\text{CO}_2$  cylinder.

**2.6. Optimization of  $\text{CO}_2$  Gas Concentration.** To study the effect of controlled atmosphere ( $\text{CO}_2$ ) against all the life stages of *C. maculatus*, the airtight containers were filled with 25kg of black grams and then infested by releasing the *C. maculatus* eggs, larva, pupa, and adults 30 each.  $\text{CO}_2$  was released at different concentrations, viz., 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% for 24, 48, 72, and 96 hr with three replications of each life stage for each treatment. The black gram grains were stabilized to the moisture content of about 12% wet basis. After releasing the desired concentration into the containers, they were made airtight by closing the valves and lid with teflon tape around the thread of couplings. Carbon dioxide concentration was maintained and monitored regularly during the treatments. Optimization of the concentration and time for achieving 100% mortality for all life stages of *C. maculatus* were investigated.

**2.7. Insect Mortality.** Mortality of different life stages of *Callosobruchus maculatus* was calculated based on the suggestion given by the author [11] as given below.

**2.7.1. Egg Mortality.** Egg mortality was observed after treating the eggs (infested grain) with elevated carbon dioxide at varying times. Treated grains were kept for 20 to 25 days for adult emergence. The emergence of adult insects after the observation period shows the survival of eggs which was determined using

$$\begin{aligned} \text{Percent egg mortality} &= 100 - \text{Percent egg survival,} \\ \text{Percent egg survival} &= \frac{\text{No. of adults emerged}}{\text{No. of eggs treated}} * 100. \end{aligned} \quad (3)$$



FIGURE 3: Experimental setup of controlled atmosphere storage bin for black gram grains.

**2.7.2. Larva Mortality.** Treated grains were kept for 14 to 19 days for adult emergence. Emergence of adult insects after the observation period shows the survival of the larva. The below-given expressions (4) and (5) were used to calculate the mortality of larva in percentage:

$$\text{Percent larva mortality} = 100 - \text{Percent larva survival,} \quad (4)$$

$$\text{Percent larva survival} = \frac{\text{No. of adults emerged}}{\text{No. of larva treated}} * 100. \quad (5)$$

**2.7.3. Pupa Mortality.** Treated grains were kept for 7 to 9 days for adult emergence. Emergence of adult insects after the observation period showed the survival of pupa. Below given expressions (7) and (6) were used for determining the pupa mortality:

$$\text{Percent egg mortality} = 100 - \text{Percent pupa survival,} \quad (6)$$

$$\text{Percent pupa survival} = \frac{\text{No. of adults emerged}}{\text{No. of pupa treated}} * 100. \quad (7)$$

**2.7.4. Adult Mortality.** The percent adult mortality was calculated using

$$\text{Percent adult mortality} = \frac{\text{No. of adults dead}}{\text{No. of adults released}} * 100. \quad (8)$$

**2.8. Silo Storage.** Grains were filled from the top of the silo to make up to 550 kg and keep the 25% headspace in the silo. And then, 300 pairs of adults [12] of *Callosobruchus maculatus* were released from the top of the silo for the experiment

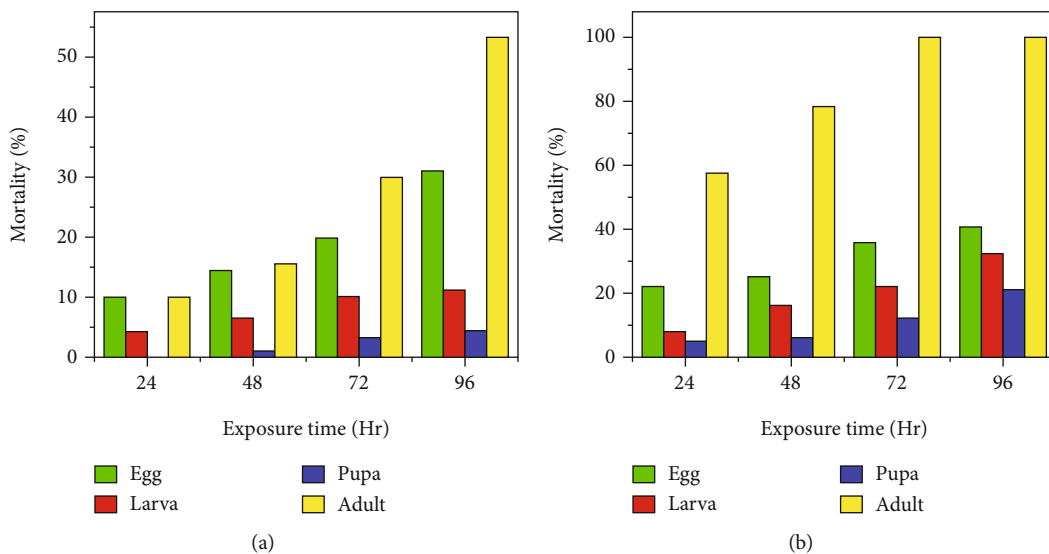


FIGURE 4: Mortality of *Callosobruchus maculatus* egg, larva, pupa, and adult at (a) 10% CO<sub>2</sub> concentration and (b) 20% CO<sub>2</sub> concentration.

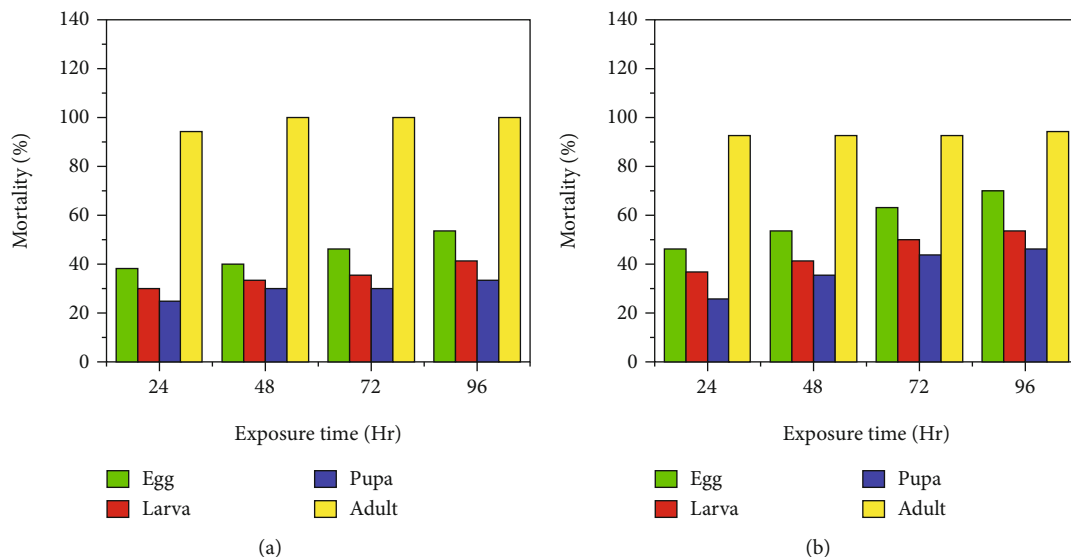


FIGURE 5: Mortality of *Callosobruchus maculatus* egg, larva, pupa, and adult at (a) 30% CO<sub>2</sub> concentration and (b) 40% CO<sub>2</sub> concentration.

as shown in Figure 3. After releasing the adult insects in the grains, the grains were kept undisturbed for 25 days to develop a uniform level of grain infestations with different stages of egg, larva, pupa, and adults. All life stages of *Callosobruchus maculatus* can be completely killed by an optimized level of carbon dioxide concentration. The carbon dioxide was then purged into the bin until 70% CO<sub>2</sub> was achieved. As a result, it is sufficient to achieve complete mortality of all life stages of *Callosobruchus maculatus*.

**2.9. Water Retention Capacity of Black Gram.** The optimal soaking conditions for black gram were determined at 10°C and 60°C using 100 g of black gram grains in 500 ml of water (1:5). At every 30-minute intervals, measurements were taken in triplicate. The weight difference between wet and dry grain was used to calculate the percentage of retention. The retention curves were created by plotting soaking time

(h) vs. water retention (percent). It was determined in the black gram grains.

**2.10. Physiological Loss in Weight (%).** The initial weight and final weight of the grains were recorded, and the percent weight loss was calculated using the formula

$$\text{Physiological loss in weight (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100. \tag{9}$$

### 3. Results and Discussion

**3.1. Effect of CO<sub>2</sub> Concentrations on Egg Mortality.** The egg mortality of *Callosobruchus maculatus* was observed when exposed to various concentrations of CO<sub>2</sub> for 4 days of

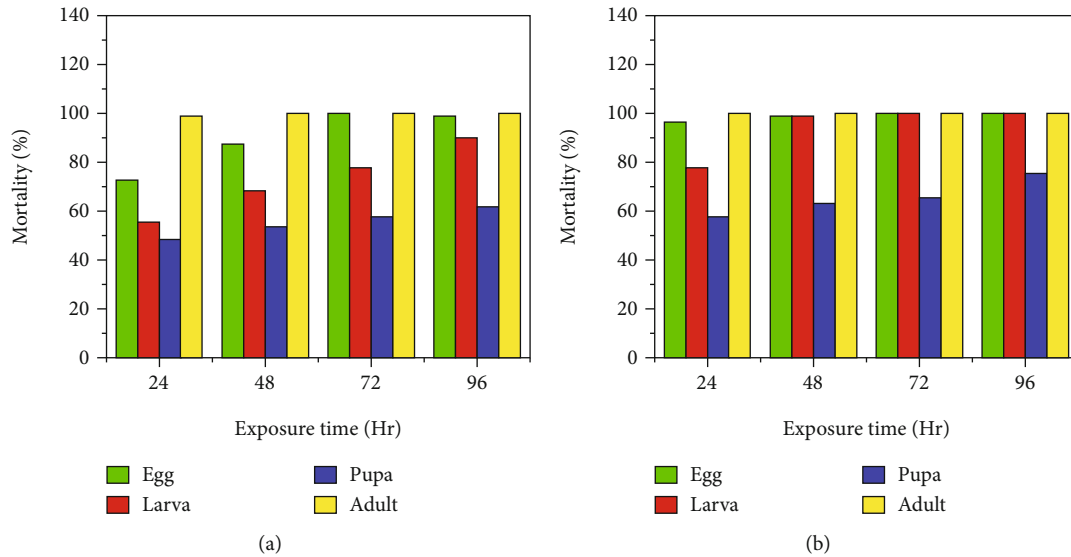


FIGURE 6: Mortality of *Callosobruchus maculatus* egg, larva, pupa, and adult at (a) 50% CO<sub>2</sub> concentration and (b) 60% CO<sub>2</sub> concentration.

exposure. The treated samples of eggs were kept separately in an ambient atmosphere to determine the mortality of the eggs by observing the total number of adult emergence after 18 to 25 days. The egg mortality increased gradually when exposed to higher concentrations with an increase in exposure days. Figure 4 shows the lowest mean egg mortality recorded at a 10% concentration of CO<sub>2</sub>. Figure 5 shows the mortality of *Callosobruchus maculatus* egg, larva, pupa, and adult at 30% CO<sub>2</sub> concentration and 40% CO<sub>2</sub> concentration. For the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> days of treatment, the mortality was found to be 10.00, 14.44, 20.00, and 31.11%, respectively. 60% CO<sub>2</sub> concentration showed a maximum percentage of egg mortality with a lesser exposure period. It was observed that 97.78% and 100% mortality was observed when the samples are exposed to 60% CO<sub>2</sub> for 24 and 48 hours of treatment as shown in Figure 6. The results obtained by authors in [13] studied that increased exposure periods are required to obtain 100% mortality among the developmental stages of the bruchids when it is exposed to 100% CO<sub>2</sub>. The eggs were most susceptible with a 100% mortality recorded within 24 h exposure. Variation in sensitivity to controlled atmosphere storage of legumes during the egg stages of the *Callosobruchus maculatus* was observed at 100% mortality when exposed to 50% CO<sub>2</sub> for 5 days and 70% CO<sub>2</sub> for 4 days, respectively [14]. Furthermore, hypercarbia sensitivity increases during periods of intense metabolic activity within phases. This is due to the fact that hypercarbia slows down respiration by suppressing the action of respiratory enzymes, preventing O<sub>2</sub> use [15].

The probit analysis of *Callosobruchus maculatus* eggs at different carbon dioxide concentrations is given in Table 1. It was observed that LC<sub>50</sub> and LC<sub>99</sub> for 95% fiducial limit at different times had different concentrations. 50% egg mortality can be achieved at the concentration of 40.55, 38.55, 38.13, and 37.17% for 24, 48, 72, and 96 hours, respectively. And 99% mortality can be achieved at the carbon dioxide concentration of 71, 63.46, 51.95, and 52.59% for 24, 48, 72, and 96 hours of exposure. It was observed that

TABLE 1: Probit analysis of CO<sub>2</sub> concentrations on different life stages of mortality of *Callosobruchus maculatus*.

Stages	Exposure period (days)	LC <sub>50</sub> (%)	LC <sub>99</sub> (%)	$\chi^2$
Egg	1	40.55	71.00	4.03
	2	38.55	63.46	1.98
	3	38.13	51.95	4.32
	4	37.17	52.59	2.28
Larva	1	44.67	86.81	1.95
	2	41.33	69.07	5.66
	3	39.53	67.02	3.48
	4	37.35	63.51	4.58
Pupa	1	53.37	107.87	3.37
	2	48.10	96.70	3.86
	3	45.07	96.82	2.88
	4	40.60	71.14	5.14
Adult	1	20.30	34.55	0.021
	2	17.62	25.57	0.08
	3	11.55	20.82	0.006
	4	9.78	16.17	0.003

\*LC: lethal concentration.

high concentration requires a lesser exposure period to attain complete mortality than lower concentration. A similar finding was also seen in other studies [16]. The results of ANOVA showed that CO<sub>2</sub> concentration and exposure period had a significant effect on mortality of *Callosobruchus maculatus* eggs at the level  $P < 0.01$ .

**3.2. Effect of Controlled Atmosphere on Larva Mortality.** The direct effect of various CO<sub>2</sub> concentrations tested in these experiments exhibited different degrees of toxicity towards *Callosobruchus maculatus* larva. 100% mortality of *Callosobruchus maculatus* larva was achieved at a CO<sub>2</sub> concentration of 60% for 48 hours of exposure period as shown in Figure 6. According to the study [17], 100% mortality of C.

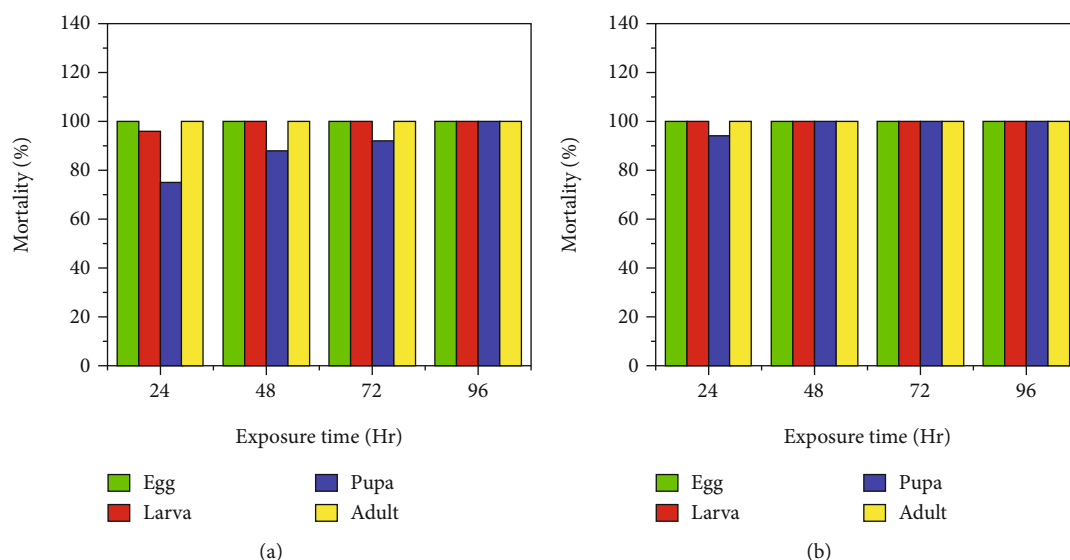


FIGURE 7: Mortality of *Callosobruchus maculatus* egg, larva, pupa, and adult at (a) 70% CO<sub>2</sub> concentration and (b) 80% CO<sub>2</sub> concentration.

TABLE 2: Percentage of eggs laid and adult emergence in bulk grain silo without CO<sub>2</sub> and gunny bag storage.

Sample	Initial—without carbon dioxide in silo			Gunny bag storage		
	No. of eggs/50 grains	Egg laid (%)	Mortality (%)	No. of eggs/50 grains	Egg laid (%)	Mortality (%)
1	14	28	3	48	96	2
2	18	36	1	50	100	4
3	18	35	3	47	94	1
4	17	33	3	41	82	5
5	16	31	3	36	72	4
6	11	22	3	46	92	9
7	16	32	3	50	100	3
8	16	32	2	47	94	6
9	14	28	3	41	82	2
10	18	35	2	37	74	2
Mean	16	31	3	44.3	88.6	3.8
SD	2.2	4.3		4.16	10.33	

*maculatus* first instar larvae was attained after 72 hours of exposure to 70 percent CO<sub>2</sub>, and the results were identical to 97.8% 1<sup>st</sup> instar and 100% 2<sup>nd</sup> instar larvae with chickpea. Only 72 to 96 hours were needed to achieve 90 to 100% mortality of larvae of the species at the 70% CO<sub>2</sub> concentrations tested. 96 hours was necessary to obtain 100% mortality for the larvae of *C. maculatus*. A similar finding is also reported by the researchers [18]. The first larval stage was recorded 48 hours after exposure, and the fourth larval instar required a 96-hour exposure period to achieve 100% mortality [19].

Carbon dioxide concentration at 60% for 48-hour exposure exhibited very strong insecticidal properties since its LC<sub>50</sub> at 95% fiducial limits towards *Callosobruchus maculatus* larva were estimated to be 41.33% CO<sub>2</sub>, shown in Table 1. For the same exposure period, LC<sub>99</sub> was calculated as 69.07% of CO<sub>2</sub> needed for achieving 99% of larva mortality. Since larva of *C. maculatus* are embedded in seed material, they may have been shielded from the external

atmosphere. It was observed that LC<sub>50</sub> and LC<sub>99</sub> progressively decreased with an increase in hours. The statistical analysis (ANOVA) for mortality of *Callosobruchus maculatus* larva shows that different CO<sub>2</sub> concentrations and exposure periods had a significant effect on the mortality of *Callosobruchus maculatus* larva at  $P < 0.01$ .

**3.3. Effect of Controlled Atmosphere on Pupa Mortality.** Complete mortality of pupa of *C. maculatus* was observed at 70% carbon dioxide after 96 hours of treatments as shown in Figure 7. It was observed that the percentage of mortality obtained when the carbon dioxide level reached 70% was 74.44, 87.78, 91.11, and 100% for 24, 48, 72, and 96 hours of exposure, respectively. Mortality rates of pupa were increased with an increase in carbon dioxide concentration, which is shown in Figures 4 and 5. Pupae are protected from CA by feeding chambers within the chickpea, but they are still susceptible. The effects of lower ambient-oxygen

TABLE 3: Percentage of eggs laid and adult emergence after 30 days in bulk grain silo with CO<sub>2</sub> and gunny bag storage.

Sample	Initial—with carbon dioxide in silo			Gunny bag storage		
	No. of eggs/50 grains	Egg laid (%)	Mortality (%)	No. of eggs/50 grains	Egg laid (%)	Mortality (%)
1	15	31	100	49	98	2
2	15	30	100	49	98	4
3	16	31	100	39	78	1
4	14	27	100	40	80	5
5	15	29	100	36	72	4
6	15	31	100	49	98	9
7	15	31	100	33	66	3
8	15	29	100	25	50	6
9	19	37	100	43	86	2
10	15	30	100	35	70	2
Mean	15	31	100	40	80	4
SD	1.3	2.6		7.94	15.88	

TABLE 4: Percentage of eggs laid and adult emergence after 60 days in bulk grain silo with CO<sub>2</sub> and gunny bag storage.

Sample	Initial—with carbon dioxide in silo			Gunny bag storage		
	No. of eggs/50 grains	Egg laid (%)	Mortality (%)	No. of eggs/50 grains	Egg laid (%)	Mortality (%)
1	16	33	100	43	86	2
2	16	31	100	49	98	4
3	14	29	100	50	100	1
4	10	21	100	40	80	2
5	14	28	100	32	64	4
6	16	32	100	49	98	1
7	15	30	100	46	92	5
8	13	25	100	49	98	5
9	14	28	100	43	86	4
10	17	33	100	32	64	9
Mean	15	29	100	43	87	4
SD	1.92	3.84		6.80	13.60	

concentrations on insect size development are observed to be proportional [20].

Most of the *C. maculatus* exposed as pupae on the black gram grains either died or were arrested as pupae and would mostly likely eventually die in that stage or as incomplete pupal-adult molts [21]. For pupae of *C. maculatus* exposed to 90% CO<sub>2</sub> for 120 hours, there were 69.4% mortality and 100% mortality after 216 hours of exposure. The pupae's high tolerance to inert atmosphere may be due to their lower metabolism than in the other stages [22].

The probit analysis of *C. maculatus* pupae at different carbon dioxide concentrations and exposure periods is given in Table 1. The LC<sub>50</sub> were achieved at the CO<sub>2</sub> concentration of 53.37, 48.10, 45.07, and 40.60% for 24, 48, 72, and 96 hours of exposure, respectively. Similarly, LC<sub>99</sub> was achieved at less concentration for a higher exposure period. The lowest concentration at 95% fiducial limit for LC<sub>50</sub> and LC<sub>99</sub> recorded at 96 h of exposure was 40.46% and 71.14%, respectively. Also, the highest LC<sub>50</sub> and LC<sub>99</sub> recorded at 24 h treatment were 53.37 and 107.87%. Results indicate that less

concentration needs more exposure time for treatments. ANOVA for the effect of CO<sub>2</sub> concentration and exposure time showed highly significant mortality of pupa stages at the level of  $P < 0.01$ .

**3.4. Effect of Controlled Atmosphere on Adult Mortality.** The mortality of *Callosobruchus maculatus* adults at different carbon dioxide and exposure times was determined. Adults of *Callosobruchus maculatus* showed 100% mortality when the CO<sub>2</sub> concentration and exposure time were increased. It was observed that 100% adult mortality was recorded with the concentration of 20% for 72 h and 30% for 48 h of the exposure period as shown in Figures 4 and 5. The results indicated that *Callosobruchus maculatus* adults are more sensitive to high concentration of carbon dioxide and would be easier to control than the other stages of insects.

Mortality of *Callosobruchus maculatus* adults was mainly due to metabolism exposed to low oxygen atmospheres found to be mainly anaerobic, and mortality occurred by anaerobic metabolism with accumulation of toxic products



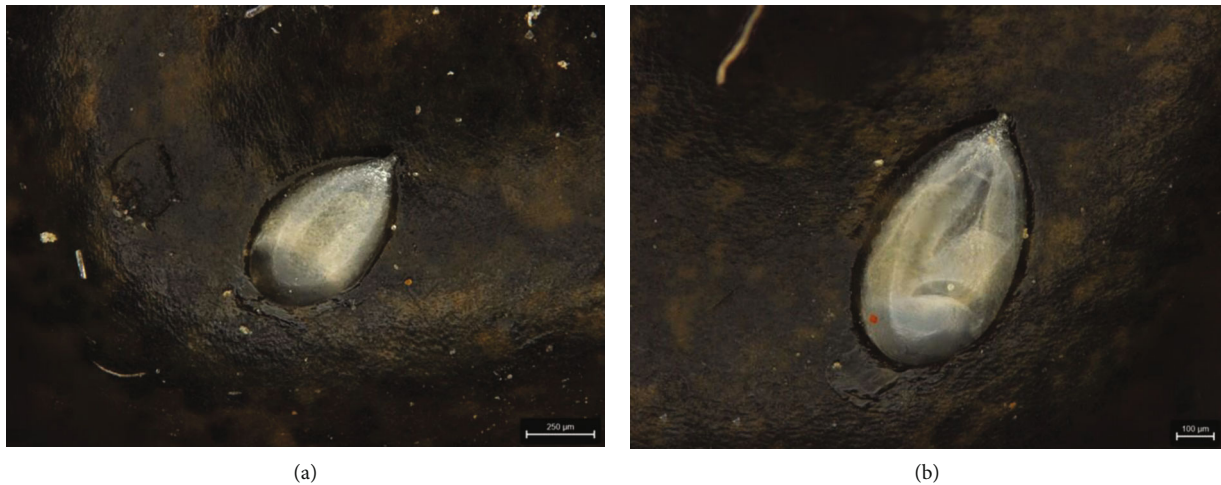


FIGURE 8: Microscopic image of eggs of *Callosobruchus maculatus* (a) before CO<sub>2</sub> treatment and (b) after CO<sub>2</sub> treatment.

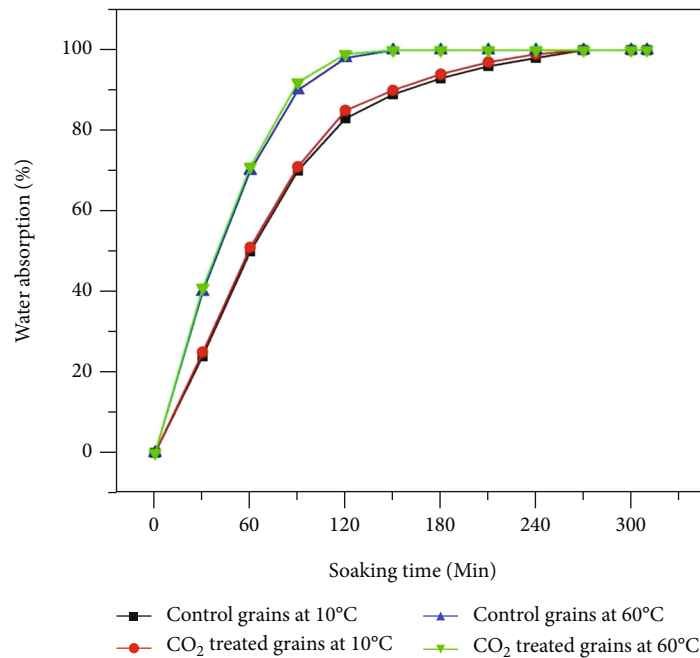


FIGURE 9: Water retention curve of black gram grain samples after 2-month storage.

[23]. Insects' respiratory systems are made up of highly branching cuticle-walled tubes that run throughout the body; it is thought to be the main entry point for poisonous gases into the body [24]. Modified atmosphere can increase the action of low humidity, by prolonging the opening of the spirals, thereby permitting rapid loss of water. Insects' ability to keep water within specified limitations (typically 50-90 percent) is an important part of their structure and physiology [25]. *C. maculatus* are the most vulnerable in terms of the developmental stages they are subjected to, and these adults exhibit high activity and hypoxia sensitivity. The addition of CO<sub>2</sub> resulted in a comparatively short developmental rates and greater adult mortality.

LC<sub>50</sub> and LC<sub>99</sub> and chi square of *Callosobruchus maculatus* adults are presented in Table 1. LC<sub>50</sub> were recorded as 20.30, 17.62, 11.55, and 9.78% for 24, 48, 72, and 96 hours, respectively. LC<sub>99</sub> were recorded as 34.55, 25.57, 15.82, and 16.17% for 24, 48, 72, and 96 hours of exposure, respectively. Significant differences were observed in the susceptibility of adults of *Callosobruchus maculatus* ( $P < 0.01$ ). From the ANOVA, it was observed that carbon dioxide concentration and exposure period had a significant effect on the mortality of *Callosobruchus maculatus* adults.

3.5. Percentage of Egg Laid on Storage Silo. The percentage of egg laid was calculated for 90 days at an interval of 30 days

TABLE 5: Physiological loss in weight of grains.

Storage methods	Initial weight (kg)	Final weight (kg)	Weight loss (%)
Silo storage	550	527.43	4.10
Gunny bag storage	200	154.88	22.56

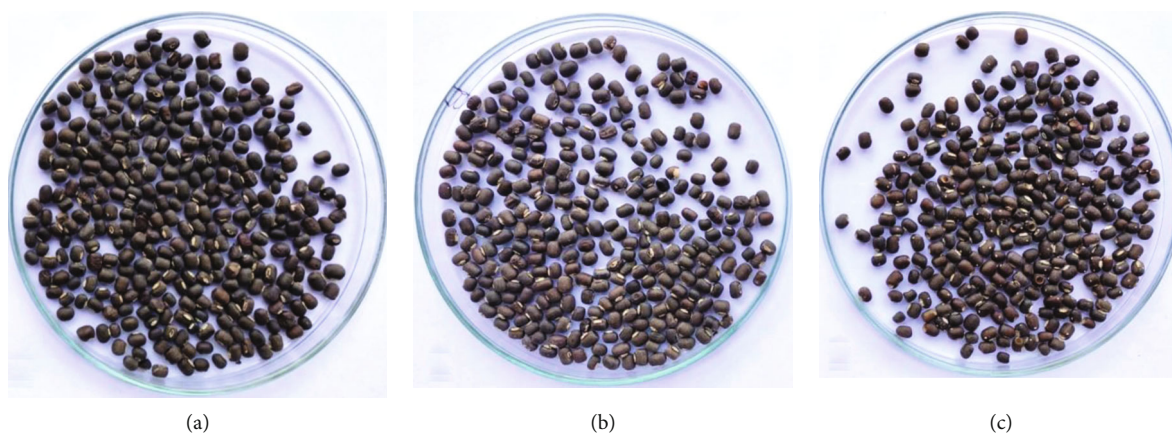


FIGURE 10: Black gram grain samples (a) before storage; (b) after controlled atmosphere storage; (c) after gunny bag storage.

during the storage period in silo and gunny bag storage. The sample from the top, middle, and bottom of the silo was taken and mixed uniformly for the quality analysis. It was observed that egg laid was 31% after 30 days of storage in a controlled atmosphere storage silo without carbon dioxide gas as mentioned in Table 2. After 30 days, carbon dioxide concentration was increased to 70% as a critical concentration for pupa mortality. Because pupa was the most tolerant stage of *Callosobruchus maculatus* insect, 70% CO<sub>2</sub> would be enough and efficient to kill all the stages of *Callosobruchus maculatus* inside the silo.

The eggs laid measured after 60 and 90 days of storage under elevated carbon dioxide are given in Tables 3 and 4. The mean value of egg laid was 31 and 29% observed after 60 and 90 days of storage under elevated carbon dioxide. Counted egg laid samples are kept separately for 20 to 28 days to determine the mortality. The egg laid percentage of grains in gunny bags was observed as 43.4, 40, and 43% after 30, 60, and 90 days of storage.

**3.6. Effect of Carbon Dioxide on Mortality of Egg inside the Silo.** Carbon dioxide effect on egg mortality was determined by the emergence of adult insects after 20 to 28 days of observation. Mortality of egg was observed as 3 and 3.8% in silo and gunny bag storage after 30 days of storage. After 30 days of storage, mortality of eggs increased significantly in silo grain samples whereas in gunny bags, the egg mortality was recorded as 4%. And the egg mortality observed after 60 days of storage was 100 and 4% in silo grains and gunny bag grains. Since the silo was filled with 70% carbon dioxide concentration after 30 days (developmental stage of *Callosobruchus maculatus*) of storage, the mortality of egg increased to 100% after 30 days and 60 days of storage. The result showed a significant increase in mortality of eggs observed when the grain was treated with carbon dioxide in bulk

grain. Gunny bag storage of black gram had maximum egg laid percentage after 90 days of storage at ambient condition. The mean adult emergence was observed as 96.2, 97.61, and 97.56% after 30, 60, and 90 days of gunny bag storage of black gram grains. A similar result was reported by authors in [26], the lethal effect of elevated carbon dioxide concentration on different life stages of *Callosobruchus maculatus* (Fab.). After 24 h of exposure, eggs and the adults attained 100% mortality whereas larvae died after 48 h of the exposure period compared to the day older larvae which needed 72 h of exposure time to achieve 100% mortality. Figure 8 depicts the microscopic image of *Callosobruchus maculatus* before CO<sub>2</sub> and after CO<sub>2</sub> treatment. The enhanced carbon dioxide treatment raised the atmospheric pressure inside the silo, which caused all of the eggs to shatter, causing 100% egg mortality.

**3.7. Effect of CO<sub>2</sub> Concentration on Water Retention Capacity of Black Gram Grains.** Figure 9 depicts the water retention vs. soaking time behaviour of black gram samples after 2 months of CA exposure. The retention curves of carbon dioxide-treated and control grains show similar behaviours in terms of achieving 100% retention.

In both control and CO<sub>2</sub>-treated grains, considerable water retention occurred within the first 1 hour at 60°C, reaching around 70% retention, and 100% retention was reached around 150 minutes later. The inhibition of the capillary outer layers of the pericarp, which promote the retention of water, can be related to the high rate of water retention at the initial stage. The high matric potential of the various elements of the grain could also be linked to a higher initial rate of water retention. There was no significant change in water retention between the control and CA-treated samples.

**3.8. Physiological Loss in Weight (%).** Physiological loss in weight of black gram measured after storing in a controlled atmosphere storage bin and gunny bag after 90 days of storage is given in Table 5. It was observed that minimum loss of 4.10% was observed in silo storage. Maximum loss of 22.56% was measured in the gunny bag storage after the storage period [27]. This may be due to moisture loss and insect infestation. Visible comparison of grain stored in the controlled atmosphere storage bin and gunny bag storage sample is shown in Table 5. Grain stored in the controlled atmosphere storage bin and gunny bags showed moisture loss which tends to decrease the mass of the individual grains resulting in weight loss in bulk grains. In a gunny bag, the grain weight loss was much higher than the grain stored in the silo. It clearly reveals that the insect infestation in the gunny bag storage caused the weight loss of bulk grains. A visible appearance of black gram grains before and after storage is shown in Figure 10. More damaged grains and eggs laid in the grains can be found in gunny bag storage. Shafique and Ahmad [28] studied the pulse beetle on grains and adult progeny developed from eggs laid on the cowpea for the resistivity study. The findings show that there was a positive and substantial association between eggs laid and grain weight loss, as well as a strong link between grain weight loss and grain damage. Murdock et al. [29] reported minimum loss when the bruchid-infested cowpea was stored for 6 months in a sealed drum. However, airtight storage was found effective in reducing quantitative loss [30].

#### 4. Conclusion

The effect of controlled atmosphere storage on different life stages of *Callosobruchus maculatus* in the black gram grain was investigated. The ideal gas concentration and time interval for *Callosobruchus maculatus* mortality at all life stages were determined. Black gram grains were stored in the silo, and their ability to absorb water as well as their physiological weight loss was examined using the optimum concentration and exposure period (70 percent CO<sub>2</sub> for 96 hours). CA has no effect on the black gram quality, as it retains its water-absorbing properties. Physiological loss in weight of black gram was observed at a minimum of 4.10% loss in silo storage. A maximum loss of 22.56% was observed in the gunny bag storage after the storage period. The use of controlled atmosphere storage (70% CO<sub>2</sub> concentration) increased *C. maculatus* mortality at all developmental stages. Carbon dioxide affects eggs and adults the most, while larvae and pupae are the least affected. The grains stored in a controlled atmosphere storage silo showed better grain quality and less weight loss compared to grains stored in gunny bags. Consequently, the technique is viewed as a possible substitute for fumigants. Due to the high CO<sub>2</sub> concentration and damage to interior tissues, the technique was successfully scaled up to treat huge volumes of grain continuously in a short amount of time, effectively killing insects.

#### Data Availability

The data used to support the findings of this study are included in the article. Should further data or information

be required, these are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

#### Acknowledgments

The authors thank Saveetha School of Engineering, SIMATS, Chennai, for the technical support to complete this research work. The authors appreciate the supports from Ambo University, Ethiopia.

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