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Effect of Elevated Levels of CO_2 on Biological Parameters of *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) in Stored Green Gram

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Authors' contributions

This work was carried out in collaboration among all authors. Author BR conceptualized the study, performed the methodology, did data curation and prepared the original draft of the manuscript. Author BVJ performed the methodology, reviewed and edited the manuscript. Authors AG, KSIL and CNR wrote and reviewed the manuscript and did statistical analysis. Authors KRM and MS edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

Green gram is a legume crop that provides essential nutrients and protein, especially in countries with a predominantly vegetarian population like India. However, post- harvest losses due to insect pests, particularly *Callosobruchus chinensis*, significantly affect stored green gram quality and quantity. In this context, an experiment was conducted to study the effect of elevated levels of CO_2 on biology of *Callosobruchus chinensis* (L.) in stored green gram. The adult beetles were exposed to eight different concentrations of CO_2 *viz*., 10, 20, 30, 40, 50, 60, 70, and 80%. The observations on fecundity, incubation period, larval-pupal period, adult emergence and adult longevity were recorded to assess the biology of *C. chinensis*. The results indicated that 80% CO_2 was the most effective treatment for controlling the pulse beetle, yielding the lowest fecundity (1.20 eggs), adult emergences (1.66 adults), and the shortest adult lifespan (3.50 days). However, no significant differences were observed across the CO_2 treatments for incubation period and larval-pupal periods.

Keywords: Callosobruchus chinensis; biology; modified atmosphere; carbon dioxide.

1. INTRODUCTION

In many developing nations, legumes play a vital role in providing dietary protein. Their cost-effectiveness has led them to describe as "economical protein source". Among the various types of legumes, mung beans (*Vigna radiata*) stand out for their exceptional protein content. These contain approximately 25% protein by weight, making them significantly more protein-dense than most grain crops. In India it is grown with an area, production and productivity of 5.13m ha^{-1}, 3.9 MT and 601 kg ha^{-1}, respectively (India stat, 2020-21). Stored grains, particularly pulses like green gram, face significant threats from various insect pests during storage and the primary pest is *Callosobruchus chinensis*. These pests can lead to substantial economic losses, affecting food security and the livelihoods of farmers. Understanding the importance of managing stored grain pests is crucial for maintaining the quality and safety of pulse crops [1] have reported seed damage levels reaching up to 93.33% in some pulse varieties, indicating severe infestation impacts. Furthermore, [2] investigated that damage caused by *C. chinensis* was particularly severe in green gram, with damage levels reported at 88.44%. Protecting food grains from insect infestation is now a days a challenge in agriculture. The management of pulse beetles, particularly *Callosobruchus chinensis*, has traditionally relied heavily on synthetic insecticides and fumigants. Excessive usage of these chemical pesticides causes a number of adverse consequences, including pest resistance, toxicity to non-target species, and impact to the ecosystem [3]. However, the chemical-based approach has increasingly come under scrutiny due to its numerous drawbacks and potential risks [4]. Continuous use of the same insecticides can lead to the development of resistance in *C. chinensis* populations [5]. This resistance reduces the effectiveness of these chemicals over time, necessitating the use of higher doses or different insecticides, which can further exacerbate environmental and economic issues. Moreover, plant-derived alternatives, though safer, often lack sufficient efficacy and common fumigants like methyl bromide and phosphine, despite their widespread use, pose risks to human health and the environment [6]. In response to these concerns, carbon dioxide has emerged as a promising alternative and it is an environmentally friendly and safe option for grain protection [7] addressing the need for effective pest control methods [8,9]. CO_2 deleteriously affects the normal body functions including digestive, respiratory, circulatory, nervous and hormonal systems of organisms [10]. Molecules of this gas permanently open the spiracles after entered in the insect body through these spiracles [11]. The use of carbon dioxide as control measure in stored grains is gaining traction due to its effectiveness, safety, alignment with organic farming practices and makes it a valuable tool in modern agricultural pest management strategies. Hence, the current study was conducted to understand the effect of different concentrations of CO_2 on the biology of *C. chinensis*.

2. MATERIALS AND METHODS

2.1 Mass Culturing of the Test Insect

The mass culturing of *C. chinensis* (L.) (Bruchidae: Coleoptera) was carried out in laboratory of Seed Research and Technology Centre (SRTC), PJTSAU, Rajendranagar, Hyderabad. To maintain the stock culture of *C. chinensis*, healthy green gram seed of variety MGG-295 was procured from Telangana State Seed Development Corporation (TSSDC), Nizamabad, Telangana. The mother culture of *C. chinensis* maintained at Seed Entomology Laboratory SRTC, Rajendranagar, Hyderabad. For mass culturing about 100 adult beetles were released with the help of aspirator into plastic containers having 1000 g of disinfested green gram seed and the containers were covered with muslin cloth held tightly with rubber bands to allow aeration and this is to allow free flow of air and also to prevent the escape of beetles. Twenty-five containers were maintained for mass culturing of test insect. The containers were kept undisturbed under laboratory conditions (32 ± 1 °C temperature and 75% relative humidity), till the emergence of F1 adults [12].

2.2 Design of Airtight Container and Injection of CO_2

To examine the impact of carbon dioxide (CO_2) treatment on *Callosobruchus chinensis*, we developed a custom-designed airtight containers (1L capacity) consist of two perforations with 3 mm diameter which serve as inlet and outlet holes. Nylon tubes of 3 mm diameter were inserted into the holes. 100 grams of thoroughly disinfested green gram seeds were placed individually into each container. This ensured a controlled environment for observing the effects of CO_2 on the target insect species. Prior to introducing carbon dioxide, flushed the existing air from the airtight container by opening the top outlet, then sealed it with a rubber cork. CO_2 was introduced using a cylinder fitted with an outlet tube, nozzle, and needle (Model BRG 0/1) at 2 kg/cm^2 pressure. Then injected the desired CO_2 concentration through the bottom inlet using a CO_2 cylinder, immediately sealing both inlet and outlet tubes with rubber corks to ensure gas retention within the container.

2.3 Determination of Carbon Dioxide Concentration in Air Tight Containers after Injection and Subsequent Experiment Conducted

To ensure whether the desired concentration of CO_2 released was maintained in the plastic container or not, it was checked by using CO_2/O_2 analyzer (PBI Dansensor, PBI 2006, Denmark). For determination of CO_2, the analyzer was calibrated with atmospheric air (20.9% O_2 & 0.03% CO_2) then the needle of the analyzer was introduced into the top outlet tube of the airtight container and the measuring button of the CO_2/O_2 analyzer was pressed. The carbon dioxide concentration present in the air tight container was displayed on screen within 10 seconds.

2.4 Assessment of Biological Parameters of *C. chinensis*

To study the effect of modified atmosphere with elevated levels of CO_2 on *C. chinensis,* twenty-seven airtight containers were filled with 100 g of disinfested green gram seed by releasing ten pairs of freshly emerged (0-24h) *C. chinensis* adults and these beetles are directly exposed to different carbon dioxide treatments *viz*., 10, 20, 30, 40, 50, 60, 70 and 80% with three replications of each treatment. After releasing the desired concentration of carbon dioxide into the containers they were made airtight by plugging them with robber corks and sealed with Teflon tape. Control was maintained by following the same procedure in plastic containers under normal laboratory conditions without exposing the seed to CO_2 concentrations. After exposure to various concentrations of CO_2, for three hours, the *C. chinensis* adults were removed and placed in plastic jars containing disinfested healthy seed. Ten specimen tubes were taken for each replication of the treatment and one grain with freshly laid eggs of *C. chinensis* was placed in tube and plugged with cotton. The data on incubation period (days), Larval-pupal period (days), adult emergence (Number) (total number of adults emerged from 45^{th} days after treatment to till the beetles cease to emerge from seeds), adult longevity (days) and fecundity were recorded [13]. The statistical methods described by Snedecor and Cochran [14] were adopted in the present investigation. The data were subjected to square root and angular transformation values wherever necessary and analysed by completely randomized design (CRD) and factorial completely randomized design (FCRD) as suggested by Panse and Sukhatme [15]. OPSTAT software was used for statistical analysis of the data.

3. RESULTS AND DISCUSSION

3.1 Effect of Elevated Levels of CO_2 on Fecundity

As observed from the Table 1 it was evident that all the CO_2 treatments were found to be significantly superior in reducing the fecundity of *C. chinensis* over the untreated control.

Significantly lowest mean number of eggs 1.20 was recorded at 80% CO_2 concentration, followed by 70% CO_2 and 60% CO_2 in which 3.33 and 3.40 eggs were recorded, which were on par with each other. However, the mean number of eggs at 50%, 40%, 30%, 20% and 10% CO_2 concentrations were observed as 4.86, 6.46, 8.13, 8.20 and 11.13, respectively. Significantly highest mean number of eggs were observed in untreated control, which resulted in 17.46 eggs. The findings are consistent with previous research where in fecundity of *C. chinensis* decreased with increasing CO_2 concentration [16]. Similarly, Emekci et al. [17] found that eggs production in *Rhyzopertha dominica* was reduced as CO_2 concentration increased, due to decrease in respiration rate. Exposure of Indian meal moth, *Plodia interpunctella* to an atmosphere with 96% CO_2 significantly reduced both egg production and egg viability [18]. Additionally, Bursell [19] noted that elevated CO_2 levels cause prolonged spiracle opening, leading to desiccation and mortality.

3.2 Effect of Elevated Levels of CO_2 on Egg Incubation Period and Larval-pupal Period

The incubation period and larval-pupal period of *C. chinensis* varied between 3.83 to 4.33 days and 24.66 to 27.16 days, respectively, across different treatments with varying CO_2 concentrations in treated seeds. However, no significant difference was observed among the treatments in both the cases. The shortest and longest incubation periods, 3.83 and 4.33 days, were observed at 10%, 60% and 80% CO_2 concentrations (Table 1). Furthermore, the shortest larval-pupal period of 24.66 days was observed in the untreated control, while the longest period of 27.16 days was recorded at a 40% CO_2 concentration. The larval-pupal period was found to follow the normal ranges as similar in untreated control. Since, *C. chinensis* is an internal feeder and the developmental of larval-pupal period probably occurs inside the grain and is protected from any CO_2 concentrations. Similar findings reported by Tejender and Sharma [20], with increase in CO_2 concentration at 350, 400 and 450 ppm, egg incubation and larval-pupal period of *Spodoptera litura* decreased at all temperatures.

3.3 Effect of Elevated Levels of CO_2 on Adult Emergence

Adult emergence was significantly reduced with minimum mean number of adults 1.66 emerged in seeds treated with 80% CO_2 concentration followed by 70% CO_2 with 4.00 adults which was on par with 60% CO_2 4.33 adults. However, 9.66, 11.33, 14.00, 25.00 and 25.33 adults in seeds treated with CO_2 concentrations at 50%, 40% 30%, 20% and 10% CO_2 concentration (Table 1). All the CO_2 treatments were found to be superior over the untreated control which recorded highest 37.00 adults. Studies conducted by Carli et al. [21] revealed that at 20% CO_2 concentration highest mean number of adult emergences was recorded and it decreased with increase in CO_2 concentration till 80%. Earlier, Carvalho et al. [22] recorded no adult emergence of *Sitophilus zeamais* and *Sitophilus oryzae* at 90% CO_2 concentration. Gunasekaran and Rajendran [23] observed the sub lethal effects of carbon dioxide against *Stegobium paniceum* and *Lasioderma serricorne* and they reported that treatment of adults with 30% and above CO_2 concentrations caused a significant reduction in progeny production and adverse effects on the multiplication potential of the survivors. Similarly, the increase in carbon dioxide concentration caused decrease in the adult emergence in many insects as reported by Mohamed et al. [4], Spratt [24], Jayashree et al. [25], Divya et al. [26]. Increase in carbon dioxide concentration caused decrease in the adult emergence of pulse beetle, *C. chinensis* it may be due to cell and mitochondrial membrane to become more permeable to cell damage and decrease in ATP production which ultimately effect on adult emergence.

3.4 Effect of Elevated Levels of CO_2 on Adult Longevity

The longevity of adults ranged from 3.50 to 7.73 days in seeds exposed to with different concentrations of CO_2 treatments. The CO_2 concentration at 80% resulted in lowest lifespan of about 3.50 days. Adult longevity of 4.33, 5.50 and 6.20 days were observed at 70%, 60% and 50% CO_2 concentrations, respectively. Adult longevity of 6.50 days was observed at 40% CO_2, which was on par with 30% CO_2 6.53 days followed by 20% CO_2 which resulted 7.00 days. However, highest adult life span of 7.66 days was observed in untreated control which was on par with 10% CO_2 i.e. 7.73 days, the treatments *viz.,* 10% CO_2 and untreated control were found to be least effective in reducing adult lifespan (Fig. 1) The results are in accordance with [11], who reported 12.00- 25.00% of *C. chinensis* adults survived two to three days when exposed to 20% CO_2 concentration in stored red gram.

Table 1. Effect of elevated levels of CO_2 on biology of *Callosobruchus chinensis*

CO_2 Concentration (%)	Incubation period (days)	Larval-pupal period (days)	Adult emergence (days)	Fecundity (eggs/female)
10	3.83 (2.19)	26.10(5.20)	25.33 (5.13)	11.13 (3.48)
20	4.16 (2.27)	26.41(5.23)	25.00 (5.09)	8.20 (3.03)
30	4.00 (2.23)	25.16 (5.11)	14.00 (3.87)	8.13 (3.02)
40	4.00 (2.23)	27.16 (5.30)	11.33 (3.50)	6.46 (2.73)
50	4.13 (2.66)	25.86 (5.18)	9.66 (3.26)	4.86 (2.42)
60	3.83 (2.19)	27.03 (5.29)	4.33 (2.30)	3.40 (2.09)
70	3.93 (2.22)	27.03 (5.29)	4.00 (2.22)	3.33 (2.08)
80	4.33 (2.30)	26.33(5.22)	1.66 (1.60)	1.20 (1.48)
Untreated Control	4.10 (2.25)	24.66 (5.06)	37.00 (6.16)	17.46 (4.29)
CD (*p*=0.05)	N. S	N. S	0.34	0.18
CV (%)	2.98	1.33	4.78	3.08

The values in parentheses are transformed values

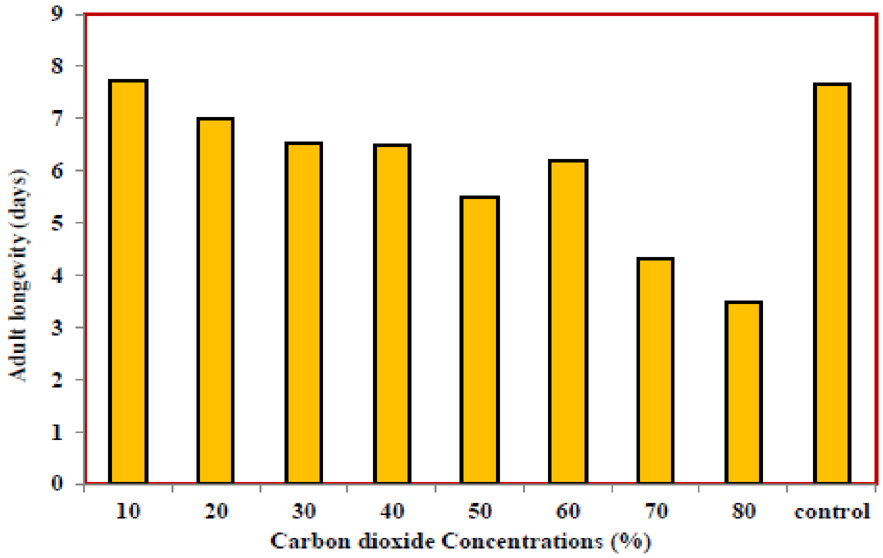

Fig. 1. Effect of elevated levels of CO_2 concentrations on adult longevity (days) of pulse beetle *C. chinenesis*

Most insects are more easily killed with higher carbon dioxide concentrations [27]. Similarly, Manojkumar et al. [28] revealed that complete mortality of adults within five days at 40% CO_2 concentration on lesser grain borer, *R. dominica* [29], observed percent mortality within six days when developmental stages of laboratory strains of *Trogoderma granarium* exposed to 60% CO_2 concentration. The increase in carbon dioxide concentration reduced the adult life span which may be due to damage of wax coat on the insect cuticle by sorption and abrasion and may also due to internal physiological effects.

4. CONCLUSION

The research clearly shows that increasing CO_2 levels, particularly at 80% concentration, substantially reduces the reproductive capacity and survival of *C. chinensis*. This is evidenced by the marked decrease in fecundity, adult emergence and adult longevity observed at higher CO_2 levels. These studies help to bridge the gap between scientific research and practical application in agriculture and food storage, ultimately contributing to more efficient and environmentally friendly pest management strategies. Future research could focus on optimizing the application of this method in various storage conditions and exploring its effectiveness against other stored grain pests.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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