Efficacy of modified atmosphere with elevated 
CO$_2$ on pulse beetle, *Callosobruchus chinensis* in 
Redgram

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**Abstract**

Investigations were carried out to evaluate the effect of modified atmosphere with carbon dioxide against pulse beetle, *Callosobruchus chinensis* was studied by directly exposing the adult bruchids to six concentrations of CO$_2$, viz., 5, 10, 20, 40, 60 and 80%. The data was recorded on adult mortality and subsequent progeny development of the live adults exposed to different elevated CO$_2$ concentrations. The results indicated that among different treatments, 60% and 80% CO$_2$ concentrations caused 100 percent mortality of adults even at one day after treatment. Though some of the adults exposed to 40% CO$_2$ concentration survived for one or two days but they become sterile and did not lay eggs further. Thus modified atmosphere with elevated CO$_2$ concentrations of 40% and above were found to be the superior treatments for the management of *C. chinensis* in stored redgram.

**Keywords:** *Callosobruchus chinensis*, modified atmosphere, carbon dioxide, redgram storage

**Introduction**

Pigeonpea, *Cajanus cajan* (Linnaeus) Milsp. is an important pulse crop grown in India next to chickpea and occupied about 92 per cent of world’s pulse production. The losses caused by storage pests are more in case of pulses compared to cereals (Huysamans, 1970) and losses due to improper storage have been reported to be higher in pigeon pea than in the other commonly grown legumes (Mukherjee et al., 1970) [2]. Gujar and Yadav (1978) [3] reported that huge losses of about 55-60% in seed weight and 45.5 to 66.3% in protein content in green gram due to bruchids and the infested seeds become unfit for human consumption. Quantitative and qualitative losses occur in pigeon pea seeds during storage, due to various insect pests and the pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) is the most destructive species of stored pulses in India (Singh and Jambunadham, 1990) [4]. The use of chemical pesticides and fumigants is the common practice to contain storage pests especially to avoid post harvest losses in stored pulses. Recently due to the withdrawal of methyl bromide fumigant in stored products to protect the earth from global warming, environmental pollution, high amounts of left over pesticide residues in stored commodities and development of phosphine resistance in major storage insect pests created an urgent need to search for the alternative strategies in stored grain pest management. In this context, use of modified atmosphere through the introduction of carbon dioxide (CO$_2$) has been considered as safe method to control storage pests. The use of this technique has been in vogue in developed countries as early as in 1975. Bailey and Banks (1975) [5] reviewed the effect of CO$_2$ atmosphere on stored product insects.

Storage of seeds with CO$_2$ rich modified atmosphere is considered as the safest method to control insects from stored grains which leave no harmful residues after the treatment of the stored grain with CO$_2$. The CO$_2$ treatment is safe, residue free and also accepted in the organic market (Bera et al., 2008) [6] and also approved by Environmental Protection Agency (EPA), USA. Though there were 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 Rajasri et al., 2015 [7,8,9], 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. Hence the current study was taken up to evaluate the efficacy of modified atmosphere storage with elevated levels of CO$_2$ on pulse beetle *C. chinensis* longevity,
fecundity and progeny production in stored redgram. More specifically to evaluate the lethal exposure period required to cause adult mortality of *C. chinensis* under 5% to 80% CO₂ replacement of air in the air tight containers.

**Materials and Methods**

The adult bruchid beetles required for conducting the studies were obtained by mass rearing the pest under laboratory conditions. The freshly emerged adult bruchids obtained from the laboratory cultures were used in the study. Thirty freshly emerged *C. chinensis* adults were transferred to plastic airtight containers which was provided with two perforations of 3 mm diameter which serve as an inlet and outlet holes and nylon tubes of 3 mm diameter were inserted into the holes. Rubber corks of 2.95 mm diameter which exactly seal the inlet and outlet tubes were used to plug them after filling the containers with desired concentrations of CO₂ and thus the entire system was made airtight after releasing the CO₂ into the containers. Adults were directly exposed to six different concentrations of CO₂ viz., 5%, 10%, 20%, 40%, 60%, and 80% and then the adults were transferred to glass rearing tubes with disinfested healthy pigeonpea seeds. The mortality of beetles were recorded at 24 hours interval for about seven days and were discarded daily. Percent adult mortality was calculated by using the following formula.

\[
\text{Percent adult mortality} = \frac{\text{Number of insects dead}}{\text{Number of insects released}} \times 100
\]

The egg laying capacity of the female adult exposed to different concentrations of CO₂ was recorded after seven days of exposure and the seed was kept under laboratory conditions for about 25-30 days for recording the F1 progeny production. The data were subjected to transformation wherever necessary and analysed by adopting CRD and factorial completely randomised design (Panse and Sukhatme, 1978) [10].

**Results and Discussion**

**Adult mortality of *C. chinensis* exposed to elevated CO₂**

The mortality of bruchid obtained with the treatment of high concentrations of CO₂ revealed that, significantly low mortality was obtained with 5% (67.48%) and 10% (69.93%) CO₂ concentrations respectively and were on parity with each other (Fig 1). Significant variation was recorded with the exposure of adults to the higher concentrations viz., 20%, 40%, 60%, and 80% with mortality of 73.84%, 80.85%, 88.47% and 96.56% respectively compared to significantly lowest adult mortality of 25.33% obtained in control. The overall mean adult mortality studies with different exposure periods and of different concentrations CO₂ (Table 1) and their interaction effect indicated that among the different concentrations, significantly lowest adult mortality was obtained with 5% (67.48%) and 10% (69.93%) concentrations and they were on par with each other. Exposure of adults to the higher concentrations viz., 20%, 40%, 60%, and 80% showed significant variation by recording 73.84% mortality with 20% concentration and it further increased to 80.85% with 40% concentration. At 60% concentration 88.47% mortality was obtained while among all the concentrations, highest adult mortality of 96.56% was obtained with 80% concentration. Exposure of adults to lowest period of 30 min recorded lowest adult mortality of 65.04% followed by 45 min with 69.85% mortality and increase in exposure periods resulted in increased mortality rates. Significantly highest mortality of 78.30% was recorded with longest exposure periods of 90 min followed by 73.78% and 71.93% mortalities obtained with 75 min and 60 min exposure periods, respectively.

The interaction effect of concentrations and exposure periods also showed significant variations, among all the interactions. Highest mortality of 100% was recorded with the exposure of adult insects to 60% and 80% concentrations respectively for 90 min while lowest adult mortality of 60.95%, 61.90%, and 64.28% was recorded by the exposure to low concentrations of 5, 10 and 20% respectively with low exposure time of 30 min. The results are in agreement with the findings of Ofuya and Reichmuth (1993) [11] who concluded that the mortality of *C. maculatus* to CO₂ exposure was significantly influenced by CO₂ concentrations. Harein and Press (1968) [12] confirmed that insect mortality of *T. castaneum* was increased with increase in CO₂ concentration. Spratt et al., (1985) [13] subjected several developmental stages of laboratory strains of *Trogoderma granarium* to 60% CO₂ and they observed 100% mortality of adults within 6 days. Mannad et al., (1999) [14] and Bera et al., (2004) [15] stated that modified atmosphere system involving CO₂ concentrations ranging from 20 to 80 % in paddy effectively controlled rice weevil and lesser grain borer. Krishnamurthy et al. (1993) [16] stated that at 80% CO₂, 100 % mortality of *T. castaneum* and *S. oryzae* adults were observed. According to Mbata et al. (2000) [17] complete mortality of *Callosobruchus subinnotatus* adults occurred when treated with hypoxic conditions. Zhou et al. (2001) [18] found that elevated CO₂ reduced the O₂ consumption of *Platynotastaltana*. The oxygen consumption rate decreased by 62% in 20% CO₂ and by 73% in 79% CO₂. The rate of respiration of *Tribolium confusum* adults as measured by CO₂ output was severely depressed during the initial hours of exposure to elevated CO₂ concentrations (Ali Nazeer, 1971). [19] Empirical mortality data have shown that levels of CO₂ toxic to insects are generally above 20% (Banks and Annis, 1990) [20] and Zhou et al., 2001 [18]. Most of the stored grain pests are more easily killed with higher CO₂ concentrations up to 100% (Jay, 1984) [21]. Divya et al., 2015 [8] reported that *C. chinensis* adults exposed to elevated Concentrations of 40% and 50% CO₂ not only checked seed infestation but also checked the progeny production of pulse beetle and reduced the weight loss of seed compared to normal atmosphere.

**Effect of different concentrations of CO₂ on fecundity of *C. chinensis* in stored redgram**

The bruchid adults exposed to low concentrations of CO₂ of 5% seems to be not affected as indicated by the survival of adults up to seven days and laid significantly more number of eggs (41.00) on par with control (56.00) as depicted in the Fig 2. The fecundity of adults was totally arrested when they were exposed to high concentrations of CO₂ (40%, 60%, 80%). From the results it was evident that the adults who could able to withstand and survive at low concentrations of CO₂ for about five days and above laid significantly more number of eggs (41 and 28 eggs/100 seeds at 5 and 10% concentrations, respectively) while those exposed to higher concentrations of 40%, 60% and 80% CO₂ died immediately within a day or two days and did not record any fecundity indicating the susceptibility of *C. chinensis* adults to high concentrations of CO₂ Gunashekaran and Rajendran (2005) [22] opined that progeny production was affected by CO₂ treatment of adults of *Stegobium panicum* and *Lastioderma serricorne*.
**Effect of different concentrations of CO\textsubscript{2} on adult emergence of C. chinensis in stored redgram**

Pulse beetle adults directly exposed to low concentrations of 5% CO\textsubscript{2} recorded low adult mortality laid more no. of eggs and showed high adult emergence (14.00) while at the next 10% higher CO\textsubscript{2} concentration, the adult emergence had significantly comedown to 9.00 and still reduced to 2.00 at 20% and all the next higher concentrations viz., 40%, 60% and 80 % were found to be fatal to the adults and no adult emergence was recorded from all the higher concentrations (Fig. 3). From the results it was clear those CO\textsubscript{2} concentrations of 40% and above were detrimental to C. chinensis which affected the fecundity and adult emergence in redgram.

The present research findings are in accordance with the reports of Rajasri et al. (2015) [9] who opined that the modified atmosphere with higher concentrations of CO\textsubscript{2} was effective in preventing the insect damage due to grain moth, S. cerealella in stored rough rice without affecting seed viability and quality of rice seed up to nine months of storage and Gunashekaran and Rajendran (2005) [18], who reported the lethal effects of CO\textsubscript{2} (with 30% and above CO\textsubscript{2} concentrations) against S. panicum and L. serricone adults caused a significant reduction in progeny development indicating the adverse effect of elevated CO\textsubscript{2} on the multiplication potential of the survivors.
Table 1: Effect of different concentrations and exposure periods of CO₂ on mean adult mortality of *C. chinensis*

<table>
<thead>
<tr>
<th>CO₂ Concentration (%)</th>
<th>30min</th>
<th>45min</th>
<th>60min</th>
<th>75min</th>
<th>90min</th>
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<td>5</td>
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<td>(51.30)</td>
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<td>68.57</td>
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<td>72.38</td>
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<td>(55.88)</td>
<td>(56.58)</td>
<td>(58.26)</td>
<td>(62.23)</td>
<td>(56.96)</td>
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<td>20</td>
<td>64.28</td>
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CD(\(\alpha=0.05\))  
Sem±  
Concentration (\(F_1\)) 1.68 0.86  
Exposure period (\(F_2\)) 1.42 0.72  
Interaction (\(F_1XF_2\)) 3.77 1.92

Conclusions
This study revealed that the minimum lethal exposure period of 90 minutes was required for 100% mortality of pulse beetle, in the modified atmosphere with high concentrations of CO₂, more than 60% in the airtight containers. The adult pulse beetles exposed to CO₂ rich atmosphere (40% and above) have become sterile which prevented the egg laying and progeny production in stored redgram. Hence, the storage of redgram seed in airtight containers under modified atmosphere storage technique with high concentration of > 40% carbon dioxide was proved to be an effective, safer alternative and eco-friendly strategy for the management of pulse beetles in stored redgram.

References


