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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) [1] 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% CO_2 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 out let 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 CO_2 and thus the entire system was made airtight after releasing the CO_2 into the containers. Adults were directly exposed to six different concentrations of CO_2 *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 CO_2 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 CO_2

The mortality of bruchid obtained with the treatment of high concentrations of CO_2 revealed that, significantly low mortality was obtained with 5% (67.48%) and 10% (69.93%) CO_2 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 CO_2 (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 CO_2 exposure was significantly influenced by CO_2 concentrations. Harein and Press (1968) [12] confirmed that insect mortality of *T. Castaneum* was increased with increase in CO_2 concentration. Spratt *et al.,* (1985) [13] subjected several developmental stages of laboratory strains of *Trogoderma granarium* to 60% CO_2 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 CO_2 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% CO_2, 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 hypercarbic atmospheres. Zhou *et al.* (2001) [18] found that elevated CO_2 reduced the O_2 consumption of *Platynotastaltana*. The oxygen consumption rate decreased by 62% in 20% CO_2 and by 73% in 79% CO_2. The rate of respiration of *Tribolium confusum* adults as measured by CO_2 output was severely depressed during the initial hours of exposure to elevated CO_2 concentrations (Ali Niazee, 1971). [19] Empirical mortality data have shown that levels of CO_2 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 CO_2 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 CO_2 on fecundity of *C. chinensis* in stored redgram

The bruchid adults exposed to low concentrations of CO_2 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 CO_2 (40%, 60%, 80%). From the results it was evident that the adults who could able to withstand and survive at low concentrations of CO_2 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% CO_2 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 CO_2. Gunashekaran and Rajendran (2005) [22] opined that progeny production was affected by CO_2 treatment of adults of *Stegobium panicum* and *Lasioderma serricorne*.

Effect of different concentrations of CO_2 on adult emergence of *C. chinensis* in stored redgram

Pulse beetle adults directly exposed to low concentrations of 5% CO_2 recorded low adult mortality laid more no. of eggs and showed high adult emergence (14.00) while at the next 10% higher CO_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 CO2 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_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_2 (with 30% and above CO_2 concentrations) against *S. panicum* and *L. serricorne* adults caused a significant reduction in progeny development indicating the adverse effect of elevated CO_2 on the multiplication potential of the survivors.

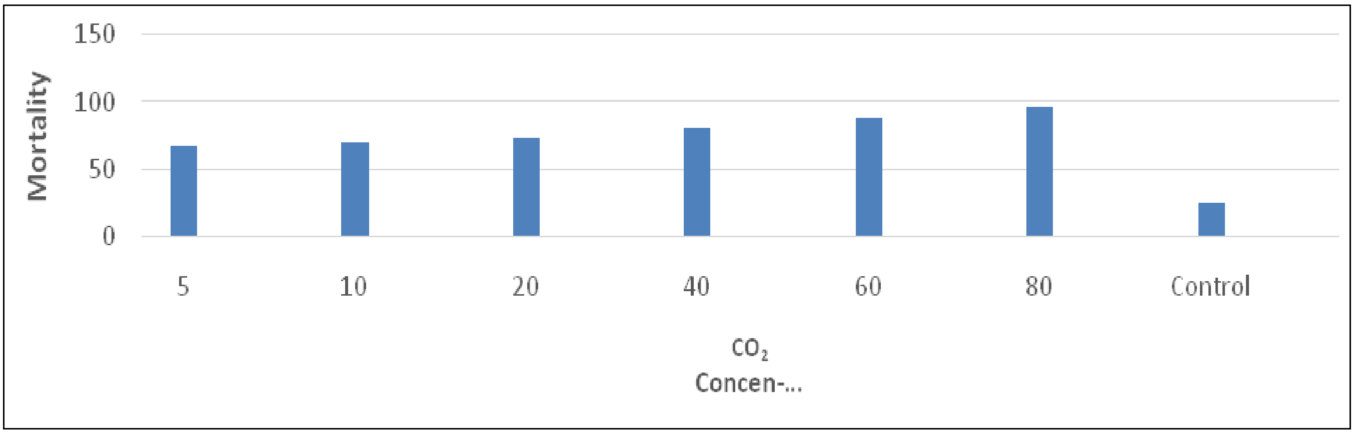

Fig 1: Effect of different concentrations of CO_2 on mean adult mortality of *C. chinensis*

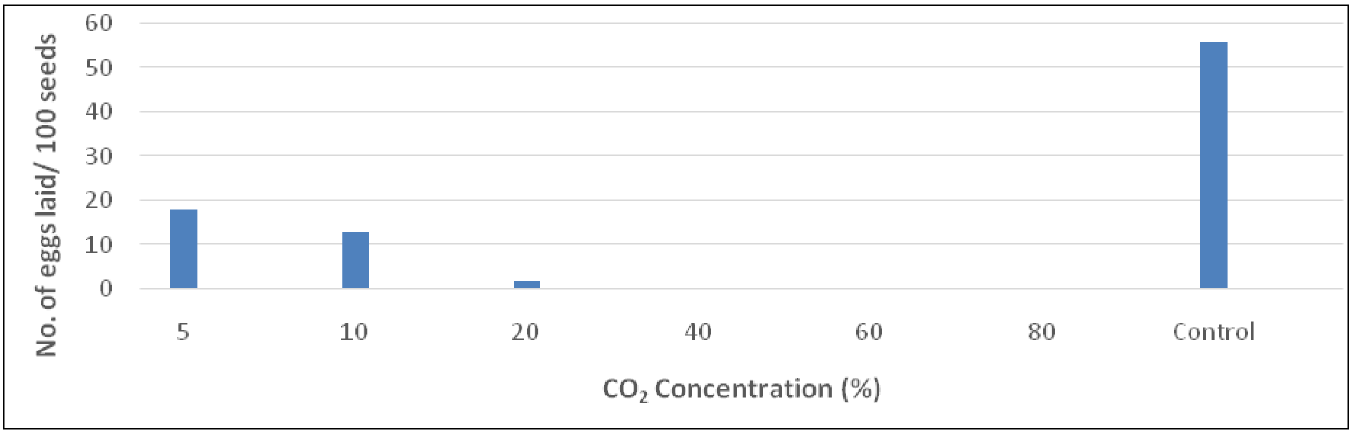

Fig 2: Effect of different concentrations of CO_2 on mean No. of eggs laid by *C. chinensis* / 100 seeds

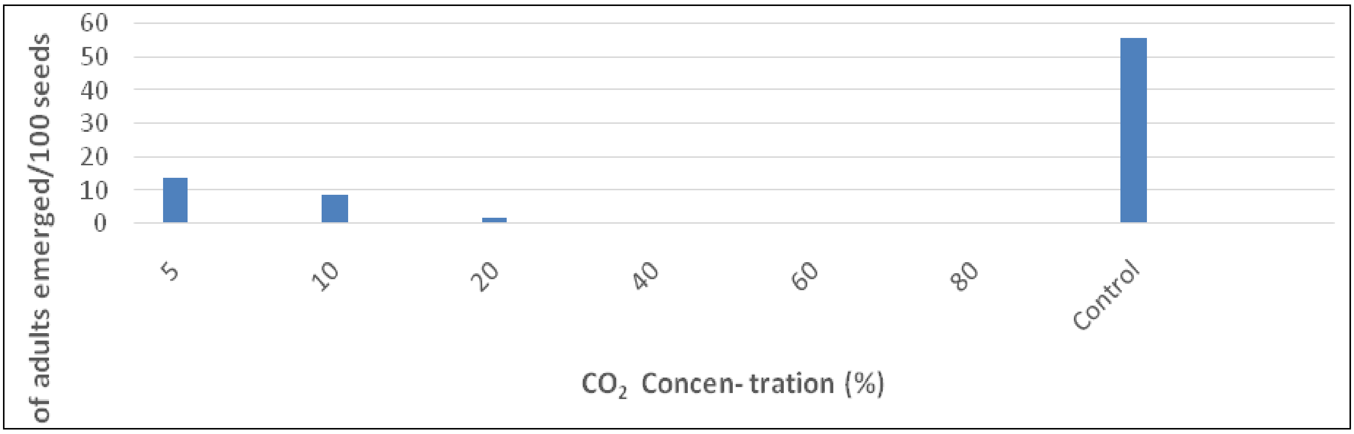

Fig 3: Effect of different concentrations of CO_2 on mean No. of adult of *C. chinensis* emerged / 100 seeds

Table 1: Effect of different concentrations and exposure periods of CO_2 on mean adult mortality of *C. chinensis*

CO_2 Concentration (%)	30min	45min	60min	75min	90min	Mean
5	60.95 (51.30)	66.51 (54.65)	66.56 (54.65)	67.61 (55.32)	75.71 (61.51)	67.48 (55.48)
10	61.90 (51.86)	68.57 (55.88)	69.71 (56.58)	72.38 (58.26)	77.13 (62.23)	69.93 (56.96)
20	64.28 (53.27)	72.85 (58.61)	73.99 (59.31)	76.66 (61.09)	81.42 (64.52)	73.84 (59.36)
40	72.37 (58.29)	78.57 (62.39)	80.71 (63.92)	84.04 (66.44)	88.57 (70.23)	80.85 (64.25)
60	76.66 (61.10)	82.38 (65.25)	90.47 (72.07)	92.85 (74.51)	100.0 (90.00)	88.47 (72.58)
80	93.80 (75.57)	94.75 (76.94)	96.66 (81.38)	97.61 (82.74)	100.0 (90.00)	96.56 (81.32)
Control	25.23 (30.11)	25.23 (30.11)	25.23 (30.11)	25.23 (30.11)	25.33 (30.11)	25.33 (30.11)
Mean	65.04 (54.5)	69.85 (57.69)	71.93 (59.71)	73.78 (61.21)	78.30 (66.94)	
		CD(P=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 CO_2 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.

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