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# Effect of solid carbon dioxide on pulse beetle, *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Chrysomelidae) in Redgram: A way to ecostore pest management

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## Abstract
The impact of solid $CO_2$ on the developmental biology of pulse beetle in red gram was carried out at the Centre for Agro-climatic Studies, College of Agriculture, Raichur, Karnataka. The results are quiet promising and clearly affected the oviposition at 90 and 70 percent $CO_2$ concentrations and persisted upto 30 days after treatment whereas, untreated check reported 51.80 eggs per 10 seeds. However, 50 percent $CO_2$ treatment was not successful in inflicting complete mortality. Similarly, lowest damage of 13.93 percent was noticed in 90 percent $CO_2$ treatment, which was on par with 70 and 50 percent $CO_2$ treatments with no seed weight loss in 90 and 70 percent $CO_2$ treatments. Based on these parameters, both 90 and 70 percent $CO_2$ concentrations were found effective in hindering the development of the pulse beetle without affecting the quality of seeds and its germination.

**Keywords:** Dry ice, developmental biology, eco-friendly management, pulse beetle and storage entomology

## Introduction
The extent of damage caused by stored product insects have always been a major concern since humanity began storing grain for future use. Post-harvest losses alone account for about 10 percent of total food grains due to unscientific storage, insects, rodents, microorganisms *etc*. Under Indian scenario, annual storage losses have been estimated to be 14 million tonnes accounting for around ₹7,000 crore out of which insects alone account for nearly ₹1,300 crores. From these post-harvest losses, storage insects alone account for 2.0 to 4.2 percent (Anon., 2023).
Pulse beetle, *Callosobruchus chinensis* (L.), is one of the important pests of grain legumes both in storage and field. As reported by Gujar and Yadav (1978) [5], around 55- 60 percent losses in seed weight and 45.5 to 66.3 percent losses in protein content was due to bruchid infestation in storage rendering them unfit for human consumption. Conventional fumigants and residual insecticides are commonly used to combat stored grain pests. In the recent years, consumer awareness pertaining to health hazard from residual toxicity and the growing problem of insect resistance to these conventional insecticides has provoked the researchers to search for alternative strategies for stored grains protection. In this context, the current investigation explores the utilization of solid carbon dioxide for managing stored grain pest as an alternative pest control technique. In the recent past, carbon dioxide ($CO_2$) has received considerable attention in disinfesting stored foodstuffs, particularly durable products (Bailey and Banks, 1980 and Bell and Armitage, 1992) [2, 3]. The present study acts as baseline study for standardization of the solid $CO_2$ concentration required to manage pulse beetle as well as impact of it on the developmental biology of test insect. Since the use of the $CO_2$ as a compressed gas in household management of storage pests is a cumbersome process, the solid form of $CO_2$ *i.e.*, dry ice was used as media to deliver the active ingredient under current experimental setup.

## Materials and Methods
The current investigation was carried out at Centre for Agro climatic studies, Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Raichur

Karnataka. The details of the material utilized and the adopted methodology is presented hereunder.

**Maintenance of pure culture of pulse beetle on red gram seeds**

The pulse beetle culture was the prime necessity for conducting the further research activities. The pure culture of pulse beetle was established by collecting adults from the infested redgram seeds which were subsequently released into transparent plastic container (2000cc) containing redgram seeds. The opening of these containers were covered with muslin cloth and fastened tightly with the help of rubber band. Fresh seeds were provided regularly and exposed separately for the multiplication of insects at room temperature of 27 °C. The adults emerged from this culture were used throughout the period of experimentation.

**Dose standardisation**

Dry ice was procured from Hyderabad in insulated container. The procured dry ice was stored in deep freezer at the temperature -80 °C for further use. The quantity of dry ice required to obtain 50, 70 and 90 percent was calculated through trial-and-error method. Dry ice was in the form of small pellets and weighed on an electronic balance. The weighed dry ice was placed in 50 ml PP or HDPE vials (5.5 cm × 4.3 cm) with perforations, which was placed in the 3000cc transparent container with redgram grains. The dry ice was placed in the small vials to avoid direct contact with the seeds in the test containers.

**Design of airtight container**

To estimate the concentration of $CO_2$ inside the containers, it is necessary for the container to be airtight. Hence, a perforation of 3 mm diameter was made on the container lid and rubber cork of 2.95mm diameter which exactly sealed the perforation was used as a plug. This granted the access for measuring the concentration of gas inside the container without much error. The same procedure was followed for Zip-lock polythene bags of 700 gauge. The rubber cork was fitted on the side of the bags and the concentration of gas inside the bag was measured.

**Determination of $CO_2$ concentration in containers after application of dry ice**

The concentration of the gas inside the container was measured by using CheckMate II gas analyzer (PBI Dansensor, PBI 260303, Denmark). For determination of $CO_2$ concentration, the instrument was calibrated with atmospheric air (20.95% $O_2$ and 0.03% $CO_2$), then the needle of the analyzer was introduced into the rubber cork sealed perforation on the lid of the airtight container and the measuring button of the analyzer was pressed. The concentration of the $CO_2$ and $O_2$ present in the container was displayed on the screen was recorded.

**Study of efficacy of solid carbon dioxide (dry ice) against pulse beetle**

Efficacy of solid carbon dioxide (dry ice) was determined against pulse beetle at three different doses - 2g, 4g and 6g - in zip-lock bags of 700 gauge. 20 pairs of adult pulse beetles were introduced into the plastic bags with 1 kg of disinfested red gram seeds and allowed for multiplication for 15 days. On the 16$^{th}$ day different doses of solid carbon dioxide *viz*., 90, 70 and 50 percent was imposed in respective bags. These treatments were compared to untreated control. These treatments were replicated five times to fit them statistically. The bag was left open till the pressure in the bag was balanced and later was sealed tight and left undisturbed. On the 31$^{st}$, 41$^{st}$, 51$^{th}$ days of release of the pulse beetles, observations pertaining to damage parameters were taken by destructive sampling.

**Observations recorded**

**Number of eggs laid and percent seed damage**

From each treatment, ten seeds were drawn randomly and number of eggs laid were counted with the help of stereo binocular microscope. Similarly, the percent seed damage was estimated using the formula

$$\text{Per cent seed damage} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds}} \times 100$$

**Percent weight loss**

The damage by the pulse beetle resulted in the weight loss, which was computed using the following formula as suggested by Harris and Limblad (1978) [6].

$$\text{Per cent seed weight loss} = \frac{\text{O.W.} - \text{C.W.}}{\text{O.W.}} \times 100$$

Where,
O.W.= Original weight on dry weight basis
C.W.= Current weight on dry weight basis

**Percent adult mortality**

A sample of 100g was drawn from the treatment containers randomly and placed in freezer to to make the adults inactive for further counting and percent adult mortality was calculated at an interval of 24, 48 and 72 hours.

**Seed germination and analysis of enzyme activity**

Seed germination test was conducted to estimate the impact of dry ice treatment on the viability of the stored seeds. Germination test was conducted using 100 seeds each from respective treatment and placed in between germination paper and incubated in germination room. The germination room was maintained at 25 ± 1 °C temperature and 90±2 percent relative humidity. At the end of seventh day, the number of normal seedlings in each replication were counted and the germination computed and expressed in percentage. (ISTA, 1999). Similarly, the activity of the dehydrogenase activity was estimated to confirm the viability of the seeds.

**Statistical analysis**

The entire experiment was implemented in completely randomized design and SPSS (IBM v22) software was used for analysing the results. The means values were transformed and subjected to the Duncans' multiple range test and summarized with standard error and critical difference.

**Results and Discussion**

The results of the current investigation revealed the efficacy of the solid $CO_2$ to effectively manage the incidence of pulse beetle under storage conditions. The impact of solid $CO_2$ on the oviposition by pulse beetle in the red gram seed revealed that, in the 90 and 70 percent $CO_2$ concentrations, egg laying was completely inhibited up to 30 days after treatment as compared to the untreated check (51.80eggs/10 seeds) (Table 1). From the data it is inferred that the 90 and 70 percent $CO_2$ concentrations were successful in causing cent percent mortality of the adult resulting in lack of oviposition by the adults. However, 50 percent $CO_2$ treatment was not successful in causing complete mortality of the insect. Subsequently, the highest percent seed damage at 10 days (Table 2) after treatment was noticed in the untreated check (23.50%) followed by least percent seed at 90 percent $CO_2$ treatment (13.80%) which was on par with 70 and 50 percent $CO_2$ treatments (13.89 and 14.33%, respectively). The same trend continued even at 20 days after treatment. At 30 days after treatment the highest seed damage was noticed in the untreated check (32.40%) and the lowest damage in the 90 percent $CO_2$ treatment (13.93%) and was on par with 70 and 50 percent $CO_2$ treatments with14.00 and 14.88 percent, respectively. The lack of pest load in the higher concentrations *i.e.*, 90 and 70 percent $CO_2$ treatments reflected in lack of seed loss. However, 0.56 percent seed weight loss was noticed in relatively lower concentrations of 50 percent. The highest weight loss was recorded in the untreated check (2.31) at 10DAT with same trend continued at 20 and 30DAT.

**Table 1:** Effect of solid carbon dioxide on oviposition by pulse beetle in red gram seeds

| Sl. No. | Treatments | Number of eggs per 10 seeds | | | |
|---|---|---|---|---|---|
| | | One day before treatment | 10 DAT | 20 DAT | 30 DAT |
| 1. | Solid $CO_2$ @ 6 g (90% $CO_2$) | 14.00 (3.72) | 14.20 (3.75)[a] | 14.40 (3.75)[a] | 14.40 (3.77)[a] |
| 2. | Solid $CO_2$ @ 4 g (70% $CO_2$) | 14.00 (3.73) | 14.40 (3.79)[a] | 14.60 (3.81)[a] | 14.60 (3.80)[b] |
| 3. | Solid $CO_2$ @ 2 g (50% $CO_2$) | 14.80 (3.83) | 20.40 (4.51)[b] | 20.60 (4.53)[b] | 21.40 (4.61)[b] |
| 4. | Untreated check | 14.20 (3.73) | 38.60 (6.21)[c] | 46.00 (6.77)[c] | 51.80 (7.19)[c] |
| S.Em± | | 0.20 | 0.14 | 0.21 | 0.19 |
| CD @ 1% | | NS | 0.56 | 0.85 | 0.79 |

**Table 2:** Effect of solid carbon dioxide on seed quality parameters of red gram seeds stored in LDPE Zip-lock bags

| Sl. No. | Treatments | Seed damage (%) | | | | Seed weight loss (%) | | | | Germination (%) | Dehydrogenase enzyme activity (OD value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | One day before treatment | 10 DAT | 20 DAT | 30 DAT | One day before treatment | 10 DAT | 20 DAT | 30 DAT | | |
| 1. | Solid $CO_2$ @ 6 g (90% $CO_2$) | 13.78 (21.60) | 13.80 (21.62)[a] | 13.89 (21.63)[a] | 13.93 (21.69)[a] | 1.84 (7.70) | 0.00 (0.00)[a] | 0.00 (0.00)[a] | 0.00 (0.00)[a] | 88.59 (69.30) | 0.685 |
| 2. | Solid $CO_2$ @ 4 g (70% $CO_2$) | 13.96 (21.67) | 13.89 (21.76)[a] | 13.93 (21.84)[a] | 14.00 (21.89)[a] | 1.87 (7.85) | 0.00 (0.00)[a] | 0.00 (0.00)[a] | 0.00 (0.00)[a] | 87.78 (68.45) | 0.665 |
| 3. | Solid $CO_2$ @ 2 g (50% $CO_2$) | 13.67 (21.59) | 14.33 (22.08)[a] | 14.60 (22.25)[a] | 14.80 (22.50)[a] | 1.84 (7.77) | 0.56 (3.27)[b] | 0.90 (4.78)[b] | 1.19 (6.17)[b] | 91.2 (68.30) | 0.654 |
| 4. | Untreated check | 14.02 (21.84) | 23.50 (28.97)[b] | 28.70 (32.37)[b] | 32.40 (34.67)[b] | 1.90 (7.90) | 2.31 (8.75)[c] | 3.11 (10.13)[c] | 4.53 (12.23)[c] | 90.51 (72.19) | 0.653 |
| S.Em± | | | 1.47 | 1.34 | 1.42 | 0.39 | 0.70 | 0.67 | 0.43 | 0.36 | 0.01 |
| CD @ 1% | | | NS | 5.53 | 5.87 | NS | 2.88 | 2.78 | 1.76 | 1.48 | 0.04 |

DAT: Days after treatment
Figures in the parentheses are $\sqrt{(x+1)}$ transformed values
Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

**Adult mortality**

The observation on the mortality of the adults was recorded at hourly intervals. At two hours of treatment of solid $CO_2$ in the LDPE zip-lock bags (Table 3), the highest adult mortality was observed in the 90 percent $CO_2$ treatment (84.00%), followed by 70 and 50 percent $CO_2$ treatments with 59.33 and 48.67 percent, respectively. Similar trend was observed at four hours after treatment where significant adult mortality occurred in the 90 percent $CO_2$ treatment (100.00%). This was followed by followed by 70 and 50 percent $CO_2$ treatments with 73.33 and 54.60 percent, respectively. The adult mortality in the untreated check remained the same with zero percent at both intervals. However, at 48 hours after treatment, 100.00 percent mortality was achieved in both 90 and 70 percent $CO_2$ treatments. 69.40 and zero percent mortality was observed in 50 percent $CO_2$ treatment and untreated check, respectively. Similarly, at 72 hours after treatment, 100.00 percent mortality was achieved in both 90 and 70 percent $CO_2$ treatments. 71.93 and zero percent mortality was observed in 50 percent $CO_2$ treatment and untreated check, respectively.

**Table 3:** Effect of solid carbon dioxide on adult mortality of pulse beetle

| Sl. No. | Treatments | Percent adult mortality at different hours of treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 2 hr | 4 hr | 6 hr | 8 hr | 24 hr | 48 hr | 72 hr |
| 1. | Solid carbon dioxide @ 6 g (90%) | 84.00 (66.44)a | 100.00 (90.00)a | 100.00 (90.00)a | 100.00 (90.00)a | 100.00 (90.00)a | 100.00 (90.00)a | 100.00 (90.00)a |
| 2. | Solid carbon dioxide @ 4 g (70%) | 59.33 (50.33)b | 73.33 (58.98)b | 84.00 (66.56)b | 90.67 (74.32)b | 96.00 (79.81)b | 100.00 (90.00)a | 100.00 (90.00)a |
| 3. | Solid carbon dioxide @ 2 g (50%) | 48.67 (44.24)c | 54.60 (47.64)c | 60.00 (50.77)c | 67.33 (55.17)c | 68.40 (55.79)c | 69.40 (56.41)b | 71.93 (58.00)b |
| 4. | Untreated check | 0.00 (0.00)d | 0.00 (0.00)d | 0.00 (0.00)d | 0.00 (0.00)d | 0.00 (0.00)d | 0.00 (0.00)c | 0.00 (0.00)c |
| S.Em± | | 0.59 | 0.73 | 0.79 | 1.81 | 1.50 | 0.38 | 0.48 |
| CD @ 1% | | 5.29 | 3.01 | 3.26 | 7.48 | 6.21 | 1.56 | 2.00 |

The literature on the effect of solid $CO_2$ on the pulse beetle is lacking to compare such studies and it seems to be the first of its kind on the use of solid $CO_2$ to manage pulse beetle. Hence, reviews pertaining to the use of $CO_2$ in Modified Atmospheric Storage are taken to compare and discuss the outcome of current investigation.

The egg count, percent seed damage, seed weight loss and adult mortality data suggest that both 90 and 70 percent $CO_2$ concentrations were able to check the growth and development of the pulse beetle. The results are in corroboration with the work of Hashem and Risha (2000) [7] who revealed that 85 percent $CO_2$ was the most efficient when the bruchid infested faba beans were exposed for 3 days and lethality occurred in all stages of the insect. They also opined that 75 percent was the second-best efficient treatment with five days of exposure and 50 and 35 percent $CO_2$ were not sufficient to prevent the infestation of the pest even when the exposure period was extended to 7 and 10 days. Jay *et al*. (1971) [9] also noticed that $CO_2$ concentration of 60 percent gave over a 95 percent control of most stored grain insects after a four-day exposure at temperatures of 27 °C. The results on adult mortality was in agreement with the work of Omar *et al*. (1995) [10] who indicated that a four-day exposure period to 75 percent $CO_2$ caused cent percent mortality of *S. oryzae* adults.

The results are also comparable to the work of Annis and Mortan (1997) [1] who opined that at higher concentrations of $CO_2$ greater than 65 percent $CO_2$ caused cent percent mortality of all life stages of *S. oryzae.*

**Percent seed germination (%)**

There was no statistically significant difference noticed in germination percentage in red gram (Table 2). The percent germination ranged from 87.78 percent in 70 percent $CO_2$ treatment to 91.2 percent in 50 percent $CO_2$ treatment. The percent germination in the untreated check was 90.51 percent. This indicated that there was no adverse effect of solid $CO_2$ on the germination of red gram seeds.

**Dehydrogenase enzyme activity (OD value)**

There was no statistically significant difference noticed in the dehydrogenase enzyme activity of red gram seeds (Table 2). The values ranged from 0.653 in untreated check to 0.685 in 90 percent $CO_2$ treatment. The results of the present investigation on the germination and dehydrogenase enzyme activity of the redgram seeds exposed to different concentrations of $CO_2$ fall in line with Bera *et al.* (2004) [4] who revealed that storage in $CO_2$ rich atmospheres irrespective of concentrations and periods showed no adverse effect on germinability and vigour of the wheat seed. Similarly, there was no change in the dehydrogenase activity and malondialdehyde content because of $CO_2$ rich atmosphere. The present findings also support the work of White and Jayas (1993) [11] who opined that prolonged controlled atmospheric storage with high levels of $CO_2$ did not affect the germination of wheat seeds.

**Conclusion**

The storage of the produced grains is one of the key aspects for the sustainable supply of the food grains and maintain food security. However, numerous insect pests were found to infest the store grains inflicting severe yield losses. Among the pulses, redgram is being severely infested by the pulse beetles. Conventional usage of the insecticide results in the contamination of the produce leading to the health hazards. In this context, the investigation was formulated to address these issues, by utilizing the solid $CO_2$ as a way of eco-friendly management of pulse beetle in stored grains. The results were quiet promising with, egg count, percent seed damage, seed weight loss and adult mortality data at both 90 and 70 percent $CO_2$ concentrations were able to check the growth and development of the pulse beetle in air tight containers. Hence, Carbon dioxide can be used as an effective store grain fumigant in relatively air tight bins.

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