EffectofelevatedlevelsofCO$_2$on bionomics of *Callosobruchuschinensis*(L.)(Bruchidae: Coleoptera) instoredgreengram

ABSTRACT

An experiment was conducted to study the effect of elevated levels of CO_2 on bionomics of *Callosobruchus chinensis* (L.) in stored green gram during 2020-21 at Seed Entomology Laboratory, Seed Research and Technology Centre, College of Agriculture, Rajendranagar, Hyderabad, India. 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 bionomics 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.

Key words: *Callosobruchus chinensis,* Bionomics, 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 *C. 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 by 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 *Callosobruchuschinensis*, has traditionally relied heavily on synthetic insecticides and fumigants. However, the chemical-based approach has increasingly come under scrutiny due to its numerous drawbacks and potential risks [3].

Continuous use of the same insecticides can lead to the development of resistance in *C. chinensis* populations[4]. This resistance reduces the effectiveness of these chemicals over time,

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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 [5].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[6] addressing the need for effective pest control methods. 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*.

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2. MATERIAL 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 greengram 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 (Plate 1) 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 greengram 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 [7].

2.2 DESIGN OFAIRTIGHTCONTAINER AND INJECTIONOF 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 twoperforations with 3 mm diameterwhich serve as inlet and outlet holes. Nylon tubes of 3 mm diameter were insertedinto theholes. For each experimental unit, 100 grams of thoroughly disinfested green gram seeds were placed into the 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.

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2.3 DETERMINATIONOFCARBONDIOXIDECONCENTRATIONINAIRTIGHTCONTAINERSAFTER INJECTION AND SUBSEQUENT EXPERIMENT CONDUCTED

To ensurewhetherthe desired concentration ofCO_2released was maintained inthe plastic container or not, it was checked by using CO_2/O_2analyzer (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 wasintroduced into the top outlet tube of the airtight container and the measuring button ofthe CO_2/O_2analyzer was pressed. The carbon dioxide concentration present in the airtight container was displayed on screen within 10 seconds.

2.4 Assessment of biological parameters of *C. chinensis*

To studythe effect ofmodified atmospherewith elevated levelsof CO_2on *C. chinensis,* twenty-seven airtight containers were filled with 100 g of disinfestedgreen gram seed by releasing ten pairs of freshly emerged *C. chinensis* beetles and thesebeetles 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 thedesired concentration of carbon dioxide into the containers they were made airtight bypluggingthemwithrobbercorksandsealedwithTeflontape.Controlwasmaintained by followingthesameprocedureinplasticcontainersundernormallaboratoryconditions without exposing the seed to CO_2 concentrations. After exposure to variousconcentrations of CO_2, for three hours, the *C. chinensis* adults were removed and placedin plastic jars containing disinfested healthy seed. Ten specimen tubes were taken foreach 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 incubationperiod (days),Larval-pupalperiod (days), adult emergence (Number) (total numberof adults emerged from 45^{th} days after treatment to till the beetles cease to emerge fromseeds),adultlongevity(days) and fecundity were recorded [8]. The statistical methods described by [9] were adopted inthepresentinvestigation.Thedataweresubjectedtosquarerootandangulartransformationvalueswherever necessaryandanalysedbycompletelyrandomizeddesign(CRD)andfactorialcompletelyrandomizeddesign(FCRD)assuggestedby[10].

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3. RESULTS AND DISCUSSION

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3.1. EFFECTOF ELEVATEDLEVELSOFCO_2ONFECUNDITY

As observedfrom theTable 1 it was evidentthat alltheCO$_2$treatmentswerefoundtobesignificantlysuperiorinreducingthefecundityof *C.chinensis*over theuntreated control. Significantly lowestmean number of eggs1.20 wasrecorded at80%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 eggsat 50 %,40 %, 30 %, 20 % and 10%CO_2 concentrations were observed as 4.86, 6.46,8.13, 8.20 and 11.13respectively. Significantlyhighestmeannumberofeggswereobservedinuntreated control,whichresultedin17.46eggs. The findings are consistent with previous research where in fecundity of *C. chinensis* decreased with increasing CO_2 concentration[11]. Similarly, [12] found that eggs production in*Rhyzoperthadominica*was reduced as CO_2concentration increased, due to decrease in respiration rate. Exposure of Indian meal moth, *P. interpunctella*to an atmosphere with 96 % CO_2 significantly reducedbothegg productionandegg viability [13].Additionally, [14] noted that elevated CO_2 levels cause prolonged spiracle opening, leading to desiccation and mortality.

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Table 1. Effect of elevated levels of CO_2 on bionomics of *Callosobruchuschinensis*

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

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3.2. EFFECTOF ELEVATEDLEVELSOFCO$_2$ ONEGGINCUBATION PERIOD AND LARVAL-PUPAL PERIOD

The incubation period and larval-pupalperiodof *C. chinensis* varied between 3.83 to 4.33 days and 24.66to27.16days, respectively,across differenttreatments with varying CO_2concentrations in treated seeds. However,nosignificantdifferencewasobserved 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 (Table1). 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 inuntreated control. Since, *C. chinensis* is an internal feeder and the developmental oflarval-pupal period probably occurs inside the grain and is protected from any CO_2concentrations. Similar findings reported by [15] with increase in CO_2 concentration on eggincubation and larval-pupal period of *S. litura.*

3.3. EFFECT OF ELEVATEDLEVELSOFCO$_2$ ONADULT EMERGENCE

Adult emergence was significantly reduced with minimum mean number of adults 1.66 emerged in seeds treatedwith 80 % CO_2concentration followed by 70 % CO_2 with4.00adults whichwason par with 60 % $CO_2$4.33adults. However,9.66, 11.33, 14.00, 25.00 and 25.33 adults in seeds treated withCO_2concentrationsat50%, 40%30 %, 20 % and 10 % CO_2 concentration (Table1). All the CO_2 treatments were found to besuperior over the untreated control which recorded highest 37.00 adults. Studiesconductedby[16] revealedthatat20%CO_2concentration highest mean number of adult emergences was recorded and it decreasedwith increase in CO_2 concentration till 80 %. Earlier, [17] recordednoadultemergenceof*S.zeamais*and*S.oryzae*at90%CO_2concentration. [18] observed the sub lethal effects of carbondioxide against *S. paniceum*and *L. serricorne* and they reported that treatment of adultswith30%andaboveCO$_2$concentrationscausedasignificantreductioninprogenyproduction andadverseeffects onthemultiplication potentialof thesurvivors. Similarly, theincrease in carbon dioxide concentration caused decreasein theadult emergence in many insects as reported by [19] [3] [20] and [21]. Increase incarbon dioxide concentration causeddecrease in theadult emergenceof pulse beetle, *C. chinensis* it may be due to cell and mitochondrial membrane

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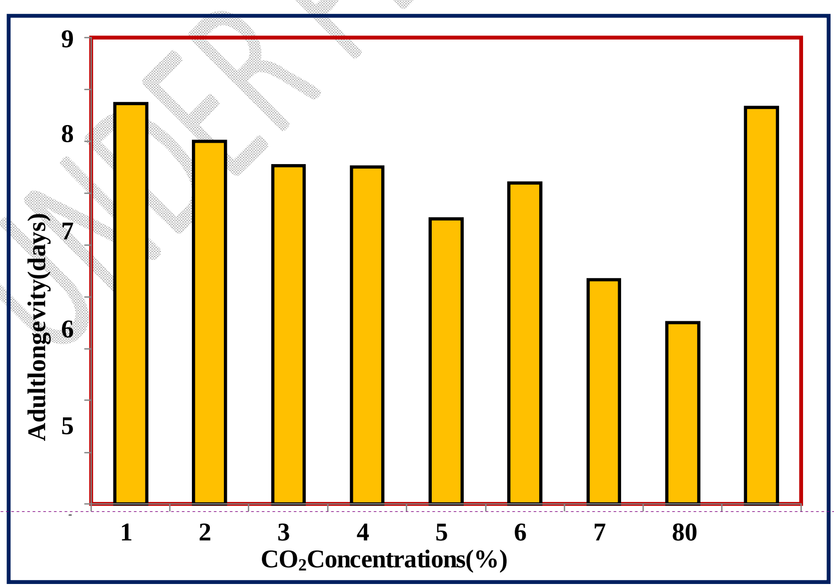
tobecomemorepermeabletocelldamageanddecreaseinATPproductionwhichultimatelyeffect on adultemergence.

3.4. EFFECT OF ELEVATEDLEVELSOFCO_2 ONADULT LONGEVITY

The longevity ofadults rangedfrom3.50to7.73daysinseedsexposed towithdifferent concentrations of CO_2 treatments. The CO_2 concentration at 80 % resulted in lowest lifespanofabout3.50days.Adultlongevityof4.33,5.50and6.20dayswereobservedat 70 %, 60% and50 %CO_2concentrations, respectively. Adult longevity of 6.50 days was observed at 40 % CO_2, which was on parwith 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 whichwas on par with 10 % CO_2 i.e. 7.73 days, the treatments *viz.,* 10 % CO_2 anduntreatedcontrol werefound to beleast effectivein reducingadultlifespan (Fig.1) Theresultsareinaccordancewith[11], whoreported12.00- 25.00 % of *C. chinensis* adults survived two to three days when exposed to 20 % CO_2 concentration in stored red gram. Most insects are more easilykilledwith higher carbondioxide concentrations [22]. Similarly, [23] revealed that completemortalityofadultswithinfivedaysat40%CO_2concentration on lesser grain borer, *R. dominica* [24] , observed percent mortalitywithinsixdays whendevelopmental stages of laboratorystrains of *Trogoderma granarium* exposed to 60 % CO_2 concentration. The increase in carbon dioxide concentration reduced the adult life span whichmay be due to damage of wax coat on the insect cuticle by sorption and abrasion andmayalso dueto internalphysiological effects.

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



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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.

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1.Ahmad S, HaqueMA, Mahmud H.. Effect of pulse beetle, *Callosobruchuschinensis* L on oviposition and damage in some important genotypes of pulse crops in Bangladesh. Biomedical Journal. 2018;2(2):2544-2548.http://dx.doi.org/10.26717/BJSTR.2018.02.000739

Comment [D18]: Author ignored all articles with DOI.
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