Evaluation ofElevatedCO_2onnutritionalqualityofGreenGramforTheManagementof*Callosobruchus chinensis* (L.) During Storage

ABSTRACT

An experiment was conducted to study the assessment of nutritional quality parameters of green gram treated with elevated levels of carbon dioxide to manage Callosobruchus infestationduring 2020-21 at Seed Entomology Laboratory, Seed Research and Technology Centre, College of Agriculture, Rajendranagar, Hyderabad, India. Nutritional quality of *Callosobruchus chinensis* infested green gram seeds exposed to fourdifferent concentrations of CO_2*viz.*, 20, 40, 60 and 80 per centwas studied. Theresults revealed that the 80 per cent CO_2 concentration maintained high carbohydratecontent (62.93 per cent) and crude fat (4.51 per cent) besides less protein (25.26 per cent) contentaftertwo months of storage. The 80per cent CO_2 treatment was consistently found to be the best treatment when it was retested in bulk quantity (25 kg of seed) after two months of storage period.High carbohydrate, crude fat content and low protein content were observed in all theCO_2 treatments(20,40, 60and 80per cent)whencomparedto untreated control.These findings suggest that using elevated CO_2 levels could be a viable alternative to synthetic pesticides chemical fumigants for preserving green gram during storage.

Key words: Nutritional, *Callosobruchus chinensis*,CO_2, Carbohydrate, protein, crude fat, Controlled atmosphere.

INTRODUCTION

Legumes, such as chickpeas, play a crucial role in human nutrition, not only supplementing the nutrients in cereal-based diets but also enhancing the taste and texture of staple dishes. Among legumes,green gram,*Vigna radiata* is a crucial pulse crop in India that significantly contributes to both nutrition and the agricultural economy. With its impressive protein content of about 25per cent, nearly triple that of cereals, this versatile legume serves as a vital source of plant-based protein while also providing substantial economic benefits to farmers across the country.In India, green gram is cultivated over an area of 5.13 million hectares, producing approximately 3.9 million tonnes, which translates to a productivity of 601 kilograms per hectare for the 2020-21 period [1]. Green gram is prone to significant qualitative and quantitative damage caused by storage pests. It is frequently infested by the multivoltine bruchid species *C. chinensis* and *C. maculatus*, which are commonly known as pulse beetles.According to [2], green gram suffers the highest average damage at 55.4per cent, followed by black gram (35.3per cent), pigeon pea (22.1per cent), cowpea (16.8per cent), gram (11.1per cent) and pea (8.8per cent).However, storing grain for long periods, usually about a year, requires multiple fumigations which can cause chemical residues to build up in the grain [3], leading to the development of resistant populations and increasing costs for farmers. It is crucial to develop natural, affordable and environmentally sustainable methods for managing insect pests in grain and stored products.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 [4] addressing the need for effective pest control methods. Controlled Atmospheres (CA) stands out as one of the most effective preservation techniques for a broad range of agricultural and food products [5]. This approach involves maintaining low oxygen and high carbon dioxide (CO_2) levels, which impact the respiration rates of pests and help prolong the shelf life of fresh produce [6].The current study was aimed to examine the nutritional quality parameters of greengram treated with elevated levels of carbon dioxide *viz.*, 20, 40, 60 and 80 per cent which involves evaluating various aspects such as carbohydrate composition, protein content and crude fat.Therefore, considering the urgent need to overcome food security challenges, mitigate storage losses due to pests and develop environmentally sustainable pest management strategies, we framed our study to investigate the utilization of carbon dioxide as an effective and eco-friendly seed protectant against the pulse beetle, *C. chinensis*, in stored green gram.

MATERIAL AND METHODS

MASS CULTURING OF THE TEST INSECT

The mass culturing of *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) was conducted in the Seed Entomology Laboratory, Seed Research and Technology Centre (SRTC) at College of Agriculture, Rajendranagar, Professor Jayashankar Telanagana State Agricultural University, Hyderabad. To establish the stock culture, healthy green gram seeds of the MGG-295 variety were sourced from the Telangana State Seed Development Corporation (TSSDC), Nizamabad, Telangana. The mother culture of *C. chinensis* was maintained at the Seed Entomology Laboratory at SRTC, Rajendranagar, Hyderabad. For mass rearing, approximately 100 adult beetles were introduced into plastic containers holding 1,000 grams of disinfested green gram seeds using an aspirator. The containers were covered with muslin cloth secured with rubber bands to ensure proper aeration and prevent the beetles from escaping. Twenty-five such containers were used for mass culturingand they were kept under laboratory conditions at a temperature of 32 ± 1 °C and relative humidity of 75per cent.

EXPERIMENTAL SETUP

To study the effect of elevated levels of CO_2 on nutritional quality parameters,fifteenairtightcontainerswerefilledwithfivekgofdisinfestedgreen gram seed.Twenty-five pairs of freshly emerged adults were released into the containers at twenty-fivedays priortocarbondioxide treatmentto ensureuniform levelof infestation. After twenty-five days, weight of the green gram seed was taken thereafter, CO_2 wasreleasedatdifferentconcentrations*viz*.,20,40,60and80per centandthreereplications of each treatment were maintained. After releasing the CO_2 concentration into the containers, they weremade airtight. Untreated control was maintained by the same procedure adopted without exposingthe seed to CO_2 concentration. Airtight containers having the disinfestedgreen gram seed exposed to different concentrations of CO_2were observed in eachreplication of the treatment.

NUTRITIONAL QUALITY ANALYSIS

The data on nutritional quality parameters *viz*., carbohydrate,total proteins and crude fat were observed at two months after treatment. The nutritional quality parameters of the best treatment and untreated control were retested in bulkquantity(25 kgseed)after twomonths of treatmentto ascertain theresults.

Carbohydrate of green gram during storage

Estimation of carbohydrates in this investigation was done by the IS 1656-2007(Difference)method.

Carbohydrates (per cent) = 100 – (A + B + C + D)

Where,A =per cent moisture

B = per cent protein

C=per cent fat

D=per centash

Total protein of green gram

The protein content of green gram during storage was determined by estimatingnitrogen inthe sample by micro-kjeldahl method as described by [7] using Kelplus auto analyser. Initially in the presence of two grams of catalyst mixture(copper sulphate and potassium sulphate in the ratio of 1: 5) and ten ml of concentratedsulphuric acid (H_2SO_4), 0.2 g of sample was digested at 420°C for two hours. Digestedsample was distilled using 40 per cent NaOH in micro-kjeldahl distillation system.Ammoniawasobservedinexcessoffourper centboricacid.Theobtaineddistillate was titrated with standard acid 0.1 N HCL till pink colour has appeared. The per centnitrogenwas calculatedas follows.

$$N2\ (\text{per cent}) = \frac{[Titre\ value\ of\ the\ sample - Titre\ value\ of\ the\ blank]\ x\ Normality\ of\ HCL\ (0.1) \times 14 \times 100}{Weight\ of\ the\ sample \times 1000}$$

The protein content was estimated in per cent by multiplying the obtained nitrogen per cent with factor 6.25 [8].

Crude fat of green gram during storage

Crude fat was determined by AOAC 922.06 – 2006 in Quality Control Laboratory, Rajendranagar, Hyderabad. Empty fat extraction beaker along with boiling stones (T) was weighed. Approximately two grams of the dehydrated seed was weighed in triplicates and packed into filter paper and placed in the thimbles provided with the instrument. Volume n – hexane taken in a beaker was recorded. Required conditions for soxtherm extraction were maintained as per software settings and thimble containing seed sample was inserted into slot provided for thimbles. The thimble containing the test portion was immersed into the boiling solvent. The intermixing of the matrix with hot solvent ensured rapid solubilisation of extractable. In the second step, the thimble was raised above the solvent and the test portion was further extracted by a continuous flow of condensed solvent for one hour 20 minutes. The solvent was evaporated and recovered by condensation. After drying, the resulting crude fat was determined gravimetrically. Extraction cups were dried at 102 ± 2 °C in hot air oven for 30 minutes to remove moisture. Excessive drying was avoided which can oxidize fat and give erroneous results. The defatted sample was cooled in a desiccator and weighed to the nearest 0.1 mg (F). The per cent crude fat was calculated as follows

per centCrudefat, Hexanes/ equivalent extract = $\frac{F-T}{S} x100$

Where, F = Weight of cup + fat residue in grams

T=Weight of emptycupin grams

S=Testweightingrams

STATISTICAL ANALYSIS

The statistical methods described by [9]were adopted in the present investigation. The data were subjected to angular transformation wherever necessary and two sample t- test was carried out to ascertain the results of effect of elevated levels of CO_2 on nutritional quality parameters by retesting the best treatment in comparison with untreated control in bulk seed.

RESULTS AND DISCUSSION

Effect of modified atmosphere with elevated levels of CO_2 on carbohydrate content of green gram seeds, during storage

The results pertaining to the effect of CO_2 treatment on carbohydrate content ofstoredgreen gramseedsarepresentedinTable1. Initially,the carbohydrate content recorded was 62.96per cent, with increase in storage duration thecarbohydrate content was found to decrease in all the CO_2 treatments. After two monthsof storage, the lowest carbohydrate content was recorded in untreated control (60.20per cent), which was found to be significantly inferior to the rest of the CO_2 treatments. Thehighest carbohydrate content (62.93per cent) was recorded in CO_2 treatment at 80 per cent after twomonthsof storage.

The present investigation is in conformity with the findings of [10] whoreportedthatundercontrolledatmosphere(upto80per centCO_2),the stored cowpeaseeds retainedthe highesttotalhydrolysablecarbohydratecontent. The initial carbohydrate content of green gram seed observed was 62.50per cent.To ascertain the results, 80 per cent CO_2 treated green gram seeds (best treatment) wereretested in bulk quantity (25 kg of seed) for further two months of treatment to test theperformance in bulk and is compared its performance with the untreated control. From the results it can be concluded that the t-calculatedvalue (24.70) is more than the t-table value (2.10) at 18 degrees of freedom and 5 per centlevel of significancehence, the 80 per centCO_2 treatment oncarbohydrate content of green gram was significantly superior over the untreated control even in thebulkquantities (Table 2). Ourresultsareinconformitywiththefindingsof [11]who reportedthat reduction in carbohydrate was not solely dependent upon the progenydevelopment.Therearesomeotherfactors,whichmayhavecontributedtowardsreductionincarbohydratecontents.Suchfactorscontributevarietalresistanceaswellasinsect preference[12]. Results revealed that carbohydrate content decreased with *C. chinensis* infestation which might be due to depletion of cellulose and starchresultedfrom themetabolicand enzymatic activityoftheinsects as reportedby[13].

Table1. Effect of carbon dioxide (CO_2) treatment on nutritional parameters of green gram aftertwo months ofstorage

S. No.	(CO_2 Concentrations)	Carbohydrates (per cent)	Total protein (per cent)	Crude fat (per cent)
1	Carbon dioxide 20 per cent	60.57 (51.08)	28.24 (32.08)	4.16 (11.77)
2	Carbon dioxide 40 per cent	60.87 (51.26)	27.08 (31.35)	4.29 (11.95)
3	Carbon dioxide 60 per cent	61.45 (51.59)	26.20 (30.77)	4.40 (12.10)
4	Carbon dioxide 80 per cent	62.93 (52.15)	25.26 (30.16)	4.51 (12.25)
5	Untreated Control	60.20 (50.86)	28.68 (32.36)	3.98 (11.51)
	SEm±	0.12	0.15	0.04
	CD (P=0.05)	0.36	0.45	0.12
	CV (per cent)	0.42	0.86	0.57

The values in parentheses are angular transformed value

Table 2. Effect of carbon dioxide (CO_2) treatment on carbohydrate content of green gram (bulk quantity 25 kg) aftertwo months ofstorage

t-Test:Two-SampleAssumingEqualVariances

	CO_2 (80 per cent)	Control
Mean	62.00	59.88
Variance	0.017	0.057
Df	18	
t Stat	24.70	
tCriticaltwo-tail	2.10	

(Initialcarbohydrate content:62.50)

Effect of modified atmosphere with elevated levels of CO_2 on total protein of green gram seeds during storage

The results pertaining to the effect of CO_2 treatment on total protein content ofstoredgreen gramseedsarepresentedinTable1.Initially,the protein contentrecordedwas21.56per cent,withincrease instoragedurationit was found to increase in all the CO_2 treatments. The lowest proteincontent of 25.26 per centwas recorded in 80 per cent CO_2 treatmentwhereas, inuntreated control 28.68 per centof protein content was recorded and which was on par with20per centCO_2concentration (28.24per cent). [14] reported that, initially the protein content in healthy green gram seed was 22.41per cent which increased to 26.77per centdueto *C.chinensis* infestation. The initial total protein content recorded ingreen gram seedwas 21.50per cent.From the results it can be concluded that the t-calculated value (38.98) is more than the t-table value (2.10) at 18 degrees of freedomand 5 per cent level of significance and hence, the 80 per centCO_2 treated green gram sampleon total protein content was significantly lowest over the untreated control even in thebulkquantities (Table 3). Present studies revealed that the total protein content increased with increase ininsect infestation, which is in conformity with the [15], who reportedtotalproteincontentincreasedby*C.maculatus*infestation in cowpea fromonemonthtothreemonths.[1 1] revealed that increase oftotal proteinsis due to the addition of non-beneficial rather harmful proteins such as cast skins,exuviates,dead insects,wings, legs andother bodypartsoftheinsects that comealong withthekhaprabeetle, *Trogoderma.granarium* infestedgrain samples.

Table 3. Effect of carbon dioxide (CO_2) treatment on total protein content of green gram (bulk quantity 25 kg) aftertwo months ofstorage

t-Test:Two-SampleAssumingEqualVariances

	CO_2 (80 per cent)	Control
Mean	26.03	28.71
Variance	0.018	0.03
Df	18	
t Stat	38.98	
tCriticaltwo-tail	2.10	

(Initialtotalproteincontent:21.50)

Effect of modified atmosphere with elevated levels of CO_2 on crude fat of green gram seeds during storage

The results pertaining to the effect of CO_2 treatment on crude fat content ofstored green gram seeds are presented in (Table 1). Initially, the crudefat content recorded was 4.66per cent, with increase in storage duration itdecreasedinalltheCO$_2$treatments.Thelowestcrudefatcontentof 3.98 per centwas recorded in untreated control. The CO_2 treatment at 80 per centrecorded4.51 per centof crudefat content aftertwo months of storage. From the results it can be concluded that the t-calculatedvalue (20.75) is more than the t-table value (2.10) at 18 degrees of freedom and 5 per centlevel of significance and hence, the 80 per centCO_2 treated green gram sample on totalcrude fat content was significantly superior over the untreated control even in the bulkquantities(Table 4). The results with respect to crude fat, decreased with increased *C. chinensis* infestation. Thepresent findings are in conformity with the [16], who observed asignificant decrease in fat content with the increase in infestation levels. [17] also reported a decline in maize fat content from 5.90to 5.30 per centafter four months of storagedueto damagebystoragepests.

Table 4. Effect of carbon dioxide (CO_2) treatment on crude fat of green gram (bulk quantity 25 kg) aftertwo months ofstorage

t-Test:Two-SampleAssumingEqualVariances

	CO_2 (80 per cent)	Control
Mean	4.54	3.86
Variance	0.004	0.06
Df	18	
t Stat	20.75	
tCriticaltwo-tail	2.10	

(Initialcrudefatcontent:4.66)

CONCLUSION

The study demonstrates that storing seeds in an 80per cent CO_2 atmosphere effectively preserves high carbohydrate and crude fat content while slightly reducing protein levels after two months. This method, particularly when applied to 25 kg of seeds, was found to be the most effective in maintaining nutritional quality. While increased infestation by *Callosobruchus chinensis* negatively impacted carbohydrates and fat, it unexpectedly increased protein content in green gram seeds. Despite the challenges of maintaining a sealed environment, CO□ application offers a sustainable alternative to chemical pesticides, preserving grain integrity.While specific studies on green gram treated with elevated levels of carbon dioxide may be limited, research this topic can provide insights into the methodologies and considerations for such assessments.Further assessment of these silica powders should be carried out against various stored grain pests across different grain commodities, under varying temperature and humidity conditions, and using diverse application methods to effectively integrate them into management systems in storage arenas.

Disclaimer (Artificial intelligence)

Option 1:

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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