Assessment of nutritional quality parameters ofgreen gram exposed to elevated levels of carbon dioxide for managing *Callosobruchuschinensis*(L.)

ABSTRACT

An experiment was conducted to study the assessment of nutritional quality parameters of green gram treated with elevated levels of carbon dioxide during 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 %was studied. Theresults revealed that the 80 % CO_2 concentration maintained high carbohydratecontent (62.93 %) and crude fat (4.51 %) besides less protein (25.26 %) content aftertwo months of storage and this treatment was also 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 80%)whencomparedto untreated control.

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

INTRODUCTION

Green gram, scientifically known as *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 25%, 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.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 Bhaduria and Jakhmola (2006), green gram suffers the highest average damage at 55.4%, followed by black gram (35.3%), pigeon pea (22.1%), cowpea (16.8%), gram (11.1%), and pea (8.8%).However, storing grain for long periods, usually about a year, requires multiple fumigations which can cause chemical residues to build up in the grain (Flora et al., 2006), 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.Controlled Atmospheres (CA) stands out as one of the most effective preservation techniques for a broad range of agricultural and food products (Jayas and Jeyamkondan, 2002). This approach involves maintaining low oxygen and high carbon dioxide (CO2) levels, which impact the respiration rates of pests and help prolong the shelf life of fresh produce (Caleb et al., 2012).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 % which involves evaluating various aspects such as carbohydrate composition, protein content and crude fat. While specific studies on greengram treated with elevated levels of carbon dioxide may be limited, research on similar topics can provide insights into the methodologies and considerations for such assessments.

MATERIAL AND METHODS

MASS CULTURING OF THE TEST INSECTAND SUBSEQUENT EXPERIMENT CONDUCTED

The mass culturing of *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) was conducted in the Seed Entomology Laboratory, Seed Research and Technology Centre (SRTC) at PJTSAU, Rajendranagar, 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 75%.

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*.,20,40,60and80%andthreereplications 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. 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 (%) = 100 – (A + B + C + D)

Where,A =% moisture

B = % protein

C=% fat

D=%ash

Total protein of green gram

The protein content of green gram during storage was determined by estimating nitrogen inthe sample by micro-kjeldahl method as described by Sadasivan and Manickam (2008)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 % NaOH in micro-kjeldahl distillation system.Ammoniawasobservedinexcessoffour%boricacid.Theobtaineddistillate was titrated with standard acid 0.1 N HCL till pink colour has appeared. The %nitrogenwas calculatedas follows.

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

The protein content was estimated in % by multiplying the obtained nitrogen % with factor 6.25 (Mariotti *et al.*, 2008).

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 % crude fat was calculated as follows

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

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

T=Weight of emptycupin grams

S=Testweightingrams

Twosamplet-testwascarriedouttoascertain the results of effect of elevated levels of CO_2 on nutritional quality parametersbyretestingthebesttreatment in comparison withuntreated control inbulk seed.

RESULTS AND DISCUSSION

Effect of modified atmosphere with elevated levels of CO2 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 62.96 %, 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 (60.20 %), which was found to be significantly inferior to the rest of the CO_2 treatments. Thehighest carbohydrate content (62.93%) was recorded in CO_2 treatment at 80 % after twomonthsof storage.

The present investigation is in conformity with the findings of Shehata *et al*.(2009),whoreportedthatundercontrolledatmosphere(upto80% CO_2),the stored cowpeaseeds retainedthe highesttotalhydrolysablecarbohydratecontent. The initial carbohydrate content of green gram seed observed was 62.50 %.To ascertain the results, 80 % 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 %level of significancehence, the 80 % CO_2 treatment oncarbohydrate content of green gram was significantly superior over the untreated control even in thebulkquantities (Table 2). Ourresultsareinconformitywiththefindingsof Muhammad*etal*.(2009)who reportedthat reduction in carbohydrate was not solely dependent upon the progenydevelopment.Therearesomeotherfactors,whichmayhavecontributedtowardsreductioni ncarbohydratecontents.Suchfactorsarevarietalresistanceaswellasinsect preferenceTripathi *et al*., 2013. Results revealed that carbohydrate content decreased with *C. chinensis* infestation which might be due to depletion of cellulose and starchresultedfrom themetabolicand enzymatic activityoftheinsects as reportedbyAllali *etal*.(2020).

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 contentrecordedwas21.56 %,withincrease instoragedurationit was found to increase in all the CO_2 treatments. The lowest proteincontent of 25.26 % was recorded in 80 % CO_2 treatment whereas, inuntreated control 28.68 % of protein content was recorded and which was on par with20% CO_2concentration (28.24 %). Gujar (1975) reported that, initially the protein content in healthy green gram seed was 22.41% which increased to 26.77% dueto *C.chinensis* infestation. The initial total protein content recorded ingreen gram seedwas 21.50 %.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 % level of significance and hence, the 80 % CO_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 Bamaiyi*et al.* (2006), who reportedtotalproteincontentincreasedby*C.maculatus*infestation in cowpea fromonemonthtothreemonths.Muhammad *et al.* (2009) 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, *T.granarium* infestedgrain samples.

Effect of modified atmosphere with elevated levels of CO2 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 4.66 %, with increase in storage duration itdecreasedinalltheCO$_2$treatments.Thelowestcrudefatcontentof 3.98 % was recorded in untreated control. The CO_2 treatment at 80 %recorded4.51 % of 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 %level of significance and hence, the 80 % CO_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 Jood and Kapoor (1993), who observed asignificant decrease in fat content with the increase in infestation levels. Farhan *et al.*(2013) also reported a decline in maize fat content from 5.90to 5.30 % after four months of storagedueto damagebystoragepests.

CONCLUSION

The study demonstrates that storing seeds in an 80% CO2 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.

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

S. No.	(CO_2 Concentrations)	Carbohydrates (%)	Total protein (%)	Crude fat (%)
1	Carbon dioxide 20 %	60.57 (51.08)	28.24 (32.08)	4.16 (11.77)
2	Carbon dioxide 40 %	60.87 (51.26)	27.08 (31.35)	4.29 (11.95)
3	Carbon dioxide 60 %	61.45 (51.59)	26.20 (30.77)	4.40 (12.10)
4	Carbon dioxide 80 %	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 (%)	0.42	0.86	0.57

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 %	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)

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 %	Control
Mean	26.03	28.71
Variance	0.018	0.03
Df	18	
t Stat	38.98	
tCriticaltwo-tail	2.10	

(Initialtotalproteincontent:21.50)

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 %	Control
Mean	4.54	3.86
Variance	0.004	0.06
Df	18	
t Stat	20.75	
tCriticaltwo-tail	2.10	

(Initialcrudefatcontent:4.66)