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Effect of modified atmosphere with elevated levels of CO_2 on seed quality parameters of greengram during storage

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Abstract
The present study was carried out at Entomology Laboratory, Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad, Telangana during 2020-2021 to study the effect of elevated levels of CO_2 on seed quality parameters of stored greengram. Seeds were artificially infested with *Callosobrochus chinensis* (L.) and exposed to different concentrations of CO_2 *viz*., 10, 20, 30 and 40 per cent with three replications of each treatment and packed in air tight plastic containers. Forty per cent CO_2 concentration checked the seed damage (2.36 per cent) and weight loss (1.97 per cent) upto six months of storage. Storage of greengram seeds in different CO_2 concentrations (10, 20, 30 and 40 per cent maintained the seed quality (viability of seed) without any detrimental effect on germination (maintained above 90 per cent), seedling vigour and moisture content (8.63 per cent) upto six months of storage.

Keywords: Modified atmosphere, carbon dioxide, pulse beetle, greengram, quality of seeds

Introduction
Pulses constitute the major source of protein in the diet developing countries. They are known as "poor man meat" as pulses are the cheapest source of dietary protein. In developing countries like India and worldwide, pulses are the major source of protein for the daily diet of low-income groups and Vegeterians. Among them greengram (*Vigna radiata* (L.) is a protein rich staple food and it contain about 25 per cent of protein, which is almost three times that of cereals.
Greengram native of India and Central Asia. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand and Indonesia. In India greengram production contributes more than 70 per cent. It is grown in India with an area, production and productivity of 4.5 m ha^{-1}, 2.50 mt and 548 kg ha^{-1}, respectively (www.indiastat.com). The area, production and productivity of greengram crop for 2019-20 in Telangana state are 0.70 lakh ha^{-1}, 0.53 Lt and 798 Kg ha^{-1}, respectively. The predominant greengram growing districts of Telangana are Mahabubabad, Sangareddy, Suryapet, Khammam, Vikarabad and Kamareddy.
Quantitative and qualitative losses occur in greengram seeds during storage due to various insect pests, among them pulse beetle, *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) is the most destructive species of stored legume seeds in India as well as in other countries. It causes 33 per cent infestation to legume seeds. Gujar and Yadav (1978) [12] reported the seed weight loss 55-60 per cent and 45.50-66.30 per cent losses in protein content due to bruchid infestation in storage. The U.S. Food Quality Protection Act (1996) focused on registered pesticides with particular attention to worker and consumer exposures to chemical residues. Pesticides are the most powerful tool available for pest control, despite these credentials, the long and indiscriminate use of pesticides has been found ecologically unsound.
Insecticides were found to cause toxic effects on the produce intended for consumption, so it is also not safe to mix insecticide with food grain for protection against insects (Bekele *et al*., 1996) [7]. At the same time plant- derived materials are not much effective.
Treatment of using carbon dioxide is environmental free do not leave any pesticide residues in stored products have been used against pests as an alternative method (Aliniazee and Lindgren, 1970; Wang *et al*., 2009) [2, 27]. Carbon dioxide has toxic effects on developmental stages and age-groups of insect. Many researchers have determined the toxic effects of carbon dioxide according to exposure time to kill (Gunasekaran and Rajendran, 2005) [13].

Modified or controlled atmosphere storage of grains as the alteration of the concentrations of the normal atmospheric gases present in storage so as to give an artificial atmosphere to prevent mould growth and quality deterioration of stored products (Banks and Annis, 1980).

Greengram is highly infested with pulse beetle *C. chinensis* (L.) during storage. Though lot of work has been done on modified atmosphere with CO_2 on various storage pests including beetle pests and moths (Riudavets *et al*., 2009) [24], very little information was available regarding its role in controlling *C. chinensis* in stored greengram. Hence there is a need to concentrate on *C. chinensis* management strategies and its impact on seed quality parameters using modified atmosphere with the non residual fumigant *i.e.*, CO_2 and its effect on seed and nutritional quality.

Material and Methods

The present study was carried out at Seed Entomology Laboratory, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagr, Hyderabad, Telangana, India during 2020-2021. To study the effect of elevated levels of CO_2 on seed quality parameters of greengram was carried out in specially design airtight containers.

Forty five airtight containers were filled with five kg of disinfested greengram seed and twenty five pairs of freshly emerged *C. chinensis* adults were released into the containers at twenty five days prior to treatment with carbon dioxide to ensure uniform level of infestation. After twenty five days, weight of the greengram seeds were taken and CO_2 was released at four different concentrations *viz*., 10, 20, 30 and 40 per cent with three replication of each treatment and after releasing the desired concentration of carbon dioxide into the containers they were made airtight by plugging them with robber corks and sealed with Teflon tape. The concentration of CO_2 was checked by using CO_2/O_2 analyzer (PBI Dansensor, PBI 2006, Denmark). Untreated control was maintained by following the same procedure adapted for CO_2 studies in plastic containers under laboratory conditions without exposing the seed to CO_2 concentration. The airtight containers containing the disinfested seeds exposed to different concentrations of CO_2 were observed after two, four and six months of storage by drawing samples from each container and the following parameters were recorded.

Seed damage (per cent)

Seed damage (per cent) was calculated by taking the random sample of 400 seeds and counted the number of seeds with bored holes made by pulse beetle and converted into percentage.

$$\text{Seed damage (\%)} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds}} \times 100$$

Weight loss due to infestation (per cent)

The count and weight method was used to determine the seed weight loss by using the following formula.

$$W (\%) = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wd \times (Nd + Nu)} \times 100$$

W = Weight loss (%)

Wu = Weight of undamaged seeds

Number of damaged seeds

Wd = Weight of damaged seeds

Nu = Number of undamaged seeds

Seed germination percentage

Germination of the seeds was tested by paper towel method by maintaining the three replication of each treatment (ISTA rules, 1999) [14]. One hundred greengram seeds were kept in moist paper towel and allowed to germinate in walk in germinator for seven days.

$$\text{Seed germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

Seedling vigour index

For determining of the seedling vigour index, seven days old ten healthy germinated greengram seeds were selected from each replication of the treatment, shoot and root length of each of ten seedlings were measured in centimetre and average length of the seedlings was calculated. Seedling vigour index was calculated by multiplying germination percentage with seedling length (cm) as suggested by Abdul Baki and Anderson (1973) [1].

Seedling length (cm) = Shoot length + Root length

Seedling vigour index (SVI) = Seed germination percentage × Seedling length (cm).

Seed moisture content (%)

Moisture content of the seed was estimated by using Dicky John moisture meter.

Statistical Analysis

The data were subjected to angular transformation values wherever necessary and analysed by factorial completely randomized design (FCRD) as suggested by Panse and Sukhatme (1978) [20].

Results and Discussion

Effect of elevated levels of CO_2 on seed damage (per cent)

The results obtained from the studies on effect of elevated levels of CO_2 treatment on seed damage (per cent) caused by *C. chinensis* are presented in Table 1.

It was clearly evident from the data that after two months of storage, all the CO_2 concentrations (10, 20, 30 and 40 per cent) were able to restrict the seed damage below the permissible limit *i.e.*, one per cent. Whereas, significantly highest seed damage per cent was recorded in untreated control (1.44 per cent). After four months of storage least seed damage (1.58 per cent) was recorded in seeds treated with 40 per cent CO_2 concentration which was on par with 30 per cent CO_2 (1.62 per cent) followed by 20 per cent CO_2 (2.49 per cent) and 10 per cent CO_2 (3.90 per cent). The highest seed damage per cent was recorded in untreated control (5.03 per cent).

Similar trend was observed after six months of storage, lowest per cent of seed damage was recorded at 40 per cent CO_2 concentration (2.36 per cent), however, 40 per cent CO_2 was on par with 30 per cent CO_2 (2.40 per cent). While, in untreated control highest per cent seed recorded was 14.57 per cent.

The mean seed damage 0.70, 2.92 and 5.44 per cent were recorded after two, four and six months of storage, respectively. Results on exposure period revealed that mean seed damage showed increasing infestation with increase in exposure periods.

From the results it was evident that all the CO_2 treatments *viz.*, 10, 20, 30 and 40 per cent were able to maintain seed damage below permissible limits (*i.e.*, 1 per cent) after two months of storage but after four and six months of storage marginally protect the seed from *C. chinensis* infestation.

The results of this study are in line with Radhika *et al*. (2014), who opined that exposure of bruchid beetle to low concentrations of CO_2 at 10 per cent protected the seed from *C. serratus* upto three months of storage but after six months of storage infestation was increased to 50.71 per cent pod damage. Bhogeesh *et al.* (2014) [10] observed the CO_2 concentration at 50 per cent recorded least groundnut pod damage 0.67, 1.08 and 2.62 per cent at three, six and nine months after storage, respectively. Krishnaveni (2012) [16] also reported that exposure of *C. chinensis* to low concentration of CO_2 at 20 per cent protected the seed upto four months, but after five months of treatment, 12.50 per cent infestation was recorded and it was increased to 17.00 per cent after six month of storage.

Studies conducted with exposure of *C. chinensis* infested greengram seeds to different concentrations of CO_2 revealed that the effect of elevated levels of CO_2 concentration at 40 per cent was effective in preventing the damage and progeny development. At lower O_2 and higher CO_2 concentrations metabolism level of insects become too low, combined with accumulation of toxic end products, which is cause of stress for insects eventually leading to death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002) [11, 17].

Effect of elevated levels of CO_2 on weight loss (per cent)

The results pertaining to the effect of elevated levels of CO_2 on weight loss (per cent) caused by *C. chinensis* are presented in Table 1. It was evident that after two months of storage, all the CO_2 treatments (10, 20, 30 and 40 per cent) could protect the seed within permissible limit of weight loss (1 per cent). After four months of storage the least weight loss per cent was observed at 40 per cent CO_2 (0.28 per cent), followed by 30 per cent CO_2 concentration (0.52 per cent) which was on par with 20 per cent CO_2 (0**.**53 per cent). However**,** 1.33 per cent of weight loss was recorded at 10 per cent CO_2 concentration, while in untreated control 1.58 per cent of weight loss was recorded.

After six months of storage the least weight loss per cent was observed at 40 per cent CO_2 (1.97 per cent), followed by 30 per cent CO_2 (2.26 per cent), however, the 30 per cent CO_2 was on par with 20 per cent CO_2 (2.32 per cent). Weight loss per cent of 2.84 was recorded at 10 per cent CO_2 concentration, while in untreated control highest weight loss was recorded as 3.02 per cent.

Significantly highest mean weight loss of 1.86 per cent was recorded in untreated control, while, significantly lowest mean weight loss per cent of 0.78 was recorded at 40 per cent CO_2 concentration.

Mean weight loss per cent of 0.38, 0.84 and 2.48 were recorded at two, four and six months after storage, respectively. The results on exposure periods revealed that weight loss goes on increasing with increase in exposure periods and decrease with increase in CO_2 concentrations.

These results are in agreement with Shivaraja *et al*. (2012) [28] reported that 9.90 per cent weight loss due to infestation caused by *C. chinensis* in pigeonpea seeds which were treated with 10 per cent concentration of CO_2 after 45 days of exposure. Sharaf (2000) [29] reported the weight loss due to infestation per cent of faba bean seed caused by *C. chinensis* decreased with increasing carbon dioxide concentration. According to Alvinda *et al.* (2006) [3] weight loss was minimized in carbon dioxide enriched storage conditions.

Germination percentage

The germination percentage of greengram seeds exposed to different concentrations of CO_2 after different months of storage (Table 2) did not show any significant variation among the concentrations after two months of storage and cent per germination was recorded in 40 per cent CO_2 and 30 per cent CO_2 treatments. Significantly lowest germination of 95.33 per cent was recorded in untreated control.

The observations recorded after four months of storage showed similar trend. Among the concentrations 40 per cent CO_2 and 30 per cent CO_2 concentrations showed highest germination 99.00 and 98.00 per cent, respectively followed by 20 per cent CO_2 concentration (97.00 per cent), while lowest germination of 94.67 per cent was recorded in untreated control.

The results obtained on germination percentage after six months of storage also showed same trend as observed with four months after storage. Among the concentrations high germination of 97.67 per cent was recorded at 40 per cent CO_2, while significantly lowest germination of 92.67 per cent was recorded in untreated control.

The highest mean germination of 98.89 per cent was recorded at 40 per cent CO_2 concentration and lowest germination of 94.22 per cent was recorded in untreated control. Mean germination per cent of 98.72, 97.00 and 95.66 was recorded after two, four and six months of storage, respectively. The results revealed that mean germination per cent was decrease with increased storage period.

Germination percentage was found to decrease with increase in storage duration due to increase in seed damage caused by *C. chinensis.*

The results are in support with Padmasri *et al*. (2017) [18, 19] studied that redgram seeds were artificially infested with *C. chinensis* and exposed to CO_2 concentrations at 30, 40 and 50 per cent. They reported that the CO_2 concentrations at 40 and 50 per cent checked the seed infestation and also maintained the seed quality without any detrimental effect on germination upto nine months of storage and all the treatments were able to maintain germination percentage as per IMSCS (>75 per cent) upto twelve months of storage. Similarly, Reddy shekar *et al*. (2018) [23] reported that storage of maize seeds in the CO_2 rich atmosphere (60 & 80 per cent) maintained the seed quality without any detrimental effect on germination (>90 per cent) upto six months of storage.

The high germination per cent of CO_2 treated greengram seeds are compared to untreated control, it could be due to decreasing the *C. chinensis* infestation with increasing the CO_2 level resulted in high germination. Shehata *et al*. (2009) [25] stated that the germination per cent of cowpea seeds stored upto six months under controlled atmospheres was higher than the untreated seeds. Rathi *et al*. (2000) [22] reported that carbon dioxide exposed to *C. chinensis* on redgram seeds had less insect damage, less mould attack and higher germination percentage when compared to normal air treatment.

Lower germination percentage was observed in untreated control in our studies could be due to insect infestation which might have damaged seed embryo.

From the result there was no adverse effect of CO_2 on treated greengram seed germination, while, in untreated control due to increased moisture content, the *C. chinensis* infestation was also more and there by the germination per cent might have reduced during prolonged storage period.

Seedling vigour index

Seedling vigour index studies conducted with greengram seeds exposed to different concentrations of CO_2 during different months of storage (Table 2) showed significant variation in vigour index. After two months of storage significantly highest vigour index was recorded at 40 per cent CO_2 (3668.00), while significantly lowest seedling vigour index was recorded in untreated control (3034.00).

The similar trend was observed after four months of storage. Highest seedling vigour index was recorded at 40 per cent (3488.00), while lowest vigour index was recorded in untreated control (2899.00). The similar trend was followed after six months of storage, highest seedling vigour index was recorded in 40 per cent CO_2 concentration (3230.00) and was on par with 30 per cent CO_2 (3140.00), while lowest vigour index was recorded in untreated control (2563.00).

The highest mean seedling vigour index was recorded at 40 per cent CO_2 concentration (3462.00) and lowest mean seedling vigour index was recorded in untreated control (2832.00). The mean seedling vigour index showed decreasing trend from two months after storage (3338.20) to six months after storage (2926.20).

The interaction effect of concentrations and months after storage on seedling vigour index indicated significant variations among the treatments. Significantly highest seedling vigour index was observed with seeds exposed to 40 per cent CO_2 concentrations (3668.00) after two months of storage and significantly lowest seedling vigour index was observed in untreated control (2563.20) after six months of storage followed by 10 per cent CO_2 (2754.00).

The CO_2 did not have any direct effect on seedling vigour index and in turn helped in control of the insects which affect the seedling vigour.

The results are line with Bera *et al.* (2004) [9], who reported that storage of wheat seeds in CO_2 reach atmosphere irrespective of concentrations and storage periods showed no adverse effect on seed vigour. Krishnaveni (2012) [16] also reported that the mean seedling vigour index of pigeonpea seeds decreased from two months after storage (2666) to six months after storage (1651) irrespective of carbon dioxide concentrations. Similarly, Padmasri *et al.* (2017) [18, 19] reported that the mean seedling vigour index of maize seeds showed decreasing trend from three months after treatment (3172) to nine months after treatment (2150) irrespective of CO_2 concentrations.

In all the treatments irrespective of the concentrations of CO_2 gradual decrease in the seedling vigour index was observed from two months to six months of storage which could be due to increase in storage period and natural ageing process.

The present study, seedling vigour index of the greengram seed have shown decreasing pattern from two months to six months because of increased moisture content and insect infestation.

Moisture content of greengram seeds

The results pertaining to the effect of elevated levels of CO_2 treatment on seed moisture content of greengram seed during different storage periods are presented in Table 2.

The moisture content of greengram seeds exposed to different CO_2 concentrations did not show any significant variation among the CO_2 treatments upto two months of storage. While, significantly highest moisture content of 8.50 per cent was recorded in untreated control.

Similar trend was recorded after four months of storage, moisture content varied from 8.09 to 8.33 per cent in different CO_2 concentrations while, in untreated control 8.77 per cent of moisture content was recorded.

Similar trend was observed even after six months of storage, which recorded 8.42 to 8.63 per cent moisture content as against 9.28 per cent moisture content recorded in untreated control.

The mean moisture per cent of 8.06, 8.31 and 8.67 was recorded at two, four and six months after storage, respectively. Results on different storage periods revealed that mean moisture per cent increased with storage period.

The interaction effect between the treatments and storage periods with respect to moisture per cent showed that the least moisture content of 7.90 per cent was recorded at 40 per cent CO_2 concentration after two months of storage and highest moisture content of 9.28 per cent was recorded after six months of storage in untreated control.

In the present investigation, moisture content of greengram seed did not show much variation even after six months after storage, because of being stored in hermetic storage condition. The present results are in conformity with the findings of Bera *et al.* (2008) [8], who reported that under modified atmosphere upto 80 per cent CO_2, paddy seed with 11.00 per cent moisture content can be stored safely upto twelve months without much reduction in seed viability. According to Bera *et al.* (2004) [9] reported that carbon dioxide rich atmosphere had no adverse effect on germinability of wheat seeds, when stored for six months with 12.00 per cent moisture content. Padmasri *et al* (2017) [18, 19] reported that the moisture content of the redgram seeds treated with different CO_2 (30, 40 and 50 per cent) showed no significant difference among the treatments was observed after nine and twelve months of storage. The effect of elevated levels of CO_2 did not show any adverse effects on germination, moisture content and seedling vigour index of greengram seeds.

Significant increase in moisture content in the untreated control and 10 per cent CO_2 concentration resulted due to increasing the seed damage and also adversely affected the germination of greengram seed.

Table 1: Effect of elevated levels of CO_2 on seed damage (per cent) and weight loss due to infestation (per cent)

CO_2 Concentrations	Seed damage (%)				Weight loss (%)			
	2 MAS	4 MAS	6 MAS	Mean	2 MAS	4 MAS	6 MAS	Mean
10% CO_2	0.93 (5.52)	3.90 (11.38)	4.56 (12.32)	3.13 (9.74)	0.34 (4.05)	1.33 (6.62)	2.84 (9.70)	1.50 (6.79)
20% CO_2	0.53 (4.16)	2.49 (9.06)	3.31 (10.47)	2.11 (7.89)	0.29 (4.05)	0.53 (4.16)	2.32 (8.76)	1.04 (5.65)
30% CO_2	0.35	1.62	2.40	1.45	0.19	0.52	2.26	0.99

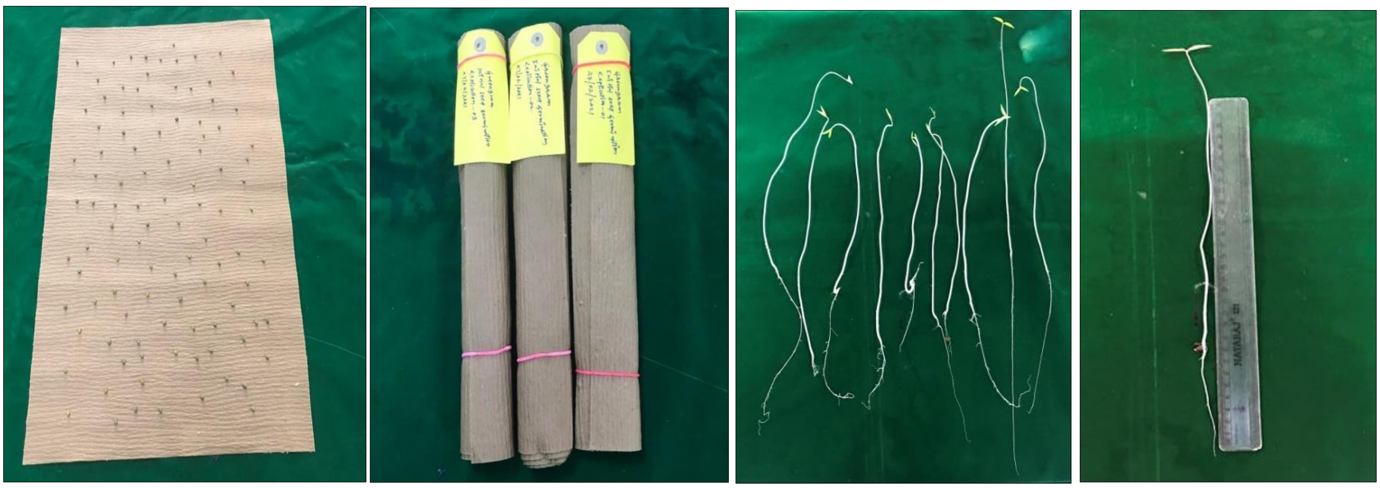
	(4.05)	(7.31)	(8.91)	(6.75)	(4.05)	(4.14)	(8.64)	(5.61)
40% CO_2	0.29 (4.05)	1.58 (7.21)	2.36 (8.83)	1.41 (6.69)	0.11 (4.05)	0.28 (4.05)	1.97 (8.06)	0.78 (5.38)
Control	1.44 (6.90)	5.03 (13.78)	14.57 (22.43)	7.01 (14.37)	1.00 (5.73)	1.58 (7.22)	3.02 (10.00)	1.86 (7.65)
Mean	0.70 (4.93)	2.92 (9.74)	5.44 (12.59)		0.38 (4.38)	0.84 (4.94)	2.48 (9.03)	
	SE(m)±		CD (P=0.05)		SE(m)±		CD (P=0.05)	
Concentrations (F1)	0.10		0.30		0.08		0.16	
Months after storage (F2)	0.08		0.23		0.06		0.12	
Interaction (F1*F2)	0.18		0.53		0.14		0.28	
CV (%)	3.53				2.69			

The values in parentheses are angular transformed values *MAS- Months after storage

Table 2: Effect of CO_2 on germination percentage seedling vigour index and moisture content of greengram seeds during storage

CO_2 Concentrations	Germination percentage				Seedling vigour index				Moisture content (%)			
	2 MAS	4 MAS	6 MAS	Mean	2 MAS	4 MAS	6 MAS	Mean	2 MAS	4 MAS	6 MAS	Mean
10% CO_2	99.00 (84.03)	96.33 (79.14)	95.00 (77.12)	96.77 (80.09)	3210.00	3093.00	2754.00	3019.00	8.03 (16.46)	8.33 (16.77)	8.63 (16.77)	8.33 (16.77)
20% CO_2	99.33 (84.82)	97.00 (80.12)	96.00 (78.52)	97.44 (81.15)	3306.00	3177.00	2944.00	3142.33	7.97 (16.39)	8.23 (16.67)	8.59 (17.03)	8.26 (16.69)
30% CO_2	100.00 (85.95)	98.00 (82.09)	97.00 (80.12)	98.33 (82.72)	3473.00	3321.00	3140.00	3311.33	7.94 (16.36)	8.16 (16.59)	8.46 (16.90)	8.18 (16.61)
40% CO_2	100.00 (85.95)	99.00 (84.03)	97.67 (81.53)	98.89 (83.83)	3668.00	3488.00	3230.00	3462.00	7.90 (16.32)	8.09 (16.52)	8.42 (16.86)	8.13 (16.56)
Control	95.33 (77.64)	94.67 (76.66)	92.67 (74.34)	94.22 (76.21)	3034.00	2899.00	2563.00	2832.00	8.50 (16.94)	8.77 (17.22)	9.28 (17.73)	8.85 (17.29)
Mean	98.72 (83.67)	97.00 (80.40)	95.66 (78.32)		3338.20	3195.60	2926.20		8.06 (16.4)	8.31 (16.75)	8.67 (17.12)	
	SE(m)±		**CD (P=0.05)**		**SE(m)±**		**CD (P=0.05)**		**SE(m)±**		**CD (P=0.05)**	
Concentrations (F1)	0.64		1.85		49.00		141.53		0.08 0.06 0.14		0.23 0.18 0.40	
Months after storage (F2)	0.49		1.45		37.95		109.63		0.06		0.18	
Interaction (F1*F2)	1.11		3.21		84.87		245.14		0.14		0.41	
CV (%)	2.38				4.66				1.45			

The values in parentheses are angular transformed values *MAS- Months after storage



A. Seeds on germination paper **B.** Rolled paper towel method **C.** Germinated seedling **D.** Seedling measurements

Fig 1: Germination test of greengram seeds (Paper towel method)

Fig 2: Damaged seeds caused by *C. chinensis* (L.)

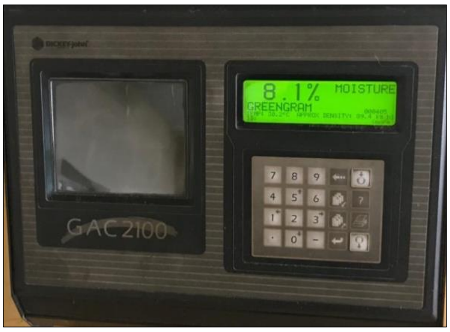


Fig 3: Dickey John Moisture Meter

Conclusions

It was evident that modified atmosphere with CO_2 treatments can be a good alternative to use of chemical treatments instead of conventional harmful fumigants. These findings important recommendation of residue free, safe and long-term storage of greengram without any detrimental effect on germination (maintained above 90 per cent), seedling vigour and moisture content (8.63 per cent) upto six months of storage.

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