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RESEARCH ARTICLE

Effect of elevated carbon dioxide on the control of lesser grain borer (*Rhyzopertha dominica*) in stored rice and its impact on seed quality

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Abstract

This study evaluated the effects of carbon dioxide (CO_2) exposure at different concentrations, seed moisture levels and number of exposures on the physiological, biochemical and seed health characteristics of rice cv ADT 43. Initially, rice seeds were treated in a deep freezer for 48 h to eliminate *Rhyzoperth dominica* infestations. Fresh insects (25 per kg of seeds) were introduced and kept for 20 days before transferring the seeds to airtight containers. The seeds were exposed to CO_2 once or thrice at 15-day intervals, using CO_2 concentrations of 30%, 40% and 50%, with 12% and 14% seed moisture contents. The effectiveness of CO_2 in controlling insect infestations was assessed, alongside its impact on seed viability, germination and overall seed health. Seeds were monitored over 8 months to track changes in seed vigor and insect damage. CO_2 exposure did not cause substantial changes in seed moisture content and maintained low metabolic activity in both seeds and insects. The best results were observed with exposure to 50% CO_2 at 12% seed moisture, especially when repeated thrice, preserved optimal seed quality, including high germination percentage (86% to 87%), longer root (20.3cm) and shoot lengths (9.2) and better dry matter production (DMP) (0.128) compared to the control group. The results of the biochemical analysis show that there is no significant difference among the treatments. CO_2 fumigation at concentrations of 30%, 40%, or 50% is an effective method that offers a promising alternative for controlling insect infestations in rice seeds pest control in seed storage without adversely affecting seed viability or quality.

Keywords

carbon dioxide; insect population; lesser grain borer; rice seed quality; seed moisture

Introduction

Rice is the staple food for more than 60% of the world's population and is mainly grown and consumed in Asia, making it one of the most important edible grains. India is the country with the most rice-growing area worldwide and it produces the second most after China. It is preferred by 65% of the Indian population and is grown under different agro-climatic conditions.

The most essential and fundamental component of sustainable agriculture is seed. The quality of the seeds greatly influences how all other inputs respond. Quality seed production and efficient distribution play a significant role in increasing food grain production. It has been projected that high-quality seeds could improve food production by 15-25%. Since quality loss during storage is unavoidable, maintaining good germination and vigor from harvest to planting is a fundamental need in the seed industry.

Over 100 insect pests infest the crop, causing a 30% production loss if left unchecked. In addition, stored-grain insects often cause as much loss as after harvest. These losses can be through direct feeding, resulting in a reduction of weight, nutritional value, germination and marketability, downgrading the quality (1). The most significant storage bug that infests various cereals, including rice, is the lesser grain borer, *R. dominica* (F.). Both adults and larvae feed within the grains and the grains are reduced to mere shells because of hidden infestation until the adults are apparent. It may also facilitate the invasion of a secondary pest. This insect is a vigorous flier and may quickly migrate from infested grain or seed to start new infestations, which are more likely to occur in "dirty" grain than clean grain. Grain dust, cracked kernels and dockage allow some insects to survive and reproduce in low-moisture environments.

The percentage of food grains lost after harvest due to mold growth and insect infestation has been conservatively estimated to be between 10% and 15%. Currently, pest control procedures in storage rely on synthetic pesticides and fumigants, the quickest and most reliable methods of control. However, indiscriminate pesticide use has resulted in the emergence of insect strains resistant to pesticides and issues with pesticide residue in food grains (2). Additionally, as insect populations become more resistant to pesticides, the effectiveness of pesticides is declining (3). In addition, nearly all fumigants, including methyl bromide and aluminium phosphide, have recently had their registrations revoked in many developed nations due to concerns about potential human cancer risks. This highlights the need to identify practical, physical and sustainable control methods that are environmentally friendly, such as MA storage, controlled atmosphere storage and cold treatments for controlling storage pests without affecting the nutritive value of grain and seed germinability.

Using a MA effectively can control insect damage (4-6). The concentrations of nitrogen (N) (78%), oxygen (O) (20.9%), CO_2 (1.1%) and other rare gases (0.03%) in the typical ambient gas mixture are changed in this storage method to a level that is lethal to pests that feed on stored products. Researchers investigated the lethality of stored-product insects exposed to MA (7, 8). Over the last few years, much work has gone into creating pest management strategies for stored products that complement or replace traditional insecticides and fumigants. Controlling insects and mites that damage a wide range of items can be done safely and sustainably with modified environment treatments (9).

Several researchers have suggested the importance of a controlled atmosphere (CA) and a MA (10, 11). Controlling the storage atmosphere using atmospheric gases is being investigated since they do not exhibit the immediate deadly effects characteristic of organic substances (12, 13). They pose little damage to man and animals and present no residue problems in treated food commodities.

The scientist reported that CO_2 is a highly desirable method for sanitizing stored durable goods and food items. As a method for insect control in bulk commodities, MA systems through increased CO_2 or decreased O atmospheres, or a combination of both, offer scope for controlling insect pests (14, 15). Hermetic storage is a usage for this technology that is rarely possible in rigid silos but is readily achieved with flexible plastic liners (16, 17). According to the authors, MA can be used in the food and agriculture sectors to protect and preserve fresh produce from harvest to end product. It is a non-toxic, environmentally friendly substitute for fumigation in controlling insects in storage facilities (18). Considering the above, experiments were carried out to understand the effect of induced CO_2 treatment on the management of lesser grain borer (*R. dominica*) in rice and their mode of action on seed quality.

Materials and Methods

Freshly harvested rice cv ADT 43 seeds obtained from Tamil Nadu Rice Research Institute, Aduthurai reconstituted the base material for the study. The test insects used for the study were lesser grain borer (*R. dominica*) for rice (Fig.1). The laboratory studies were carried out at the Department of Genetics and Plant Breeding laboratory.

Design of airtight container for exposure to carbon dioxide

The procedure to inject CO_2 and the design of an airtight container were adopted (19). Two perforations of 3 mm diameter were made for the inlet and outlet in airtight plastic containers of 1 L volume and nylon tubes of 3 mm diameter were inserted. Rubber corks of 3 mm diameter, which seal the inlet and outlet tubes exactly, were used to plug the tubes after filling CO_2. The entire system was made airtight with teflon tape and superglue.

Estimation of CO_2 to be injected for various concentrations

Carbon dioxide cylinder consisting of 2 gauges, one for measuring cylinder pressure and another for the outlet valve pressure with an initial pressure of 150 kg cm^{-2} was used to obtain the required volume and concentration of CO_2. The volume of CO_2 to be injected to obtain different concentrations in the plastic containers was calculated (20).

The volume of CO_2 to be injected in mL (V_c)

$$V_C = \frac{D_C}{100-D_C} \times Va \qquad \text{(Eqn.1)}$$

Where,

V_c = Volume of CO_2 to be added in mL

D_c = Desired concentration of CO_2 (%)

V_a= Volume of free space in a container above the seeds, including intergranular space (mL)

The time required for obtaining different concentrations of CO_2 was calculated using a flow meter to record the flow rate of CO_2 and was indicated as standard liters per minute (SLPM) when outlet pressure is 1 kg cm^{-2}.

The time required in seconds (T_c)

Where, $$T_C = \frac{V_C}{1000} \times 60 \quad \text{(Eqn.2)}$$

V_C = Volume of CO_2 to be added in mL

T_C = Time required in seconds to open CO_2 cylinder at a flow rate of 1 SLPM (standard liter per minute) to achieve the desired CO_2 concentration.

The volume of CO_2 to be injected (V_C) for various concentrations (D_c) and the time required for injection (T_C) were calculated using the table designed (Table 1) and the concentrations of CO_2 were test verified using CO_2 analyzer.

Table 1. Volume and time of injection of CO_2 for obtaining desired concentrations of CO_2

Desired concentration of CO_2 (%)	Volume of CO_2 to be added (mL)*	Time of injection of CO_2 at 1 SLPM(Sec)
D_C	V_C	T_C
0	0	0
5	46	3
10	97	6
15	154	9
20	219	13
30	375	23
40	583	35
50	875	53

* VC calculated taking Va= 0.8751(Volume of free space in the container above 100 g seeds)

Injection of CO_2 into an airtight container

Carbon dioxide was allowed into the airtight containers through the 3 mm inlet hole (bottom of the containers), keeping the outlet (top of the containers) open to flush out air from the container as per the required concentration of CO_2 to the time required for injection. After injecting the gas, the inlet and outlet were closed at one stroke using rubber corks to prevent the escape of CO_2 from the container (Fig.1)

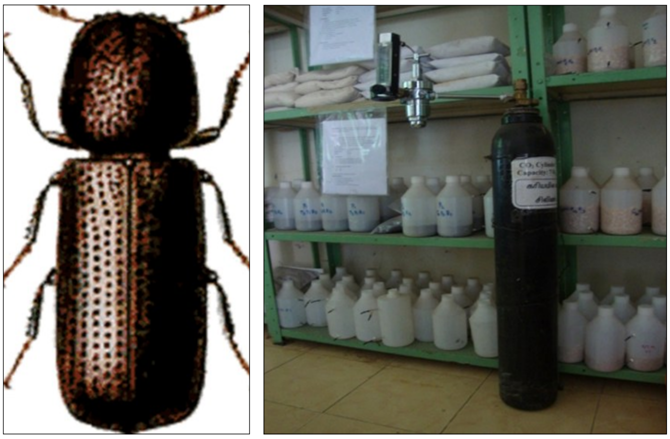

Fig. 1. Test insects and storage room with CO_2 cylinders and airtight containers. A: *R. dominica* ; B:CO_2 cylinder and airtight containers.

Concentrations of carbon dioxide and seed moisture on insect infestation and seed quality

Seed samples were dried at 35 °C for varying durations to obtain a moisture content of 12 and 14% for rice. The seed moisture content to a pre-determined value was adjusted using the formula (21) and the calculated quantity of water was added to the sample to bring it to the required moisture content.

$$Ww\ (g) = \frac{RMC-IMC}{100 - RMC} \quad \text{(Eqn.3)}$$

Where,

Ww - Weight of water to be added

RMC - Required Moisture Content in percentage

IMC - Initial Moisture Content in percentage

The rice seeds were disinfested with the required moisture content by keeping them at 20 °C for 48 h in a deep freezer in a moisture-proof container to kill all existing insects. The seeds were artificially infested with *R. dominica* at 25 insects kg^{-1} of rice seeds of cv. ADT 43 was kept as such for 20 days and the initial observations on pretreated seeds (0 months) were recorded. The inoculated seeds were transferred to airtight containers of 1 L capacity with 500 g of rice and subjected to a single exposure to CO_2 of different concentrations *viz,* 30, 40 and 50%. They were stored in the same airtight containers under ambient conditions for 8 months, adopting Factorial CRD with 4 replications. Samples were drawn at bimonthly intervals and observed for physiological and biochemical characteristics of seed health. However, after 8 months, uric acid content and field emergence were observed for best-performing treatments and control. (Table 2)

Concentrations of carbon dioxide and number of exposures to insect infestation and seed quality

Another set of experiments was conducted using the same procedure at a seed moisture content of 12.5%. Seeds were exposed to CO_2 at different concentrations, *viz.,* 30, 40 and 50% once and thrice at an interval of 15 days and stored under ambient adopting Factorial CRD with 4 replications. Samples were drawn at bimonthly intervals and observed for seed health, physiological and biochemical characteristics. However uric acid content and the field emergence were observed after 8 months for best-performing treatments and control. (Table 2)

Seed health

Live adults: The population of live adults in 100 g of seeds was counted and the mean was expressed as live adults for 100 g.

Insect infestation (%): The emergence hole from 100 seeds was observed for insect infestation and the mean was expressed in percentage (22).

Pathogen infection (%): The blotter method detected pathogens. Twenty five seeds were placed in 16 replicates equidistantly on 3 layered moistened blotters in sterilized petri plates. The seeds were then incubated at 20 ± 2 °C for 7 days with alternate cycles of 12 h in near ultraviolet light (NUV) and the remaining 12 h in darkness. On the 7th day, the seeds were inspected for signs of fungal infection. The mean

Table 2. Details of CO_2 concentrations, exposures and moisture levels for rice storage

Particulars	Rice
CO_2 concentrations (%)	30, 40, 50
Number of exposures	Once and thrice
Moisture content (%)	12 and 14

was expressed as a percentage (23).

Physiological seed quality characters

Seed moisture content (%): Rice seed moisture content was evaluated at 130 ± 1 °C for 2 h using a high constant oven technique. After drying, the seed samples were placed in calcium chloride desiccators for 30 min before being weighed. The % moisture content was estimated using the formula.

$$MC\ (\%) = \frac{M_2 - M_3}{M_2 - M_1} \times 100 \qquad \text{(Eqn. 4)}$$

Where,

MC = Moisture content

M_1= Weight of empty moisture bottle along with lid (g)

M_2= Weight of moisture bottle along with sample before drying (g)

M_3= Weight of moisture bottle along with sample after drying (g)

Germination (%): According to the ISTA (23) procedure, the germination test was conducted using paper (Between paper) medium. Four replicates of 100 rice seeds each were germinated in a germination room maintained at 25 ± 2 °C temperature and 95 ± 3% relative humidity. At the end of the prescribed period (14 days for rice), the number of normal seedlings in each replication was counted and the mean was expressed in percentage.

The root and shoot length were measured in cm and the mean values were calculated during the final count. The seedlings after measuring the root and shoot length, DMP was calculated and expressed in g 10 seedlings^{-1}.

Biochemical analysis

Quantification of starch (mg g^{-1}) : Five hundred mg of seed sample was homogenized in hot ethanol 80% to extract starch and centrifuged for retention of the residue. The residue was washed repeatedly with hot 80% ethanol and dried over a bath. 5.0 mL of water and 6.5 mL of 52% perchloric acid were added and kept at 0 °C for 20 min centrifuged again and saved the supernatant. The extraction was repeated with fresh perchloric acid. The supernatants were pooled and made up to 100 mL. From this 0.1 mL was taken and made up to 1.0 mL with water. Four mL of anthrone reagent was added and heated for 8 min. in a boiling water bath. The intensity of the dark green color was read at 630 nm by an Optima UV-VIS spectrophotometer (Model SP-3000). The starch content was expressed as mg g^{-1} in the sample (24).

Quantification of α-Amylase activity (mg maltose min^{-1}) : Enzymes were extracted from 500 mg of pregerminated rice seed samples by homogenizing them in 1.8 mL of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuging the mixture for 20 min at 20,000 rpm. 1mL of 0.067% starch solution was added to 0.1 mL of enzyme extract. 1 mL of iodine HCl solution (60 mg KI and 6 mg I2 in 100 mL of 0.05 N HCl) was added to stop the reaction after 10 min of incubation at 25 °C.

Color change was measured at 620 nm. The activity was

$$\alpha\text{ - Amylase activity} = \frac{\text{OD value}}{\text{Volume of sample Pipetted out}} \times \frac{1000}{500}$$

calculated and expressed as mg maltose min^{-1} (Eqn.5) (25).

Free amino acid (µg 50/seeds 50/mL): 50 rice seeds were soaked in 50 mL distilled water for 12 h. In a boiling water bath, one mL of 0.2% ninhydrin was combined with 1 mL of seed leachate and allowed to boil for 15 min. After that, it was cooled in running water and diluted to 10 mL. The color generated was then measured at 620 nm using an Optima UV-VIS spectrophotometer (Model SP-3000) against a leucine standard curve and the result was expressed in µg50 seeds-150 mL^{-1} (26).

Uric acid content (mg/100g)

Fifty grams of sample was finely ground to obtain a sub-sample of 4-5 g powder and soaked in 200 mL water. The sample was allowed to stand for 2 h and centrifuged. To 100 mL of supernatant, 10 mL of sodium tungstate solution was added and mixed. This was followed by adding 10 mL standard sulphuric acid solution to precipitate the proteins in the extract. An aliquot of the filtrate in a 50 mL volumetric flask was taken and 2% sodium hydroxide and 2% phosphotungstic acid reagent were added. Ten mL of standard uric acid solution containing 0.2 mg in a 50 mL flask was prepared and diluted after 5 min. The blue color intensity was measured in a UV spectrophotometer at 510 nm and expressed in mg 100g^{-1} (27).

Field emergence (%)

Hundred seeds in each of rice cv. ADT 43 was replicated 4 times and sown in a nursery by adopting FRBD. The number of seedlings germinated was counted on the 14th day and the mean was expressed in percentage.

Statistical analysis

The data obtained from different experiments were analyzed for the 'F' test of significance following the methods described by the scientist (28). Wherever necessary, the percentage values were transformed to angular (arc-sine) values before analysis. Similarly, number of live adults was transformed to square root values. The critical differences (CD) were computed at the 5% probability level. A statistical significance test was performed on the data. The letter NS was meant for non-significant (NS) if the F test was non-significant.

Results

Concentrations of carbon dioxide and seed moisture on storability

Highly significant differences were obtained with the concentrations of CO_2 (T) except for amylase content (Table 10), free amino acid (Table 11) and starch. Similarly, highly significant differences were obtained with seed moisture (M) except for moisture content (Table 4), root length (Table 6), shoot length (Table 7) and amylase content and with periods of storage (S) except for moisture content (Table 4) and starch (Table 9). The interaction effect between

S × T was highly significant except for moisture content (Table 4), free amino acid (Table 11) and starch (Table 21). Between T×M and S×M, most of the parameters are NS.

Seed health

Live adults: The number of live adults decreased with storage periods (SP) from 9.5 to 8.6. Carbon dioxide at 50% concentration and 12% seed moisture content showed fewer live adults (1.8) than the control (21.2). Carbon dioxide at 50% concentration at 12% seed moisture registered a lower number of adults (6.6) than 14% (7.8) (Table 3).

Insect infestation (%) : The insect infestation increased with SP from 0.0 to 8.7%. Carbon dioxide at 50% concentration registered the lower infestation (0.2) while controlling the higher infestation (19.2). Exposure to 12% moisture content recorded the minimum infestation (4.4) and 14% moisture (5.8). Carbon dioxide at 50% concentration recorded lower infestation at any SP (Table 4).

Pathogen infection (%): Seeds with 12% moisture content recorded the lowest pathogen infection (12.1) compared to 14% (16.3). Carbon dioxide exposed at 12% moisture registered lower infection (7.1) than the control (21.6). However, with advances in the S, it increased from 12.4 to 18.0%. At all SP seeds with 12% moisture content recorded the lowest pathogen infection (12.1) (Table 5).

Physiological seed quality characters

Moisture content (%) : The moisture content was 13.06 % at 50 % concentration and 13.11% at 30 % concentration (Table 6).

Table 3. Concentrations of carbon dioxide and seed moisture on live adults *(R. dominica)* of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
(Pretreatment)	**9.0 (3.08)**	**10.0 (3.24)**	**9.5 (3.16)**	**9.0 (3.08)**	**10.0 (3.24)**	**9.5 (3.16)**	**9.0 (3.08)**	**10.0 (3.24)**	**9.5 (3.16)**	**9.0 (3.08)**	**10.0 (3.24)**	**9.5 (3.16)**	**9.0 (3.08)**	**10.0 (3.24)**	**9.5 (3.16)**
2	17.6 (4.25)	20.4 (4.57)	19.0 (4.41)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	4.4 (2.21)	5.1 (2.36)	4.7 (2.28)
4	22.6 (4.80)	27.2 (5.26)	24.9 (5.03)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	5.6 (2.46)	6.8 (2.70)	6.2 (2.58)
6	26.6 (2.71)	29.4 (29.9)	28.0 (5.33)	0.0 (0.70)	2.0 (1.58)	1.0 (1.22)	0.0 (0.70)	0.6 (1.04)	0.3 (0.89)	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)	6.6 (2.66)	8.0 (2.91)	7.3 (2.79)
8	30.6 (5.57)	33.0 (5.78)	31.8 (5.68)	0.0 (0.70)	3.0 (1.87)	1.5 (1.41)	0.0 (0.70)	1.4 (1.37)	0.7 (1.09)	0.0 (0.70)	0.8 (1.14)	0.4 (0.94)	7.6 (2.84)	9.5 (3.16)	8.6 (3.01)
Mean	21.2 (4.65)	24.0 (4.94)	22.6 (4.80)	1.8 (1.51)	3.0 (1.87)	2.4 (1.70)	1.8 (1.51)	2.4 (1.54)	2.1 (1.61)	1.8 (1.51)	2.1 (1.61)	1.9 (1.54)	6.6 (2.66)	7.8 (2.91)	

CD	SEd	CD
S	(0.276)	(0.546)
T	(0.247)	(0.488)
M	(0.175)	(0.345)
S × T	(0.553)	(1.093)
T × M	(0.350)	(0.691)
S × M	(0.391)	NS
S × T × M	(0.782)	NS

(Figures in parentheses are square root transformations); CD – Critical difference, SEd – Standard Error of difference, NS- Non-Significant

Table 4. Concentrations of carbon dioxide and seed moisture on insect (*R. dominica*) infestation (%) of rice cv. ADT 43 during storage

qPeriods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**
2	16.2 (23.73)	19.8 (26.42)	18.0 (25.10)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	4.0 (11.53)	4.9 (12.78)	4.5 (12.24)
4	20.4 (26.85)	23.4 (28.93)	21.9 (27.90)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	5.1 (13.05)	5.8 (13.93)	5.4 (13.43)
6	23.2 (28.79)	27.8 (31.82)	25.5 (30.33)	0.0 (0.41)	3.2 (10.30)	1.6 (7.26)	0.0 (0.41)	1.2 (6.28)	0.6 (4.44)	0.0 (0.41)	0.8 (5.13)	0.4 (3.62)	5.8 (13.93)	8.2 (16.64)	7.0 (15.34)
8	28.4 (32.20)	32.8 (34.94)	30.6 (33.58)	0.0 (0.41)	4.6 (12.38)	2.3 (8.72)	0.0 (0.41)	2.4 (8.91)	1.2 (6.28)	0.0 (0.41)	1.4 (6.79)	0.7 (4.79)	7.1 (15.45)	10.3 (18.72)	8.7 (17.15)
Mean	**17.6 (24.80)**	**20.7 (27.06)**	**19.2 (25.98)**	**0.0 (0.41)**	**1.5 (7.03)**	**0.7 (4.79)**	**0.0 (0.41)**	**0.7 (4.79)**	**0.3 (3.13)**	**0.0 (0.41)**	**0.4 (3.62)**	**0.2 (2.56)**	**4.4 (12.10)**	**5.8 (13.93)**	

CD	SEd	CD
S	(0.287)	(0.566)
T	(0.256)	(0.506)
M	(0.181)	(0.358)
S × T	(0.574)	(1.496)
T × M	(0.363)	(0.716)
S × M	(0.405)	(0.801)
S × T × M	(0.811)	NS

(Figures in parentheses are arc sine transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 5. Concentrations of carbon dioxide and seed moisture on pathogen infection (%) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	**10.4 (18.81)**	**14.4 (22.30)**	**12.4 (20.61)**	**10.4 (18.81)**	**14.4 (22.30)**	**12.4 (20.61)**	**10.4 (18.81)**	**14.4 (22.30)**	**12.4 (20.61)**	**10.4 (18.81)**	**14.4 (22.30)**	**12.4 (20.61)**	**10.4 (18.81)**	**14.4 (22.30)**	**12.4 (20.61)**
2	21.0 (27.27)	23.2 (28.79)	22.0 (27.97)	9.0 (17.45)	14.4 (22.30)	12.0 (20.26)	6.0 (14.17)	11.2 (19.55)	8.4 (16.84)	3.2 (10.30)	9.0 (17.45)	6.0 (14.17)	10.0 (18.43)	14.0 (21.97)	12.0 (20.26)
4	23.2 (28.79)	28.0 (31.94)	25.6 (30.39)	10.4 (18.81)	15.2 (22.94)	13.0 (21.13)	6.4 (14.65)	14.0 (21.97)	10.0 (18.43)	6.0 (14.17)	12.0 (20.26)	9.0 (17.45)	12.0 (20.26)	17.0 (24.35)	14.0 (21.97)
6	26.0 (30.65)	30.0 (33.21)	27.6 (31.69)	15.2 (22.94)	16.4 (23.88)	16.0 (23.57)	10.0 (18.43)	15.0 (22.78)	12.2 (20.44)	7.2 (15.56)	12.0 (20.26)	10.0 (18.43)	12.0 (20.26)	16.0 (23.57)	14.0 (21.97)
8	28.0 (31.94)	33.0 (35.06)	30.4 (33.46)	16.0 (23.57)	18.0 (25.10)	17.0 (24.35)	11.2 (19.55)	16.0 (23.57)	14.0 (21.97)	9.0 (17.45)	13.2 (21.30)	11.0 (19.37)	16.0 (23.57)	20.0 (26.56)	18.0 (25.10)
Mean	**21..6 (27.69)**	**25.7 (30.46)**	**23.6 (29.06)**	**12.2 (20.44)**	**15.7 (23.34)**	**14.1 (22.05)**	**8.8 (17.25)**	**14.1 (22.05)**	**11.4 (19.73)**	**7.1 (15.45)**	**12.1 (20.35)**	**9.7 (18.14)**	**12.1 (20.35)**	**16.3 (23.81)**	**14.1 (22.05)**

CD	SEd	CD
S	(1.279)	(2.527)
T	(1.144)	(2.260)
M	(0.809)	(1.598)
S × T	(2.559)	(5.054)
T × M	(1.618)	NS
S × M	(1.809)	NS
S × T × M	(3.619)	NS

(Figures in parentheses are arc sine transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 6. Concentrations of carbon dioxide and seed moisture on moisture content (%) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	**12.00**	**14.00**	**13.00**	**12.00**	**14.00**	**13.00**	**12.00**	**14.00**	**13.00**	**12.00**	**14.00**	**13.00**	**12.00**	**14.00**	**13.00**
2	12.09	14.07	13.08	12.07	14.08	13.07	12.06	14.05	13.06	12.05	14.03	13.04	12.07	14.06	13.06
4	12.14	14.12	13.13	12.13	14.10	13.11	12.09	14.08	13.08	12.07	14.05	13.06	12.11	14.08	13.10
6	12.18	14.18	13.18	12.17	14.16	13.16	12.13	14.11	13.12	12.11	14.08	13.09	12.15	14.13	13.14
8	12.24	14.23	13.23	12.21	14.19	13.20	12.18	14.17	13.17	12.15	14.12	13.13	12.19	14.17	13.18
Mean	**12.13**	**14.12**	**13.12**	**12.12**	**14.11**	**13.11**	**12.09**	**14.08**	**13.09**	**12.08**	**14.06**	**13.06**	**12.10**	**14.09**	

CD	SEd	CD
S	(0.068)	NS
T	(0.060)	NS
M	(0.043)	NS
S × T	(0.096)	NS
T × M	(0.086)	NS
S × M	(0.136)	NS
S × T × M	(0.192)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Germination (%) : The germination percent decreased with S from 90 to 73%. Carbon dioxide at 50% concentration recorded the maximum germination (87), compared to the control (78). The germination was higher at 12% seed moisture (86) compared to 14% (81). Seeds at 12% moisture registered higher germination at all SP (Table 7).

Root length (cm) : Carbon dioxide at 50% concentration recorded the longest root length of 20.3 cm, followed by other concentrations and control (18.6). Seeds with 12% moisture registered longer root length (20.0) than 14% moisture (19.0), decreased with SP and recorded 18 cm after 8 months. The reduction was less after 2 months (0.8) and more after 8 months (1.2) (Table 8).

Shoot length (cm): Carbon dioxide at 50% concentration recorded the longest shoot length (9.2) compared to control (8.4). Seeds with 12% moisture registered longer shoot lengths (9.2) than those with 14% (8.6). The shoot length decreased with periods of storage from 10.0 to 8.1 cm. At all SP, CO_2 was at 50% concentration and seeds with a moisture content of 12% had the longest shoot length (Table 9).

Dry matter production (g 10 seedlings $^{-1}$) : The dry matter decreased with SP from 0.143 to 0.109g. Carbon dioxide at 50% concentration at 12% seed moisture recorded higher DMP (0.128) than control (0.118). Exposure to 50% CO_2 at 12% seed moisture registered maximum dry matter (0.129) than 14% (0.118). At lower seed moisture content, the DMP was always higher at all SP (Table 10).

Biochemical seed quality characters

Starch : The data in Table 9 evaluates the effects of CO_2 concentrations and seed moisture levels on starch content

Table 7. Concentrations of carbon dioxide and seed moisture on germination (%) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	92 (73.57)	89 (70.63)	90 (71.56)	92 (73.57)	89 (70.63)	90 (71.56)	92 (73.57)	89 (70.63)	90 (71.56)	92 (73.57)	89 (70.63)	90 (71.56)	92 (73.57)	89 (70.63)	90 (71.56)
2	86 (68.02)	83 (65.65)	85 (67.21)	89 (70.63)	86 (68.02)	87 (68.86)	90 (71.56)	87 (68.86)	88 (69.73)	91 (72.54.)	88 (69.73)	90 (71.56)	89 (70.63)	86 (68.02)	88 (69.73)
4	82 (64.89)	81 (64.15)	82 (64.89)	86 (68.02)	85 (67.21)	85 (67.21)	89 (70.63)	86 (68.02)	88 (69.73)	90 (71.56)	87 (68.86)	89 (70.63)	87 (68.86)	85 (67.21)	86 (68.02)
6	74 (59.34)	72 (58.05)	73 (58.69)	84 (66.42)	81 (64.15)	82 (64.89)	86 (68.02)	82 (64.89)	84 (66.42)	89 (70.63)	83 (65.65)	86 (68.02)	83 (65.65)	80 (63.43)	81 (64.15)
8	66 (54.33)	54 (47.29)	60 (50.76)	82 (64.89)	67 (54.94)	74 (59.34)	82 (64.89)	72 (58.05)	77 (61.34)	86 (68.02)	76 (60.66)	81 (64.15)	79 (62.72)	67 (54.94)	73 (58.69)
Mean	80 (63.43)	76 (60.66)	78 (62.02)	87 (68.86)	82 (64.89)	84 (66.42)	88 (69.73)	83 (65.65)	85 (67.21)	90 (71.56)	85 (67.21)	87 (68.86)	86 (68.02)	81 (64.15)	

CD	SEd	CD
S	(1.205)	(2.381)
T	(1.078)	(2.129)
M	(0.762)	(1.506)
S × T	(2.411)	(4.762)
T × M	(1.525)	NS
S × M	(1.705)	(3.367)
S × T × M	(3.410)	NS

(Figures in parentheses are arc sine transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 8. Concentrations of carbon dioxide and seed moisture on root length (cm) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	**22.7**	**21.3**	**22.0**	**22.7**	**21.3**	**22.0**	**22.7**	**21.3**	**22.0**	**22.7**	**21.3**	**22.0**	**22.7**	**21.3**	**22.0**
2	19.2	18.2	18.7	19.7	18.9	19.3	19.9	19.6	19.7	21.1	20.2	20.6	20.0	19.2	19.6
4	18.9	18.2	18.5	19.4	18.4	18.9	19.7	19.3	19.5	20.7	19.9	20.3	19.7	18.9	19.3
6	17.6	17.0	17.3	19.1	18.1	18.6	19.3	18.6	19.0	19.9	18.9	19.4	19.0	18.2	18.6
8	16.8	15.9	16.4	18.7	17.0	17.8	19.1	18.1	18.6	19.7	18.6	19.2	18.6	17.4	18.0
Mean	**19.0**	**18.1**	**18.6**	**19.9**	**18.7**	**19.3**	**20.1**	**19.4**	**19.8**	**20.8**	**19.8**	**20.3**	**20.0**	**19.0**	

CD	SEd	CD
S	(1.205)	(2.381)
T	(1.078)	(2.129)
M	(0.762)	(1.506)
S × T	(2.411)	(4.762)
T × M	(1.525)	NS
S × M	(1.705)	(3.367)
S × T × M	(3.410)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 9. Concentrations of carbon dioxide and seed moisture on shoot length (cm) of rice cv.ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	10.5	9.5	10.0	10.5	9.5	10.0	10.5	9.5	10.0	10.5	9.5	10.0	10.5	9.5	10.0
2	9.0	8.8	8.9	9.7	9.0	9.4	9.9	9.3	9.6	10.0	9.5	9.7	9.6	9.2	9.4
4	8.2	8.0	8.1	8.8	8.5	8.6	9.1	8.6	8.9	9.5	8.7	9.1	8.9	8.5	8.7
6	8.0	7.4	7.7	8.7	8.3	8.5	8.8	8.5	8.6	9.1	8.6	8.8	8.6	8.2	8.4
8	7.9	6.7	7.3	8.5	7.9	8.2	8.6	8.0	8.3	8.8	8.3	8.5	8.4	7.8	8.1
Mean	**8.7**	**8.1**	**8.4**	**9.2**	**8.6**	**8.9**	**9.4**	**8.8**	**9.1**	**9.6**	**8.9**	**9.2**	**9.2**	**8.6**	

CD	SEd	CD
S	(0.098)	(0.193)
T	(0.087)	(0.173)
M	(0.062)	(0.122)
S × T	(0.196)	(0.387)
T × M	(0.124)	NS
S × M	(0.138)	(0.274)
S × T × M	(0.277	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 10. Concentrations of carbon dioxide and seed moisture on dry matter production (g 10 seedlings[-1]) of rice ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0(Pretreatment)	**0.145**	**0.142**	**0.143**	**0.145**	**0.142**	**0.143**	**0.145**	**0.142**	**0.143**	**0.145**	**0.142**	**0.143**	**0.145**	**0.142**	**0.143**
2	0.127	0.117	0.122	0.132	0.121	0.126	0.138	0.123	0.130	0.142	0.126	0.134	0.135	0.122	0.128
4	0.123	0.110	0.116	0.127	0.115	0.121	0.129	0.118	0.123	0.135	0.121	0.128	0.128	0.116	0.122
6	0.115	0.100	0.107	0.121	0.111	0.116	0.123	0.112	0.117	0.128	0.114	0.121	0.122	0.109	0.115
8	0.109	0.093	0.101	0.114	0.102	0.108	0.118	0.105	0.111	0.121	0.107	0.114	0.115	0.102	0.109
Mean	**0.124**	**0.112**	**0.118**	**0.128**	**0.118**	**0.123**	**0.131**	**0.120**	**0.125**	**0.134**	**0.122**	**0.128**	**0.129**	**0.118**	

CD	SEd	CD
S	(0.000)	(0.001)
T	(0.000)	(0.001)
M	(0.000)	(0.000)
S × T	(0.001)	(0.002)
T × M	(0.000)	NS
S × M	(0.001)	(0.002)
S × T × M	(0.002)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

(mg g[-1]) of rice variety ADT 43 during storage over different periods. Across all SP, the starch content remained relatively stable, with minor variations observed within treatments.

No significant differences were detected for the main factors (SP, CO_2 concentrations and seed moisture levels) or their interactions, as indicated by the NS and CD values at p=0.05. Overall, the treatments and storage durations did not significantly affect the starch content of rice seeds (Table 11).

α-Amylase activity : Table 10 presents the influence of CO_2 concentrations and seed moisture levels on the α-amylase activity (mg maltose min[-1]) of rice variety ADT 43 during storage. A gradual decrease in α-amylase activity is observed across all storage durations, irrespective of the treatment. Differences in α-amylase activity between CO_2 concentrations and moisture levels are minor, ranging from 1.174 to 1.146 mg maltose min[-1]. The standard error (SEd) and CD values indicate NS effects of the main factors (S, T and M) or their interactions on α-amylase activity at p=0.05. The α-amylase activity in rice seeds slightly declined over time but was not significantly affected by CO_2 concentrations or seed moisture levels during storage (Table 12).

Free amino acid: Free amino acid content consistently increased over the SP across all treatments. Variations in CO_2 concentrations and moisture levels caused slight differences in amino acid content. The highest mean values were observed at 14% seed moisture levels and 50% CO_2 concentration, while the lowest occurred at 12% seed moisture levels with the control. Analysis of variance indicated NS of SP, CO_2 concentration (T), seed moisture (M), or their interactions on free amino acid content at p=0.05, as shown by the NS CD values. Free amino acid content increased from 22.7 to 25.4 with advances in the SP. Similarly, an increase in seed moisture content increased free amino acids (23.8 µg and 24.4 µg) (Table 13).

Uric acid : During the initial period, the control and CO_2 50% treatments showed identical uric acid content at 3.63 mg/100 g. After 8 months of storage, the control treatment resulted in a significantly higher uric acid content (8.72 mg/100 g) than the CO_2 50% treatment (6.98 mg/100 g). The overall mean uric acid content across treatments and time was 7.85 mg/100 g for the 8 months and 6.17 mg/100 g for the Control treatment, while the CO_2-treated samples averaged 5.30 mg/100 g. The SEd values were 0.051 for treatments (T) and SP and 0.072 for their interaction (S × T). The CD at p=0.05) values were 0.109 for T, 0.108 for S and 0.154 for S × T, highlighting significant interaction effects. This data demonstrates that CO_2 treatment effectively reduces uric acid accumulation in rice seeds during storage compared to untreated controls (Table 14).

Field emergence: At 12% moisture content, the field emergence was higher in the CO_2 50% treatment (87%) compared to the Control (61%), with respective values in

Table 11. Concentrations of carbon dioxide and seed moisture on starch (mg g[-1]) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2
2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2
4	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2
6	9.1	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.1
8	9.1	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.1
Mean	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	

CD	SEd	CD
S	(0.038)	NS
T	(0.034)	NS
M	(0.024)	NS
S × T	(0.076)	NS
T × M	(0.048)	NS
S × M	(0.053)	NS
S × T × M	(0.107)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 12. Concentrations of carbon dioxide and seed moisture on a-Amylase activity (mg maltose min^{-1}) of rice cv. ADT43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	1.174	1.172	1.173	1.174	1.172	1.173	1.174	1.172	1.173	1.174	1.172	1.173	1.174	1.172	1.173
2	1.165	1.162	1.163	1.170	1.168	1.169	1.171	1.168	1.170	1.170	1.170	1.170	1.169	1.167	1.168
4	1.160	1.157	1.159	1.167	1.163	1.165	1.167	1.163	1.165	1.168	1.164	1.166	1.165	1.162	1.164
6	1.155	1.153	1.154	1.164	1.159	1.162	1.165	1.160	1.162	1.167	1.162	1.165	1.163	1.159	1.161
8	1.149	1.146	1.148	1.161	1.150	1.155	1.162	1.153	1.157	1.164	1.154	1.159	1.159	1.151	1.155
Mean	1.161	1.158	1.159	1.167	1.162	1.165	1.168	1.163	1.165	1.169	1.164	1.167	1.166	1.162	

CD	SEd	CD
S	(0.005)	NS
T	(0.005)	NS
M	(0.003)	NS
S × T	(0.011)	NS
T × M	(0.007)	NS
S × M	(0.008)	NS
S × T × M	(0.016)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 13. Concentrations of carbon dioxide and seed moisture on free amino acid (μg 50 $seeds^{-1}$ 50 mL^{-1}) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and seed moisture (M)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean	12%	14%	Mean
0 (Pretreatment)	**9.3**	**9.2**	**9.2**	**9.3**	**9.2**	**9.2**	**9.3**	**9.2**	**9.2**	**9.3**	**9.2**	**9.2**	**9.3**	**9.2**	**9.2**
2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2
4	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2
6	9.1	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.1
8	9.1	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.1	9.2	9.1	9.2	9.2	9.1	9.1
Mean	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	9.2	9.3	9.2	

CD	SEd	CD
S	(0.038)	NS
T	(0.034)	NS
M	(0.024)	NS
S × T	(0.076)	NS
T × M	(0.048)	NS
S × M	(0.053)	NS
S × T × M	(0.107)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 14. Concentration of carbon dioxide (50 %) and seed moisture (12%) on uric acid content (mg 100 g^{-1}) of rice cv. ADT 43 after 8 months of storage

Treatments (T)	Periods of storage (S)		
	Initial	8 months	Mean
Control	3.63	8.72	6.17
CO_2 50%	3.63	6.98	5.30
Mean	3.63	7.85	
	T	S	S × T
SEd	**0.051**	**0.051**	**0.072**
CD (p=0.05)	**0.109**	**0.108**	**0.154**

CD - Critical difference, SEd - Standard Error of difference

parentheses representing the angular transformation (68.86 and 51.35). At 14% moisture content, the CO_2 50% treatment also outperformed the Control, with field emergence of 77% and 51%, respectively and angular transformation values of 61.34 and 45.57. The overall mean field emergence for CO_2 50% treatment was 82% (64.89 angulars), while the Control treatment recorded only 56% (48.44 angulars). The average field emergence across treatments and moisture levels was 74% at 12% moisture and 64% at 14%, with a mean of 69% (59.34 angular). The statistical analysis indicates that The SEd for treatments (T) and moisture content (M) was 0.699, while the interaction (T × M) was 0.946. The CD at p = 0.05 for T and M was 1.458, while the interaction (T × M) was NS. These results suggest that treating seeds with CO_2 50% significantly improved field emergence compared to untreated seeds across both moisture levels. Additionally, seeds stored at 12% moisture content performed better than those at 14%, indicating a role of moisture in seed viability during storage. (Table 15).

Concentrations of CO_2 and the number of exposures on storability : Highly significant differences were obtained with concentrations (T) of CO_2 except for free amino acids and starch. Similarly, highly significant differences were obtained with the number of exposures (N) except for moisture, amylase and starch. For SP all the parameters showed highly significant differences.

The interaction effect between S × T was highly significant except for moisture content, shoot length, free amino acid and starch. In the same way, the interaction effect

Table 15. Concentration of carbon dioxide (50 %) and seed moisture on field emergence (%) of rice cv. ADT 43 after 8 months of storage

Treatment (T)	Moisture content (M %)		
	12%	14%	Mean
Control	61 (51.35)	51 (45.57)	56 (48.44)
CO_2 50%	87 (68.86)	77 (61.34)	82 (64.89)
Mean	74 (59.34)	64 (53.13)	69
	T	M	T×M
SE d	**0.699**	**0.699**	**0.946**
CD (p=0.05)	**1.458**	**1.458**	**NS**

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

between T × N was highly significant except for moisture content, germination, shoot length, amylase content, free amino acid and starch. The interaction effect between S ×N is also highly significant except for several live adults, insect infestation, moisture content, germination, shoot length, dry matter production, amylase content, free amino acid and starch.

Seed health

Live adults : Live adults were nil at the second evaluation month when CO_2 was exposed thrice and once compared to control (18.7). The number of adults increased during storage (9.0 to 9.1). Higher CO_2 concentrations (50%) reduced (Table 16) the number of adults (0.0) compared to control (36.5).

Insect infestation (%) : Exposure to CO_2 at 50% concentration thrice recorded the lowest infestation (0.0) compared to control (18.7). The infestation increased with period of storage (0.0 to 8.6). At any period, CO_2 exposure once and thrice was recorded as the lowest infestation (Table 17).

Physiological seed quality characters

Moisture content (%) : Moisture content was reduced with increased CO_2 concentration. With 30% CO_2, the moisture was 12.69%. It was 12.63% at 50% CO_2 (Table 18).

Germination (%): With the advancement of storage, there was a decline in germination percentage (96 to 83). A higher concentration of CO_2 (50%) maintained the germination percentage at a higher level (91). Exposure to CO_2 thrice was effective (89) compared to one (87). At any SP, 50% CO_2 exposed thrice recorded maximum values (Table 19).

Root length (cm): Carbon dioxide exposure thrice recorded the longest root length (20.2) than once (18.0). Among concentrations, 50% registered the longest root length (20.7), compared to control (18.9) and the same decreased with SP from 22.5 to 18.3 cm. Carbon dioxide was exposed thrice at 50% and the maximum value was recorded at all periods of storage (Table 20).

Shoot length (cm): Carbon dioxide at 50% concentration registered the longest (9.1) shoot length compared to control (8.5). The shoot length decreased with periods of storage (9.5 to 8.3). When exposed thrice, longer shoot length (8.9) was observed than once (8.8). Irrespective of CO_2 concentrations, at any period of storage, CO_2 exposed thrice at 50% concentration was recorded (Table 21) as the maximum value compared to control (8.7 to 7.9).

Dry matter production (g 10 seedling^{-1}): The dry matter production decreased with SP (0.143 to 0.102). When exposed to 50% concentration, it was 0.125 g compared to the control at 0.107 g. Exposure thrice registered higher DMP (0.121) than once (0.115). After 8 months of storage, the dry matter was 0.114 g compared to the control (0.087) when exposed thrice at 50% concentration (Table 22).

Biochemical seed quality characters

Starch (mg g^{-1}): With advances in SP, starch content decreased from 9.3 to 9.1 mg g^{-1} (Table 23).

α-Amylase activity (mg maltose min^{-1}): Carbon dioxide at

Table 16. Concentrations of carbon dioxide and number of exposures on live adults (*R. dominica)* of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	9.0 (3. 08)	9.0(3.08)	9.0 (3.08)	9.0(3.08)	9.0(3.08)	9.0 (3. 08)	9.0(3.08)	9.0(3.08)	9.0(3.08)	9.0 (3. 08)	9.0 (3. 08)	9.0 (3. 08)	9.0(3.)	9.0 (3. 08)	9.0 (3. 08)
2	18.6 (4. 37)	18.8 (4.39)	18.7 (4.38)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0(0.70)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0 (0. 70)	0.0(0.)	4.6 (2. 25)	4.7 (2. 28)	4.6 (2. 25)
4	23.4 (4. 88)	23.0 (4.84)	23.2 (4.86)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0(0.70)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0 (0. 70)	0.0 (0. 70)	5.8 (2. 50)	5.7 (2. 48)	5.8 (2. 50)
6	31.6 (5. 66)	31.4 (5.64)	31.5 (5.65)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0(0.70)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0 (0. 70)	0.0 (0. 70)	7.9 (2. 89)	7.8 (2.)	7.8 (2. 88)
8	36.6 (6. 09)	36.4 (6.53)	36.5 (6.08)	0.0(0.70)	0.0(0.70)	0.0 (0 70)	0.0(0.70)	0.0(0.70)	0.0(0.70)	0.0 (0. 70)	0.0 (0.)	0.0 (0. 70)	9.1 (3. 09)	9.1 (3. 09)	9.1 (3. 09)
Mean	**23.8 (4. 92)**	**23.7 (4.91)**	**23.7 (4.91)**	**1.8 (1. 51)**	**1.8 (1.51)**	**1.8 (1. 51)**	**1.8 (1.51)**	**1.8 (1.51)**	**1.8 (1.51)**	**1.8 (1. 51)**	**1.8 (1.51)**	**1.8 (1. 51)**	**7.3 (2. 79)**	**7.2 (2. 77)**	
CD	**SEd**							**CD**							
S	(0.237)							(0.468)							
T	(0.212)							(0.418)							
M	(0.150)							NS							
S × T	(0.474)							(0.936)							
T × M	(0.300)							NS							
S × M	(0.335)							NS							
S × T × M	(0.670)							NS							

(Figures in parentheses are square root transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 17. Concentrations of carbon dioxide and number of exposures on insect (*R. dominica)* infestion (%) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
(Pretreatment)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)
2	14.8 (22.62)	14.4 (22.30)	14.6 (22.46)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	3.7 (11.09)	3.6 (10.93)	3.6 (10.93)
4	20.8 (27.13)	20.4 (26.85)	20.6 (26.99)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	5.2 (13.18)	5.1 (13.05)	5.1 (13.05)
6	24.2 (29.46)	23.8 (29.20)	24.0 (29.33)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	6.0 (14.17)	5.8 (13.93)	5.9 (14.05)
8	34.6 (36.03)	34.2 (35.79)	34.4 (35.91)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	0.0 (0.41)	8.6 (17.05)	8.5 (16.95)	8.6 (17.05)
Mean	**18.8 (25.69)**	**18.4 (25.40)**	**18.7 (25.62)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**0.0 (0.41)**	**4.7 (12.52)**	**4.6 (12.38)**	

CD	SEd	CD
S	(0.178)	(0.352)
T	(0.159)	(0.315)
M	(0.112)	NS
S × T	(0.357)	(0.705)
T × M	(0.225)	NS
S × M	(0.225)	NS
S × T × M	(0.504)	NS

(Figures in parentheses are arc sine transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 18. Concentrations of carbon dioxide and number of exposures on moisture content (%) of rice cv. ADT 43 during storage

Period of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54	12.54
2	13.00	13.08	13.04	12.70	12.62	12.66	12.64	12.60	12.62	12.62	12.58	12.60	12.74	12.72	12.73
4	13.10	13.10	13.10	12.72	12.66	12.69	12.70	12.64	12.67	12.66	12.60	12.63	12.79	12.75	12.77
6	13.18	13.20	13.19	12.80	12.70	12.75	12.76	12.66	12.71	12.70	12.64	12.67	12.86	12.80	12.83
8	13.20	13.24	13.22	12.88	12.70	12.79	12.84	12.70	12.77	12.76	12.70	12.73	13.22	12.83	12.87
Mean	**13.00**	**13.03**	**13.02**	**12.73**	**12.64**	**12.69**	**12.70**	**12.63**	**12.66**	**12.66**	**12.61**	**12.63**	**12.83**	**12.73**	

CD	SEd	CD
S	(0.069)	(0.137)
T	(0.062)	(0.122)
M	(0.043)	NS
S × T	(0.138)	NS
T × M	(0.087)	NS
S × M	(0.098)	NS
S × T × M	(0.196)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 19. Concentrations of carbon dioxide and number of exposures on germination (%) of rice cv. ADT 43 during storage

Periods of storage (S) (months)	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)	96 (78.46)
2	85 (67.21)	85 (67.21)	85 (67.21)	86 (68.02)	90 (71.56)	88 (69.73)	89 (70.63)	91 (72.54)	90 (71.56)	90 (71.56)	94 (75.82)	92 (73.57)	87 (68.86)	90 (71.56)	90 (71.56)
4	82 (64.89)	83 (65.65)	83 (65.65)	86 (68.02)	89 (70.63)	87 (68.86)	86 (68.02)	90 (71.56)	88 (69.73)	88 (69.73)	94 (75.82)	91 (72.54)	86 (68.02)	89 (70.63)	87 (68.86)
6	78 (62.02)	78 (62.02)	78 (62.02)	83 (65.65.)	87 (68.86)	85 (67.21)	85 (67.21)	89 (70.63)	87 (68.86)	86 (68.02)	92 (73.57)	89 (70.63)	83 (65.65)	87 (68.86)	85 (67.21)
8	75 (60.00)	77 (61.34)	76 (60.66)	82 (64.89)	86 (68.02)	84 (66.42)	83 (65.65)	87 (68.86)	85 (67.21)	85 (67.21)	90 (71.56)	88 (69.73)	81 (64.15)	85 (67.21)	83 (65.65)
Mean	**83 (65.65)**	**84 (66.42)**	**84 (66.42)**	**87 (68.86)**	**90 (71.56)**	**88 (69.73)**	**87 (68.86)**	**91 (72.54)**	**89 (70.63)**	**89 (70.63)**	**93 (74.66)**	**91 (72.54)**	**87 (68.86)**	**89 (70.63)**	

CD	SEd	CD
S	(0.846)	(1.678)
T	(0.760)	(1.50)
M	(0.530)	(1.60)
S × T	(1.699)	(3.356)
T × M	(1.074)	NS
S × M	(1.201)	NS
S × T × M	(2.404)	NS

(Figures in parentheses are arc sine transformations); CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 20. Concentrations of carbon dioxide and number of exposures on root length (cm) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
2	19.5	19.5	19.5	20.8	21.0	20.9	21.4	21.7	21.6	21.8	22.1	22.0	20.9	21.1	21.0
4	18.1	18.0	18.1	18.6	19.5	19.0	19.0	20.7	19.8	19.2	21.0	20.1	18.7	19.8	19.2
6	17.7	17.2	17.5	18.1	19.0	18.6	18.7	19.4	19.0	19.0	20.1	19.5	18.4	19.0	18.6
8	17.0	17.0	17.0	18.1	18.6	18.4	18.6	19.1	18.9	18.7	19.7	19.2	18.1	18.6	18.3
Mean	**19.0**	**18.8**	**18.9**	**19.6**	**20.1**	**19.9**	**20.0**	**20.7**	**20.4**	**20.2**	**21.0**	**20.7**	**18.0**	**20.2**	

CD	**SEd**	**CD**
S	(0.101)	(0.199)
T	(0.090)	(0.178)
M	(0.063)	(0.126)
S × T	(0.202)	(0.399)
T × M	(0.127)	(0.252)
S × M	(0.142)	(0.282)
S × T × M	(0.285	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 21. Concentrations of carbon dioxide and number of exposures on shoot length (cm) of rice cv.ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
2	8.7	8.6	8.7	9.0	9.3	9.1	9.0	9.5	9.3	9.0	9.6	9.4	8.9	9.3	9.1
4	8.1	8.3	8.2	8.9	9.0	9.0	9.0	9.1	9.0	9.0	9.2	9.1	8.8	8.9	8.8
6	8.0	8.0	8.0	8.3	8.7	8.5	8.5	8.7	8.6	8.6	8.9	8.8	8.4	8.6	8.5
8	7.9	7.9	7.9	8.1	8.5	8.3	8.4	8.5	8.5	8.5	8.9	8.7	8.2	8.4	8.3
Mean	**8.4**	**8.5**	**8.5**	**8.8**	**9.0**	**8.9**	**8.9**	**9.0**	**9.0**	**8.9**	**9.2**	**9.1**	**8.8**	**8.9**	

CD	**SEd**	**CD**
S	(0.101)	(0.199)
T	(0.090)	(0.178)
M	(0.063)	(0.126)
S × T	(0.202)	NS
T × M	(0.127)	NS
S × M	(0.143)	NS
S × T × M	(0.286)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 22. Concentrations of carbon dioxide and number of exposures on dry matter production (g 10 seedlings^{-1}) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
2	0.110	0.107	0.109	0.115	0.128	0.121	0.121	0.131	0.126	0.124	0.140	0.132	0.117	0.127	0.122
4	0.102	0.103	0.103	0.109	0.119	0.114	0.113	0.124	0.119	0.117	0.130	0.123	0.110	0.119	0.115
6	0.096	0.092	0.094	0.106	0.112	0.109	0.108	0.117	0.113	0.110	0.120	0.115	0.105	0.110	0.108
8	0.088	0.087	0.088	0.099	0.107	0.103	0.101	0.110	0.106	0.106	0.114	0.110	0.099	0.105	0.102
Mean	**0.108**	**0.106**	**0.107**	**0.114**	**0.122**	**0.118**	**0.117**	**0.125**	**0.121**	**0.120**	**0.129**	**0.125**	**0.115**	**0.121**	

CD	**SEd**	**CD**
S	(0.001)	(0.003)
T	(0.001)	(0.003)
M	(0.001)	(0.002)
S × T	(0.003)	(0.007)
T × M	(0.002)	(0.004)
S × M	(0.002)	NS
S × T × M	(0.005)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

50% concentration recorded higher activity (1.161) than control (1.150). Exposure thrice had more enzyme activity (1.158) than once (1.155) (Table 24).

Free amino acid (µg 50 seeds $^{-1}$ 50 mL $^{-1}$): When the number of exposures increased, there was a reduction in free amino acids (24.2). With periods of storage, the leakage was higher (from 22.7 to 25.9) at 8 months of storage (Table 25).

Uric acid (mg 100 g $^{-1}$): After 8 months of storage, the uric acid content was the least (3.68 mg) when exposed thrice to 50% carbon dioxide. For the same period, the untreated control recorded 6.69 mg (Table 26).

Field emergence (%): Carbon dioxide exposure thrice at 50% concentration registered the highest field emergence (87) when compared with control (62). Exposure thrice at 50% concentration registered high germination of 76% compared to one (74) (Table 27).

Discussion

Maintaining seed quality until sowing is mandatory, as the irreversible degenerative changes would lead to loss of quality (29). External factors accelerate the invasion of storage insects and diseases and further reduce the quality. Seed production is scientific, aiming at high quality, including seed health (30). In this scenario, insects play an important role in determining the seed's storage life due to their shorter life span. Because of this, the minimum certification standards for insect infestation in rice have been fixed as 0.5.

A good storage program should attempt minimal use of insecticides and fungicides and preventive practices with lower residual effects while ensuring quality. Considering the post-harvest losses, the biological agents would account for 10% of world production and need attention for effective control measures (31). Another important consideration

Table 23. Concentrations of carbon dioxide and number of exposures on starch content (mg g^{-1}) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3
2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
4	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.2	9.2	9.1	9.2	9.2	9.1	9.2	9.1
6	9.1	9.0	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.2	9.2	9.1	9.1	9.1
8	9.0	9.0	9.0	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.2	9.1	9.1	9.1	9.1
Mean	**9.1**	**9.1**	**9.1**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	**9.2**	

CD	SEd	CD
S	(0.034)	(0.067)
T	(0.030)	NS
M	(0.021)	NS
S × T	(0.068)	NS
T × M	(0.043)	NS
S × M	(0.048)	NS
S × T × M	(0.096)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 24. Concentrations of carbon dioxide and number of exposures on a-Amylase activity (mg maltose min^{-1}) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171	1.171
2	1.152	1.149	1.150	1.157	1.160	1.159	1.159	1.164	1.161	1.162	1.167	1.164	1.157	1.160	1.159
4	1.149	1.145	1.147	1.153	1.159	1.156	1.155	1.160	1.157	1.156	1.165	1.160	1.153	1.157	1.155
6	1.144	1.142	1.143	1.147	1.153	1.150	1.150	1.156	1.153	1.153	1.161	1.157	1.149	1.153	1.151
8	1.139	1.139	1.137	1.141	1.150	1.145	1.145	1.153	1.149	1.148	1.157	1.152	1.143	1.148	1.146
Mean	**1.151**	**1.149**	**1.150**	**1.154**	**1.159**	**1.156**	**1.156**	**1.161**	**1.158**	**1.158**	**1.164**	**1.161**	**1.155**	**1.158**	

CD	SEd	CD
S	(0.004)	(0.008)
T	(0.003)	(0.007)
M	(0.002)	NS
S × T	(0.008)	NS
T × M	(0.005)	NS
S × M	(0.006)	NS
S × T × M	(0.012)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 25. Concentrations of carbon dioxide and number of exposures on free amino acid (µg 50 seeds $^{-1}$ 50mL $^{-1}$) of rice cv. ADT 43 during storage

Periods of storage (S) in months	Concentrations of carbon dioxide (T) and Number of exposures (N)														
	Control			CO_2 30%			CO_2 40%			CO_2 50%			S × T		
	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean	Once	Thrice	Mean
0 (Pretreatment)	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7
2	23.7	23.6	23.7	23.6	23.4	23.5	23.6	23.4	23.5	23.5	23.3	23.4	23.6	23.4	23.5
4	24.7	24.7	24.7	24.6	24.3	24.5	24.5	24.2	24.4	24.5	24.1	24.3	24.6	24.3	24.5
6	25.4	25.2	25.3	25.3	25.0	25.1	25.2	24.9	25.1	25.4	24.5	25.0	25.3	24.9	25.1
8	26.2	26.2	26.2	26.1	25.9	26.0	25.9	25.6	25.8	25.8	25.5	25.7	26.0	25.8	25.9
Mean	**24.5**	**24.5**	**24.5**	**24.5**	**24.3**	**24.4**	**24.4**	**24.2**	**24.3**	**24.4**	**24.0**	**24.2**	**24.4**	**24.2**	

CD	**SEd**	**CD**
S	(0.125)	(0.248)
T	(0.112)	NS
M	(0.079)	(0.156)
S × T	(0.251)	NS
T × M	(0.158)	NS
S × M	(0.177)	NS
S × T × M	(0.355)	NS

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

Table 26. Concentration of carbon dioxide (50%) and number of exposures (thrice) on uric acid content (mg 100 g^{-1}) of rice cv. ADT 43 after 8 months of storage

Treatments (T)	Periods of storage (S)		
	Initial	8 months	Mean
Control	3.63	6.68	5.16
CO_2 50%	3.63	3.68	3.65
Mean	3.63	5.18	
	T	S	T× S
SEd	**0.045**	**0.045**	**0.063**
CD (p=0.05)	**0.095**	**0.095**	**0.135**

CD - Critical difference, SEd - Standard Error of difference

Table 27. Concentration of carbon dioxide and number of exposures on field emergence (%) of rice cv. ADT 43 after 8 months of storage

Treatments (T)	Number of exposures (N)		
	Once	Thrice	Mean
Control	62	63	62
CO_2 50%	86	88	87
Mean	74	76	75
	T	N	T×N
SE d	**0.777**	**0.777**	**1.100**
CD (p=0.05)	**1.694**	**1.694**	**NS**

CD - Critical difference, SEd - Standard Error of difference, NS- Non-Significant

regarding using insecticides/fumigants in seed storage is the reuse of seeds once the utility is questionable and in line with the required standard for seed storage. Application of non-toxic control measures without harming the seed, user and environment will be highly preferred.

Aeration systems to ventilate the bulk grain/seed to prevent insect multiplication are widely used, along with non-residual treatments like irradiation or heating, for the maintenance of quality (16). Seed storage also demands pest control methods that are eco-friendly and cost-effective for controlling storage pests and preserving quality characters (32).

Modified or controlled atmosphere storage offers a safe and pollution-free alternative to conventional fumigants, leading to residual problems. Because atmospheric gases have a lower deadly impact than pesticides, they are used more frequently. In this strategy, insect control involves altering the quantity of typical atmospheric gases, primarily N, O, CO_2 and other uncommon gases (33).

Studies were conducted on the influence of CO_2 on the storability of rice. Initial observations were recorded after 20 days on insect activity physiological and biochemical seed quality characters. Seeds were transferred to airtight plastic containers for CO_2 exposure at different concentrations, moisture levels and number of exposures at an interval of 15 days, as detailed and were stored for 8 months in the same container and observed for seed quality.

Influence of carbon dioxide on insect activity

After 8 months of storage in rice, the live adults increased from 9 to 36.5 in control, while it was nil when exposed thrice at a concentration of 50%. At all SP, even a minimum concentration of CO_2 had a knockdown effect on the number of adult insects. This may be due to CO_2 accumulation in insect blood, decreasing the pH. There has been a report that prolonged excessive acidity in the blood can disrupt the osmotic equilibrium in the insect system, resulting in mortality even in a normal atmosphere (34). Carbon dioxide concentrations effectively reduced insect populations (rice weevil, *Sitophilus oryzae* and lesser grain borer, *R. dominica*) and damage to rice seed (35). Due to the lack of haemoglobin in insects, at lower concentrations of CO_2, the consumption of O through trachea and spiracles by the insect was less. In contrast, higher CO_2 concentration could inhibit respiration and development of pupae, reducing the infestation percentage (36). A positive correlation between the period of exposure to CO_2 and mortality percentage was observed in all the life stages of *R. dominica, Sitophilus oryzae, Oryzaephilus surinamensis* and *Plodia interpunctella* (37). Overdosage of CO_2 was known to be poisonous to many insects, causing mortality (38, 39).

In contrast, complete control of *R. dominica* and *Corcyra cephalonica* in storage using 20% CO_2 without any impact on germinability and vigor in wheat accompanied by higher dehydrogenase activity (40). Carbon dioxide at 5 and 10% concentrations also decreased the insect population and seed quality damage compared to normal room storage. The insect infestation was zero with 50% concentration of CO_2, but the same increased with control. Even a minimum number of exposures effectively controlled the adult emergence.

Effect of CO_2 and seed moisture content

The fluctuations in the average moisture content were very minimal and the difference was between 0.14 to 0.28%, irrespective of the concentration of CO_2 in both (12% or 14%) seeds, which can be attributed to CO_2 and air-tight storage. Also, there was no insect build-up at different concentrations of CO_2 and a similar result was reported in another study.

This can also be assigned to the minimum respiratory action of seeds as well as the metabolic activity of insects. Even at higher seed moisture (14%), CO_2, at all concentrations, may act as a metabolic repressor, reducing the activity of insects and seeds (41).

Effect of CO_2 on seed quality characters

Seed, the most important input for crop production, is used immediately or after considerable storage for sowing. Post-harvest operations in most seed crops require time, during which the aging process starts and seeds deteriorate with time (42) and several factors are associated with aging (43).

Although a controlled atmosphere has been proven for effective insect control and viability maintenance, its commercialization is still limited and is only practiced in other countries (44). In the recent study, after 8 months of storage using 50% CO_2, the decline in germination was minimal (6%) compared to control (20 %) when exposed 3 times. Lower germination was observed when infested with *Callosobruchus chinensis* in mung bean (45, 46). However, the reduction was less with increased CO_2 concentration. The same trend was reflected in root, shoot and DMP (Fig. 3). The rate of decline was higher at increased seed moisture. Detrimental effects expected at higher moisture levels because of induced respiration could not be observed in the present study using higher CO_2 concentration (Fig. 2). Similar findings (47-49) were reported for reduced seed quality due to insect infestation.

Effect of CO_2 on biochemical characters

The biochemical characteristics, associated with insect infestation can manifest in terms of loss of quality. No changes in starch content were observed. The biochemical attributes related to seed viability showed a decline in free amino acid, amylase, when exposed to CO_2 more than once. The same observation at a higher magnitude was evident at high seed moisture content. Scientists have established a positive association between enzyme activity and viability (50). Uric acid concentration after 8 months of storage was lower in CO_2 exposure and the same was true for seeds stored at lower moisture content. In the control sample, the uric acid content increased several folds after 35 months of storage, consequent to insect protein metabolism. It can be used as an index for measuring population density and

Fig. 3. Carbon dioxide exposure and seed moisture on seedling length after 8 months of storage. A: Control; B: CO_2 at 50% concentration at 12% seed moisture.

Fig. 2. Carbon dioxide exposure and seed moisture on insect infestation after 8 months of storage. A: Control; B: CO_2 at 50 % concentration at 12 % seed moisture.

indirectly to loss in viability.

Effect of CO_2 on performance of stored seed

When assessed for field emergence, the stored seed performance recorded higher values. Seed stored at lower moisture with greater exposures to CO_2 registered higher emergence (Fig. 4).

Effect of CO_2 on response of crops to seed deterioration

The pattern of deterioration with a period of storage was almost similar (96%). When stored in a CO_2 atmosphere, the decline was slower irrespective of concentration, number of exposures and seed moisture.

Conclusion

In conclusion, using CO_2 as a storage method has shown significant effects on insect control and seed quality preservation. High concentrations of CO_2, particularly at 50%, effectively reduced insect populations, such as *Sitophilus oryzae*, *Tribolium castaneum* and *Oryzaephilus surinamensis*, by disrupting their metabolism and respiration, leading to mortality. This controlled atmosphere also minimized seed deterioration, maintaining germination rates and biochemical integrity better than traditional storage methods. While CO_2 at lower concentrations (5-10%) did not negatively impact seed quality, it still reduced insect infestations and preserved seed vitality. The minimal fluctuation in seed moisture and the reduction in metabolic activities in seeds and insects indicated that CO_2 is an effective metabolic repressor. Thus, CO_2 storage provides a promising, user-friendly, eco-friendly method for pest control and maintaining seed quality, the findings will be applicable for bulk storage. CO_2 with less moisture content maintains seed vigor and viability.

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Authors' contributions

VV carried out the physiological, biochemical and storage studies, PS participated in setting experimental design and layout, sequence alignment and drafted the manuscript. KDK participated in the sequence alignment and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

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Fig. 4. Carbon dioxide exposure and seed moisture on field emergence after 8 months of storage. A: Control; B:CO_2 at 50% concentration at12% seed moisture.

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