Original Article

EFFECT OF CARBON DIOXIDE ON QUALITY OF RICE SEEDS

EFEITO DO DIÓXIDO DE CARBONO NA QUALIDADE DE SEMENTES DE ARROZ

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ABSTRACT: In this study the effect of carbon dioxide on the physiological and sanitary quality of rice seed during storage is reported. The experimental design was completely randomized in a 2x3x3 factorial arrangement, with two cultivars (Irga 423 and 424), three concentrations of carbon dioxide (0, 25 and 50%) and three storage periods (15, 30 and 45 days). According to the results, it was found that carbon dioxide maintains germination and vigor, and reduces mycoflora associated with the seed. The incidence of *Aspergillus* sp., *Fusarium* sp. and *Rhizoctonia* sp. was reduced respectively in proportions of 50%, 75% and 100%, when the seeds were submitted to the a 50% concentration of carbon dioxide for 45 days storage. As regards seed-seedling transmission, only the fungus was able to promote *Bipolaris* sp. disease in seedlings from seeds treated with carbon dioxide. In this context, carbon dioxide is a viable alternative for maintaining the quality of rice seeds during storage.

KEYWORDS: *Oryza sativa* L. Seeds. Physiological Quality. Sanitary. Storage.

INTRODUCTION

Rice (*Oryza sativa* L.) is grown on all continents and is notable for the size of its production and cultivation area, playing a strategic role in both the economic and the social sphere (EMBRAPA, 2012). Annually, the acreage planted reaches 150 million hectares, with a production of 600 million tons (AZAMBUJA et al., 2004; TUNES et al., 2013). In this context, Brazil is a major producer of rice, harvesting 8.05 million tons in 2012/2013, with the state of Rio Grande do Sul being the major producer (CONAB, 2013). Tocantins state ranks fourth in national production, with 565,700 tons in 119,100 hectares of planted area, ranking second in total grain production (IBGE, 2013).

The use of high quality seeds is increasingly being prospected for rice growers as the most effective means to minimize production costs. The seeds should form a good initial stand, seeking better profitability of the crop (JUGRAN et al., 2010).

Fungi are major pathogens associated with seeds and directly influence their viability. In this context, about 50 species of fungi are associated with rice seeds, causing serious damage to germination, vigor, and seed-seedling transmission (SCHUCH et al., 2006). Storing seeds is considered a key step that deserves special attention from the seed agribusiness sector. Inadequate conditions favor the development of pathogens and reduce viability (DRUVEFORS; SCHNÜNRER, 2005; TELO et al., 2012.). Among the major biotic and abiotic factors associated with the preservation of seeds is intergranular atmosphere, associated with the gas composition, temperature and humidity (VIEIRA et al., 2000; MARINI et al., 2013).

The longevity of seeds is influenced by storage conditions and their packaging (DA SILVA et al., 2010). Although the environmental conditions of the stock may be artificially controlled, the cost of this operation is not economically viable, which means that almost the entire volume of seed produced in Brazil is stored at ambient temperature and relative humidity. There is a need for studies to determine the period in which the seeds of cultivated species maintain an acceptable level of quality in the various geographical areas of Brazil.

Thus, various alternatives aimed at conserving seed quality have been proposed, such as the use of controlled temperature, airtight storage, and modifications that can facilitate the maintenance of seed viability (MARINI et al., 2012; AGUIAR et al., 2012).This study aimed to evaluate the effect of carbon dioxide on preserving the quality of rice seed.

MATERIAL AND METHODS

Rice seeds

Seeds of rice cultivars Irga 423 and 424 were obtained from seed agro-industries located in the municipalities of Formoso do Araguaia (11º47'48 S latitude S, 49º31'44 " longitude W) and Lagoa da Confusão (10º47'37" latitude S, 49°37'25"

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longitude W), state of Tocantins, Brazil. The seed samples were randomly collected, totaling 10 sub-samples of 300 g for each batch, and then a representative sample was obtained for each cultivar. From this, the content of seed water was initially determined, standardizing to 12%, with the aid of a Farmex MT-16 ® moisture meter as instructed by the manufacturer. Then the seeds were placed in airtight chambers.

Carbon Dioxide

After conditioning the seeds in their respective chambers, a concentration of 25 and 50% was obtained by direct injection of CO_2 (with minimum degree of purity 99.8%), after sealing the chambers. The gas concentration was measured by a "Checkpoint" gas analyzer for modified atmospheres (Model O_2/CO_2, Dansensor) coupled to the chambers. In all bioassays, when the percentage of 25 and 50% of CO_2 into the metal chamber was established, the injection was interrupted by closing the valves and injection safety catch. The control was constituted of air (78% N_2, 0.03% CO_2 and 21% O_2).

Germination Test

Four replications of 50 seeds were placed on moistened germitest paper with sterile distilled water at a ratio of 2.5 times the weight of the paper, and put in a heated chamber at 25 °C. At 14 days after sowing, a final count was done, determining the percentage of normal seedlings (BRASIL, 2009).

Length and dry weight of seedlings

Evaluation of seedlings was carried out according to the methodology described by Vanzolini et al. (2007). Forty seedlings obtained from the germination test were randomly chosen and divided into four replicates of 10 seedlings. We evaluated the length of shoots and roots of seedlings, with the aid of a millimeter ruler. The average length of shoot and root was obtained by summing the measurements of each repetition and dividing by the number of normal seedlings; the results were expressed in centimeters. To determine dry mass, four replicates of ten normal seedlings were placed in paper bags and taken to the greenhouse, maintaining a temperature of 70 ºC until constant weight. Weighing was performed on a 0.001 g precision balance and the data were expressed in grams, using an average weight of 10 seedlings per replication.

Seedling emergence

Sowing was performed according to the methodology used by Fleck et al. (2003) using plastic trays with dimensions of 50x25x15 cm. The seeds were sown in 10-cm-thick properly sterilized sand. At the end of each storage period (15, 30 and 45 days), four replicates of 100 seeds of each cultivar were used. The counting of emerged seedlings was performed after five periods (6, 8, 10, 12 and 14 DAE) and the results expressed as a percentage of normal seedlings. For the Emergence Speed Index (ESI), we used the formula of Maguire (1962), in which ESI = N1/D1 N2/D2 + + ... + Nn / Dn, where: ESI = seedling emergence speed index; N = number of emerged seedlings, computed from the first to the last count; D = number of days after sowing from first to last count.

Identification of the mycoflora associated with seed

Using the method of filter paper or "blotter test" (BRASIL, 2009), the seeds were immersed in 70% ethanol for 60 seconds and then in sodium hypochlorite 1% for 40 seconds and washed in sterile distilled water for 60 seconds. Then the seeds were placed in Petri dishes (Ø 120 mm) with filter paper and moistened with sterile distilled water used. Four replicates of 50 seeds were used for each treatment. Plates with seeds were incubated at 25 ± 2 ° C and photoperiod 12 hours light/12 hours dark for seven days. After the incubation period, the effect of fungi associated with rice seed was evaluated and identification of fungi was carried out according to the methodology of Barnett and Hunter (1972). Evaluation of incidence of fungi on seeds was conducted under a stereoscopic and optical microscope and results were expressed as percentage of fungal incidence.

Transmission of fungi via seed-seedling

The methodology followed that of Correa et al. (2008). The percentage transmission was calculated according to the rate of transport of mycoflora associated with a corresponding number of seeds and seedlings that developed symptoms of fungal diseases. Seedlings showed that symptoms were selected and the pathogens identified by Koch's postulates. To check the fungal transmission from the seed to seedling, we used the following formula adapted from Teixeira (2003), in which TT (%) = CFI (%) .100/TSI (%), where: TT (%) = Transmission rate; CFI = rate of infected plants (with symptoms); TSI = Rate of Seeds Infected with each fungal genus.

Experimental design

The experimental design was a completely randomized factorial design (2x3x3), including two cultivars (Irga 423 and Irga 424), three CO_2 concentrations (0, 25 and 50%) and three storage periods (15, 30 and 45 days). The obtained data were processed in the mycoflora arc sine √ x +1. Comparison of means was done by Tukey test ($P \leq 0.05$) using the statistical program SISVAR 5.0 (FERREIRA, 2003). The regression analyses and graphs were generated using the program SIGMAPLOT 10.0.

RESULTS AND DISCUSSION

An assay of seed germination in cultivars Irga 423 and Irga 424 was conducted; the average germinations were of 90% and 87%, respectively. After the treatment with carbon dioxide, it was possible to observe that the seeds presented germination rates higher than 90%, especially under the concentration of 50% of carbon dioxide at all periods (15, 30 and 45 days). Regarding the non-treated seed, the germination averages were around 74% for Irga 423 and 77% for Irga 424, but it was still demonstrated that the results were similar for both varieties (Table 1).

It was also found that, regardless of treatment, the germination remained stable and got higher during the storage period, although it is noted that the seeds treated with carbon dioxide presented higher germination in relation to the untreated seeds (Table 1). This fact occurred probably due to the break of dormancy, since rice seeds are generally exposed to it; and the behavior of the seeds is different, depending on the cultivar and the cultivation system, being more intense in floodplain system (ROBERTS, 1963).

The storage acts slowly on dormancy break of postharvest rice seeds, volatilizing phenolic and other germination inhibitors present in the endosperm, embryo and shell, which reduce the availability of O2 to embryo, favoring, thus, overcoming numbness and hence the germination (TANAKA et al., 2001).

According to Mussi, (2005), storing sunflower seeds in a CO_2-rich atmosphere drastically reduces the rate of seed respiration, and the reduction in metabolism keeps up the germination capacity over time. Santos et al., (2011) observed that the concentration of CO_2 was determinative in maintaining the germination of buffel grass cv. Aridus with 47% germination at 550 ppm CO_2 and 32% germination with 360 ppm of CO_2.

Table 1. Germination, seedling emergence and emergence speed index of rice seeds (cv. Irga 423 and 424) as a function of carbon dioxide concentration (0, 25 and 50% CO_2) and storage period (15, 30 and 45 days).

Cultivar	15 days			30 days			45 days		
	0%	25%	50%	0 %	25%	50%	0 %	25%	50%
Germination (%)									
Irga 423	64b	88a	90a	76b	90 a	92a	81b	92a	96a
Irga 424	75b	81b	91a	77b	84 ab	90a	79b	95a	97a
CV %	4.7	5.4	6.3	5.1	7.1	5.9	6.5	3.5	4.5
Seedling emergence (%)									
Irga 423	46c	87a	90a	63b	79a	94a	68b	80a	96a
Irga 424	78a	81a	82a	74a	83a	89a	71b	85a	95a
CV %	6.0	10.4	8.8	7.3	5.7	7.9	8.0	4.8	9.1
Emergence speed index (%)									
Irga 423	44c	83b	88b	60c	77b	90a	67b	76b	94a
Irga 424	75b	76b	79b	67b	78b	88a	60b	81ab	83a
CV %	8.2	7.4	5.8	5.3	3.6	5.6	5.4	6.5	7.4

Means followed by the same lower case letter in the line do not differ by Tukey test ($P \leq 0.05$).

The carbon dioxide concentration of 50% CO_2 influenced seed emergence in both varieties and, as in the germination test, there was also an increase in the percentage of germination during storage period, especially for treated seeds (Table 1). The same was observed for the emergence speed of seeds stored for 30 and 45 days and exposed to carbon dioxide test. Similar results were obtained by Santos et al. (2011), who observed that the concentration of 550 ppm of carbon dioxide had the highest rate of emergence for seeds of buffel grass cv. Aridus, and seeds treated at this concentration showed a higher rate of emergence (29%) compared to the 360 ppm treatment.

According to Müller et al. (2009), when the seedlings develop quickly, either by treatment or by natural factors, this gives a higher percentage of seedling emergence. This fact influences initial growth, because the plant manages to take rapid advantage of the soil nutrients, resulting in increased productivity. Thus, the result obtained with carbon dioxide, increasing the speed of emergence, is a positive factor for the rice crop, because it influences the initial stand and consequently produces healthier and better nourished plants.

Table 2. Length of radicle and shoot of rice seeds (cv. Irga 423 and 424) as a function of carbon dioxide concentration (0, 25 and 50% CO_2) and storage period (15, 30 and 45 days).

Cultivar	15 days			30 days			45 days		
	0 %	25%	50%	0 %	25%	50%	0%	25%	50%
Radicle length (cm)									
Irga 423	5.0b	5.2b	5.3b	4.9b	5.5b	6.3b	4.4b	7.0a	7.2a
Irga 424	4.0b	4.5b	4.4b	5.6b	6.3b	6.5b	4.5b	6.7b	8.0a
CV %	8.1	5.3	4.3	4.5	6.8	3.1	2.9	3.4	6.2
Aerial part length (cm)									
Irga 423	5.5a	5.6a	5.7a	5.4a	5.6a	5.8a	5.3a	5.9a	6.0a
Irga 424	5.0a	5.1a	5.1a	5.0a	5.2a	5.4a	5.2a	5.3a	5.5a
CV %	5.0	4.4	5.6	5.1	6.2	3.9	4.0	4.8	6.2

Means followed by the same lower case letter in the line do not differ by Tukey test ($P \leq 0.05$).

As described in the literature, during the period of storage the seeds maintain their metabolism. Being in the presence of oxygen and/or a high temperature may make the process of seed deterioration begin by almost imperceptible oxidation in mitochondria. This results in premature aging of seeds, which confers a physiological loss in quality over time (SANTOS et al., 2005; WRASSE et al., 2009). In this context, the results obtained with carbon dioxide are beneficial to the physiological maintenance of rice seeds during storage for a long period, favoring seedling development, impacting directly on seed vigor (SARAVIA et al., 2007; FAROOQ et al., 2010; VIJAYAKUMAR; GOWDA, 2012).

Regarding the length of the radicles of seedlings, it is observed that at 15 and 30 days of exposure to carbon dioxide, there were no significant differences between treatments ($P \leq 0.05$). However, for the seeds subjected to CO_2 for 45 days, the radicle was bigger, especially at the higher concentration of 50% CO_2 with respect to seedlings of untreated seeds (Table 2). However, it was observed that carbon dioxide did not influence the development of shoots of rice seedlings (Table 2). The same was observed in weight ratio of the total mass of the radicles of seedlings of both cultivars, with no significant difference ($P \leq 0.05$) between treatments (Table 3). In this regard, the effect of carbon dioxide can be directly associated with the activities of amylase enzymes, dehydrogenases and others involved in germination, allowing greater mobilization of the reserves of the seeds for the formation of rootlets of seedlings (CARVALHO et al., 2012).

Table 3. Weight of radicle and aerial part of rice seeds (cv. Irga 423 and 424) as a function of carbon dioxide concentration (0, 25 and 50% CO_2) and storage period (15, 30 and 45 days).

Cultivar	15 days			30 days			45 days		
	0%	25%	50%	0%	25%	50%	0%	25%	50%
Weight of radicle (g)									
Irga 423	0.024a	0.027a	0.029a	0.023a	0.025a	0.027a	0.022a	0.025a	0.028a
Irga 424	0.017a	0.019a	0.019a	0.016a	0.020a	0.022a	0.023a	0.024a	0.025a
CV %	8.1	7.4	8.1	6.8	5.5	5.4	3.8	4.0	4.7
Weight of Aerial Part (g)									
Irga 423	0.022a	0.024a	0.024a	0.025a	0.026a	0.028a	0.026a	0.028a	0.029a
Irga 424	0.024a	0.023a	0.026a	0.024a	0.025a	0.026a	0.025a	0.027a	0.028a
CV %	3.9	5.0	4.9	8.0	7.6	5.6	8.3	5.8	6.3

Means followed by the same lower case letter in the line do not differ by Tukey test ($P \leq 0,05$).

In general, there was no significant effect of CO_2 on the incidence of fungi in seeds of both cultivars. However, at 45 days of storage, seeds treated with carbon dioxide and also the untreated seeds had decreased the incidence of fungi *Helmintosporium* sp., *Phoma* sp. and *Rhizopus* sp in seeds (Tables 4 and 5). For *Aspergillus* sp., *Bipolaris* sp. *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizoctonia* sp., CO_2 is not enough to completely inhibit the incidence of these fungi in seed (Table 4 and 5). The incidence of *Aspergillus* sp., *Fusarium* sp. and *Rhizoctonia* sp. was reduced respectively in proportions of 50%, 75% and 100%, when the seeds were submitted to the a 50% concentration of carbon dioxide for 45 days storage.

Table 4. Effect of carbon dioxide at different concentrations (0, 25 and 50% CO_2) on the mycoflora associated with seeds (%) of rice cultivar Irga 423 depending on the storage period (15, 30 and 45 days).

Fungus	Periods of Storage								
	15 days			30 days			45 days		
	0%	25%	50%	0%	25%	50%	0%	25%	50%
Aspergillus sp.	7.0d	2.5b	1.5a	7.5e	2.5b	2.0b	5.0c	3.0b	2.5b
Bipolaris sp.	0.5a	1.5b	1.0a	2.0b	0.0a	0.0a	1.0a	0.5a	0.0a
Cladosporium sp.	1.0a	0.0a	0.0a	1.0b	0.0a	0.0a	0.5a	0.0a	0.0a
Curvularia sp.	4.5c	1.5b	1.5ab	2.5b	1.0a	1.5b	3.5c	1.0a	0.5a
Fusarium sp.	4.0 c	2.0b	2.0b	3.0b	2.0ab	1.5ab	2.0b	1.0a	0.5a
Helminthosporium sp.	1.5b	0.5a	0.5a	0.5a	0.0a	0.0a	0.0a	0.0a	0.0a
Penicillium sp.	3.5c	2.5b	2.5b	3.0b	2.5b	1.0a	4.0c	2.0b	1.0a
Phoma sp.	1.0a	0.0a	0.0ab	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Rhizoctonia sp.	2.0b	2.0b	2.0b	4.5c	2.5b	2.0b	6.0d	1.0a	0.0a
Rhizopus sp.	1.0a	0.0a	1.0a	0.5a	0.0a	0.0a	0.0a	0.0a	0.0a
CV(%)					19.8				

Means followed by the same lowercase letter in the line do not differ by Tukey test ($p \leq 0.05$). * Data previously transformed into arcsine $\sqrt{x}+1$; the data being expressed with the original values.

Table 5. Effect of carbon dioxide at different concentrations (0, 25 and 50% CO_2) on the mycoflora of seeds of rice (%) cultivar Irga 424 depending on the storage period (15, 30 and 45 days)

Fungus	Periods of Storage								
	15 days			30 days			45 days		
	0%	25%	50%	0%	25%	50%	0%	25%	50%
Aspergillus sp.	8.5e	2.0b	2.0b	6.0d	4.0c	1.0a	10.0f	2.5b	0.5a
Bipolaris sp.	0.0a	1.0a	0.0a	0.0a	0.5a	0.0a	2.0b	0.0a	0.0a
Curvularia sp.	5.0c	0.5a	1.0a	4.0c	0.0a	1.0a	5.5d	3.0b	0.5a
Fusarium sp.	5.0c	1.5b	1.5ab	6.0d	2.0b	0.0a	6.0d	2.0b	0.0a
Helminthosporium sp.	1.0a	0.5a	0.0a	1.0a	0.0a	0.0a	0.5a	0.0a	0.0a
Penicillium sp.	7.5e	1.5ab	2.0b	7.0d	2.0ab	1.0a	5.0c	1.0a	0.5a
Phoma sp.	2.0ab	1.0a	0.0a	2.0ab	0.0a	0.0a	1.0a	0.0a	0.0a
Rhizoctonia sp.	3.0b	1.5ab	1.0a	5.0c	1.0a	1.5b	5.5d	1.0a	0.5a
Rhizopus sp.	0.5a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	1.5b
CV (%)					21.2				

Means followed by the same lowercase letter in the line do not differ by Tukey test ($p \leq 0.05$). * Data previously transformed into arcsine $\sqrt{x}+1$; the data being expressed with the original values.

Accordingly, the incomplete removal of the fungal species associated with seeds by carbon dioxide may be related to the low concentration of oxygen present in the intergranular space among the seeds. As described in the literature, there are rare species of fungi that obtain energy by fermentation (WEINBERG et al., 2008). Therefore, low oxygen concentrations, associated or not with high concentrations of carbon dioxide, influence the respiration and consequently the sporulation and fungal growth among the seeds (BORÉM et al., 2001). Moreover Navarro et al., (2012) argue that environments with high concentrations of CO_2 associated with low levels of O_2 usually maintain the quality of grain stored for long periods.

These results are similar to those obtained by Brackmann et al. (1996), in a study that found a strong effect of carbon dioxide on the development of *Penicillium expansum*, preserving seeds from deterioration in storage. In an analysis by Taniwaki (2010), the environment enriched with 80% CO_2 promotes effect on the sporulation of *Mucor plumbeus, Fusarium oxysporum, Byssochlamys fulva, Byssochlamys nivea, Penicillium commune, Penicillium roqueforti, Aspergillus flavus, Eurotium chevalieri* e *Xeromyces bisporus*, inhibiting these fungi from spreading through the store.

Concentrations (20, 40, 60 and 80%) of CO_2 for periods of four, eight and twelve months in storage of rice seeds with high humidity (20%), were studied in India by Bera et al., (2007). They observed that CO_2 caused a reduction of approximately 90% in the incidence of *Aspergillus* sp., *Helminthosporium* sp., *Phoma* sp., *Alternaria* sp., *Fusarium* sp., *Penicillium* sp. and *Nigrospora* sp. Their results corroborate the toxic effect of CO_2 for fungi associated with rice seeds.

For fungus transmission via seeds treated with carbon dioxide, only *Bipolaris* spp. were identified as causing leaf spots in seedlings (Figure 1A and 1B). Moreover, it was observed that the toxicity was dependent on the concentration of carbon dioxide used.

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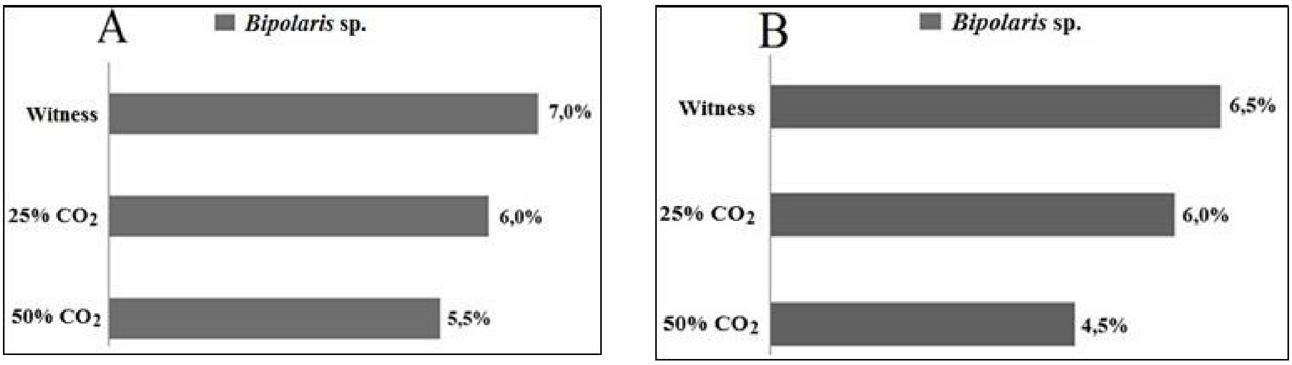

Figure 1. The effect of carbon dioxide on the percentage of transmission of the fungus *Bipolaris* spp from rice seed to seedling. **A -** Irga 423 and **B** – Irga 424, after 45 days of storage.

The non-eradication of this fungus in the seeds may be associated with its colonization of their innermost tissues, eliminating only the inocula present in the superficial layers of the seeds. Accordingly, it becomes feasible to use carbon dioxide in the conservation of rice seeds by inhibiting fungal growth and to preserve the physiological quality rice seeds

There is a need to maintain the quality of rice seeds in producing regions in Brazil, and it is essential to use new technologies to ensure the viability of the seeds. In this context, it can be inferred that carbon dioxide is an important alternative for use in the maintenance of rice seeds by the agricultural industry.

CONCLUSIONS

Carbon dioxide is a promising alternative for use in the maintenance of the physiological quality of seeds at storage without losing viability.

The carbon dioxide efficiency in maintaining vigor, germination and antifungal activity in rice seeds depends on the time of exposure and concentration of the gas.

RESUMO: Neste estudo foi avaliado o efeito do dióxido de carbono na qualidade fisiológica e sanitária de sementes de arroz durante o armazenamento. O delineamento experimental usado foi inteiramente casualizado em arranjo fatorial 2x3x3, sendo duas cultivares (Irga 423 e 424), três concentrações de dióxido de carbono (0; 25 e 50 %) e três períodos de armazenamento (15, 30 e 45 dias). De acordo com resultados, observou-se que dióxido de carbono mantém a germinação e vigor, e reduz a micoflora associada às sementes. A incidência de *Aspergillus* sp., *Fusarium* sp. e *Rhizoctonia* sp. foi reduzida respectivamente, nas proporções de 50%, 75% e 100%, quando as sementes foram submetidas à concentração de 50% de dióxido de carbono durante 45 dias de armazenamento. Para a transmissão semente-plântula, apenas o fungo *Bipolaris sp* foi capaz promover doença nas plântulas de sementes tratadas com dióxido carbono. Neste contexto, o dióxido de carbono torna-se uma alternativa viável para a manutenção da qualidade de sementes de arroz durante o armazenamento.

PALAVRAS-CHAVE: *Oryza sativa* L. Sementes. Qualidade fisiológica. Sanidade. Armazenamento.

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