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Modified Atmosphere Technology in Seed Health Management: Laboratory and Field Assay of Carbon Dioxide Against Storage Fungi in Paddy

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Abstract: An experiment was conducted during 2006-2009 to study whether modified atmosphere with varying carbon dioxide concentration can protect seed from fungal infestation. Lower CO_2 concentration upto 40% was ineffective in the control of seed mycoflora, however high concentrations of carbon dioxide reduced fungal incidence but none of the carbon dioxide concentrations tested, completely controlled fungal infestation in paddy seed or rice grain. CO_2 at 60-80% concentrations (v/v) reduced the incidence of storage fungi viz., *Curvularia lunata, Cladosporium* sp., *Rhizopus stolonifer* and *Alternaria alternata* on stored paddy seed. But 80% CO_2 was required to control *Aspergillus flavus,* an aflatoxin producing fungi. Modified atmosphere with oxygen at 5% concentration resulted in higher incidence of storage fungi (52.0%) as compared to 48.0% in basmati rice exposed to modified atmosphere with 2% O_2 concentrations and with CO_2 concentrations varying from 0-20%.

Key words: Carbon dioxide, storage fungi, *Aspergillus flavus,* paddy, rice

INTRODUCTION

Large number of storage pests namely insects, microbes (mainly fungi), rodents, birds etc., infests stored products including seeds. Annual post harvest losses caused by insect damage, microbial deterioration and other factors are estimated to be in the order of 10-20% worldwide. Higher moisture content in seeds/grains during storage increases the incidence of fungi. The most common storage fungi are species of *Rhizopus*, *Aspergillus*, *Penicillium* etc. The incidence of *Aspergillus flavus* in paddy is of utmost importance as it produces aflatoxins which are highly carcinogenic and causes cancer and thus is also a limiting factor in the export of rice. Maintenance of seed quality from harvest to planting in the next sowing season is the primary aim of good seed storage technology. Chemical fumigation, low temperature, dehumidification or low humidity conditions and controlled atmosphere are technologies that are used to protect stored product from insect pests and microorganisms. As an alternative to conventional fumigants, modified or controlled or altered atmosphere is another way of preserving stored products from pests. High carbon dioxide (hypercarbia) or low oxygen (anoxia) conditions have been reported lethal to several stored grain insect pests and microbes.

Modified Atmosphere (MA) using carbon dioxide (CO_2) is one of the methods which have been successfully used to preserve the food grains and seeds from deterioration by insect-pests and microbes (Jayas and Jeyamkondan, 2002). It also preserves grain quality and maintains high level of germination in the stored grain (Banks, 1981; Bera *et al*., 2008). Low O_2 and elevated CO_2 atmospheres have been used for many years to control insect pests in grains (Bell, 2000) but its effect against fungi needs elucidation. MA storage has no toxicological risk and is environmentally clean. CO_2, being a natural component of the atmosphere is a safe chemical and has been permitted for use as an additive to many types of drinks and foods. The influence of CO_2 on the growth of *Aspergillus flavus* and the incidence of storage fungi of paddy seed and rice grain during storage was evaluated in the present study.

MATERIALS AND METHODS

Bio-assay of carbon-dioxide gas on the growth of *Aspergillus flavus*, an aflatoxin producing fungus: Aflatoxin producing toxigenic fungi, *Aspergillus flavus* (strain 1654) was obtained from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi. The strain was cultured and maintained on Potato-Dextrose-Agar (PDA) medium for further studies.

A spore suspension was prepared from a fresh culture of *A. flavus* in 10 mL of 0.01% solution of Tween 80. The spore suspension (0.01 mL) was placed in

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Fig. 1: Specially designed glass

the centre of petri plate (5 cm diameter) containing potato-dextrose-agar medium under sterile conditions. The plates were covered and incubated in BOD incubator at 25±1°C for 2 days. The plates were then transferred to 1250 mL capacity specially designed wide mouth glass containers used as storage structure. Lid of each container was fitted with one inlet port, one outlet port (made of silicone tube), one rubber septum and the containers were made air tight using petroleum jelly (Fig. 1). Gas mixture from gas blender was introduced through inlet port and displaced gas came out through outlet port. The CO_2 gas was flushed into these jars at different concentration (0, 20, 40, 60, 80% v/v) in three replications and then incubated for 0, 5, 10, 15 and 20 days. The concentration of CO_2 inside the container was checked by using gas chromatograph taking a 1 mL gas sample through rubber septum. The containers were kept in a room under ambient condition. The size of fungal colony was measured in cm in different treatments after 5, 10, 15 and 20 days of incubation.

Bio-assay studies to evaluate efficacy of CO_2 gas on mycoflora associated with paddy seed under laboratory conditions

Seed conditioning to prepare seed lots of required moisture level: To raise seed moisture content of paddy (cv. Pusa basmati No.1) to the desired level, method described by Matthew and Powell (1981) was used with modification. The seeds were spread on a polythene sheet in thin layer and pre-determined amount of water was sprayed in the form of fine mist along with mixing. Thereafter, the seed was filled in 700 gauge polythene packets and opening was closed excluding free air space as much as possible. This was kept at 20°C for 4 days and moisture content was checked by hot air oven method. If needed, the process was repeated to get the required moisture level. The seed moisture of paddy before conditioning was 12.7%, which was equilibrated to 15%.

Inoculation of fungus: One half of the seed was treated with spore suspension of fresh culture of *Aspergillus flavus*. Eighteen samples (450 g) of each treated and untreated seeds having 15% MC were filled in the specially designed glass containers of 1250 mL capacity as above. These containers were sealed to make them airtight. Required amount of air was withdrawn from the container with the help of a 50 mL syringe and the same amount of CO_2 was injected to create different CO_2 concentrations viz., 0 (normal air), 20 and 40% (v/v). CO_2 gas at 0, 20 and 40% were flushed into six containers each of untreated and treated seeds, respectively. Pre and post-treatment observations on seed moisture, germination and vigour were recorded following ISTA (1999). Seed health test was carried out using standard blotter method to determine the percent incidence of different fungi. Plated seeds were incubated at 22±1°C temperature and incubated for 8 days. The seeds were examined under Stereo Binocular microscope for the presence of associated fungi. Total number of seeds infected by specific fungus was scored to determine the percent seed infection. Observations on seed mycoflora and incidence of aflatoxin producing fungus *Aspergillus flavus* was recorded after 10 and 20 days of incubation under hermetic condition at room temperature.

Efficacy of modified atmosphere on the moisture and associated mycoflora in basmati rice grains during hermetic storage: Rice grain of basmati type was collected from a rice sheller. The grain material was treated with spore solution of aflatoxin producing fungus *Aspergillus flavus*. The treated grain was incubated in airtight packaging for 3 days at 25±1°C. Samples were drawn from both treated and untreated seed lots and packed in packets prepared from 700 gauge polythene sheets (Fig. 2). These packets were flushed with different concentrations of CO_2 gas (0, 5, 10 and 20%) at two oxygen levels (2 and 5%) in three replications and stored for different durations (0, 5, 10 and 15 days) under room temperature. The effect of MA was assessed on grain moisture and grain mycoflora including *Aspergillus flavus* after 0, 5, 10 and 15 days of hermetic storage.

Field evaluation of different concentrations of CO_2 on mycoflora of paddy seed var. PB No. 1 under hermetic storage: Paddy seed of variety Pusa Basmati No. 1 were collected and divided into two equal lots. One seed lot was inoculated with fresh fungal culture of

Fig. 2: Rice grains packed

Fig. 3: Field evaluation of containers used for bioassay under modified atmosphere

Aspergillus flavus and the other lot was left untreated. Both these lots were sub-divided into eight equal seed samples and placed in wide mouthed jars. The mouths of these jars were tied with muslin cloth to allow CO_2 to enter. These jars, one of each type were placed inside large Sintex bins of 500 l capacity (Fig. 3). These bins were made airtight by sealing them using clay as the sealant material. Carbon dioxide gas was flushed in each bin by weight at controlled flow rate. The 250 g of carbon dioxide gas was flushed in five bins, 300 g of gas was flushed in two bins and no gas was flushed in one bin which contained atmospheric air only and served as control. These bins were kept in seed stores for 10 days. Concentration of carbon dioxide gas inside the different bins was assessed daily by gas chromatograph and an average concentration of the gas was calculated over the period of storage. After 10 days, the samples were withdrawn from each bin and assessed for the associated mycoflora by blotter technique and seed germination and vigour were assessed using paper towel method.

RESULTS

Bio-assay of carbon-dioxide gas on the growth of *Aspergillus flavus*: The results revealed that the size of the fungal colony was insignificantly affected after 5 days of incubation but it reduced significantly after 10 and 20 days of incubation under CO_2 atmosphere (Table 1).

After 5 days of incubation, the size of the fungal colony at 0% CO_2 concentration was statistically at par with CO_2 concentrations at 20, 40 or 60% but it was significantly different from CO_2 concentration at 80%. After 10, 15 or 20 days of inoculation, the size of the fungal colony at 20, 40 or 60% CO_2 concentrations was statistically at par and significantly different from 0 and

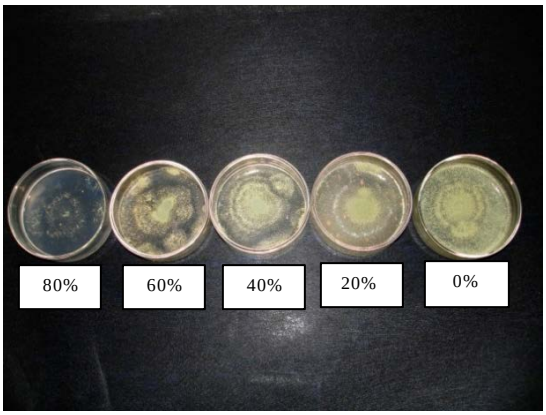

Fig. 4: Growth of *Aspergillus flavus* as affected by different concentrations (%) of carbon dioxide

Table 1: Effect of CO_2 concentration and exposure time on growth of *Aspergillus flavus*

CO_2 concentration (%)	Colony size (cm) at different incubation period (days)			
	5	10	15	20
0	2.80^{a}#	3.20^{a}	3.00^{a}	3.00^{a}
20	2.40^{ab}	2.73^{b}	2.30^{b}	2.10^{b}
40	2.33^{ab}	2.70^{b}	2.30^{b}	2.20^{b}
60	2.30^{ab}	2.90^{ab}	2.37^{b}	1.93^{bc}
80	1.97^{b}	1.97^{c}	1.57^{c}	1.67^{c}
CD (p = 0.05)	NS	0.42**	0.46**	0.36**

#Column followed by same letter's is not significantly different, Colony size at 0 period storage = 1.9 cm

80% CO_2 concentrations. However, after 20 days of incubation, the effect of CO_2 at 60 and 80% on the size of the fungal colony was statistically at par. The size of the fungal colony was significantly reduced at 80% concentration of CO_2 gas after 20 days of incubation (Fig. 4). The plates, which were not exposed to CO_2 gas, showed maximum growth of the fungus irrespective of

Table 2: Effect of CO_2 concentration and exposure period on seed moisture, seed germination, vigour and total seed mycoflora vis., *Aspergillus flavus* in paddy cv PB No. 1

	Observation after 10 days				Observation after 20 days			
CO_2 concentration (% v/v)	MC (%)	G (%)	Seed vigour	TF (Af)	MC (%)	G (%)	Seed vigour	TF (Af)
0 CO_2	14.8	90	1349	17.7 (0)	14.4	92	1695	17.3 (0)
0 CO_2+*A. flavusz*	14.2	87	1656	17.4 (0.4)	13.7	90	1733	23.9 (1.3)
20 CO_2	14.5	87	1672	13.3 (0)	13.4	92	1833	24.3 (0)
20 CO_2+*A. flavus*	13.9	91	1719	12.7 (0)	13.6	92	2001	25.4 (1.7)
40 CO_2	14.3	92	1846	13.7 (0)	13.1	90	1859	26.5 (0.3)
40 CO_2+*A. flavus*	13.8	90	1885	20.0 (0)	12.7	89	1986	28.6 (0.2)

At zero period storage: Seed moisture = 15%: Seed germination = 94%; Seed vigour = 1934, *Paddy seed treated with spore suspension of *Aspergillus flavus* (Af); TF = total fungi

incubation intervals. Thus, the growth of fungus remained unaffected at lower concentrations of carbon dioxide but the fungal growth got reduced at higher concentrations of the gas.

The loss of CO_2 gas from the glass containers was minimum upto 15 days of storage. After 20 days of storage, there was slight loss of the gas, however, maximum loss of gas was observed in containers flushed with CO_2 at 60 and 80% concentrations. Thus, the containers having higher concentration of CO_2 lose gas at fast pace.

Bio-assay studies to evaluate efficacy of CO_2 gas on mycoflora associated with paddy seed under laboratory conditions: The results revealed that the moisture content of paddy seeds decreased with increase in the concentration of CO_2 and also with increase in storage period. Seeds treated *Aspergillus flavus* had comparatively low moisture than the untreated seeds. The effect of CO_2 was not apparent on seed germination and seed vigour (Table 2), though, there was slight decrease in both germination and seed vigour i.e., by about 4 and 8%, respectively as against control after 10 and 20 days of storage and the seeds treated with *Aspergillus flavus* had higher seed vigour than untreated ones and the vigour of the seeds also increased with increase in storage duration. Five fungi viz., *Rhizopus stolonifer*, *Penicillium* spp., *Curvularia lunata*, *Alternaria alternata* and *Aspergillus flavus* were found associated with paddy seed in varying concentrations. Carbon dioxide concentrations upto 40% were ineffective against seed mycoflora and *Aspergillus flavus* in particular after 10 and 20 days after exposure. Thus, in the present bioassay studies, lower concentrations of CO_2 were ineffective against fungi associated with paddy seed. The total mycoflora on the seeds increased after 10 and 20 days of incubation irrespective of treatments as against zero period storage.

Efficacy of modified atmosphere on the status of moisture and associated mycoflora on basmati rice grains during hermetic storage: Eleven fungi were found associated with rice grains stored under hermetic conditions under various MA treatments (Table 3). At 0 period storage only *Rhizopus stolonifer* and *Aspergillus flavus* were found associated with rice grains but with increase in storage duration, other fungi appeared on the grains in varying incidence. With increase in the storage period, there was increase in the number and incidence of mycoflora, irrespective of the treatments. The maximum number of fungi (10) was found associated with rice grains after 15 days of storage. Amongest different fungi, *A. flavus* accounted for 44% of the total mycroflora associated with rice grains.

The moisture content of rice grains was almost similar in all the treatments upto 10 days of storage but slight increase in MC was observed in all the treatments after 15 days of storage. MA, with 5% O_2, supported higher number and incidence of the fungi (52%) as against 2% O_2 (48%), irrespective of CO_2 concentrations. At 2% O_2 and CO_2 concentrations of 5, 10 and 20%, the incidence of *Rhizopus stolonifer* got restricted but there was no effect of MA on the incidence of *A. flavus* which increased with increase in the storage period. However, at 2% O_2 concentration, the incidence of different fungi decreased initially after 5 days and then increased after 10 days of storage irrespective of CO_2 concentrations. But such trend was not observed in MA treatments having 5% O_2 concentration, where the fungal incidence increased after 5 days of storage and then decreased marginally but it was higher than the fungal incidence at 0 period storage.

Effect of CO_2 concentration on fungi associated with paddy seed cv PB-1 stored in airtight bins: The results revealed that concentration of CO_2 was 9% in bin kept as untreated control. In bins where 250 g of CO_2 was flushed, the concentration of CO_2 varied from 30.5-34.3% (average 32%) and the bins flushed with 300 g of CO_2, the resultant concentration in the bin varied from 35.5-37.9% (average 37%). Though, the atmospheric air contains 0.03% CO_2 but higher concentration of gas was assessed in the control bin probably because the bins contained

Table 3: Effect of MAP on seed moisture and incidence of fungi associated with basmati rice grains during storage

		Incidence of fungi (%)										
MAP treatment	MC (%)	*Rhizopus*	*Aspergillus*	*Aspergillus*	*Aspergillus*	*Penillium*	*Curvularia*	Fusarium	Alternaria	Dreschlera	*Cladospo*	*Epicoccu*
0-Day storage												
2% O_2+0% CO_2	13.5	35.5	49.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+5% CO_2	13.9	16.5	51.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+10% CO_2	13.7	9.5	52.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+20% CO_2	13.9	11.7	58.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5% O_2+0% CO_2	13.9	3.3	48.7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5% O_2+5% CO_2	13.9	6.0	33.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5% O_2+10% CO_2	13.8	4.0	38.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5% O_2+20% CO_2	14.0	0.0	33.7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5-Day storage												
2% O_2+0% CO_2	13.9	7.3	46.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+5% CO_2	13.9	6.0	33.7	Nil	Nil	Nil	0.3	Nil	Nil	Nil	Nil	Nil
2% O_2+10% CO_2	13.9	4.0	41.7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+20% CO_2	13.8	2.7	45.0	Nil	Nil	Nil	0.3	0.3	Nil	Nil	Nil	Nil
5% O_2+0% CO_2	13.9	18.0	90.0	Nil	0.3	Nil	0.3	Nil	1.0	Nil	Nil	Nil
5% O_2+5% CO_2	14.0	16.7	92.3	Nil	0.3	Nil	Nil	Nil	0.7	Nil	Nil	Nil
5% O_2+10% CO_2	13.9	17.7	90.7	0.3	Nil	Nil	0.7	Nil	0.3	Nil	Nil	Nil
5% O_2+20% CO_2	13.8	10.0	90.7	Nil	0.3	Nil	Nil	Nil	0.3	Nil	Nil	Nil
10-Day storage												
2% O_2+0% CO_2	13.9	5.5	63.0	Nil	0.3	26.0	Nil	0.7	0.3	Nil	Nil	Nil
2% O_2+5% CO_2	13.9	6.3	60.0	Nil	Nil	35.7	Nil	Nil	Nil	Nil	Nil	Nil
2% O_2+10% CO_2	14.1	8.7	63.7	Nil	Nil	10	Nil	Nil	Nil	0.3	Nil	Nil
2% O_2+20% CO_2	14.1	9.0	59.3	Nil	Nil	12	0.7	Nil	Nil	Nil	Nil	Nil
5% O_2+0% CO_2	14.0	14.7	78.0	Nil	Nil	12.3	Nil	Nil	Nil	Nil	0.3	Nil
5% O_2+5% CO_2	14.1	7.7	85.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.3	Nil
5% O_2+10% CO_2	13.9	14.0	79.0	Nil	Nil	2.0	Nil	0.3	Nil	Nil	Nil	Nil
5% O_2+20% CO_2	14.1	33.7	80.0	Nil	Nil	0.7	Nil	Nil	Nil	Nil	Nil	Nil
15-Day storage												
2% O_2+0% CO_2	14.6	15.7	86.0	0.3	Nil	3	Nil	0.7	0.7	Nil	0.3	Nil
2% O_2+5% CO_2	14.6	15.7	84.0	0.7	Nil	3.7	Nil	Nil	0.7	Nil	Nil	Nil
2% O_2+10% CO_2	14.6	7.7	91.3	1.0	0.3	2.7	Nil	Nil	1.0	Nil	0.3	Nil
2% O_2+20% CO_2	14.6	40.3	86.0	1.0	Nil	Nil	Nil	Nil	2.0	Nil	1.0	Nil
5% O_2+0% CO_2	14.5	15.7	77.5	Nil	Nil	Nil	Nil	Nil	1.0	Nil	2.0	0.3
5% O_2+5% CO_2	14.6	28.7	83.0	0.3	Nil	Nil	1.0	0.7	1.3	Nil	0.7	0.7
5% O_2+10% CO_2	14.4	26.0	65.0	Nil	Nil	Nil	Nil	0.3	0.7	Nil	Nil	Nil
5% O_2+20% CO_2	14.5	14.7	58.3	Nil	Nil	Nil	Nil	1.0	1.3	Nil	1.0	Nil

insect infested seed material and seeds and insect being living entity also respire. During the process of respiration, CO_2 and water are liberated thereby increasing the concentration of CO_2 and moisture inside the airtight bins.

Five fungi namely *Rhizopus* sp., *Penicillium* sp., *Curvularia lunata*, *Aspergillus flavus* and *A. niger* were found associated with untreated paddy seed. In lots, where paddy seed was inoculated with *A. flavus* prior to storage, *A. niger* was not detected on the seed but *Chaetomium* sp. and *Alternaria alternata* were found associated with paddy seed. In bins having 9% CO_2 (control), the fungal incidence was high in seeds treated with *Aspergillus flavus* but at 32 and 37% CO_2, the fungal incidence on treated seeds was lower than untreated seeds, i.e., there was increase in the fungal incidence in untreated seeds under MA conditions (Fig. 5).

Thus, it appeared that CO_2 gas upto 37.9% concentration was not able to effect the growth of

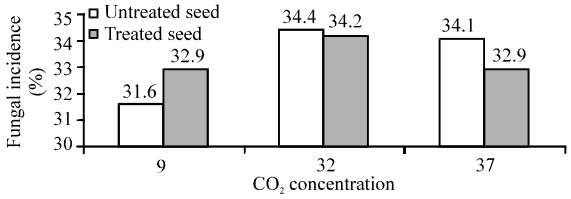

Fig. 5: Effect of CO_2 on total fungi associated with paddy seed (untreated and treated with *Aspergillus flavus* prior to storage) under hermetic storage

different fungi significantly even under hermetic storage conditions both in untreated (uninoculated) and treated (inoculated) paddy seed.

DISCUSSION

In general, incidence of all the fungi except *Aspergillus* reduced at 60% CO_2. Reduction was sharp

when CO_2 concentration reached 80%. In case of *Aspergillus* spp. 60% CO_2 was unable to reduce its incidence but at 80% CO_2, the incidence reduced significantly. None of the CO_2 concentration was able to arrest fungal incidence completely. It is clear from the present study that 20-40% CO_2 concentrations were not capable of reducing the fungal incidence on paddy seed/rice grain. Hypercarbic atmosphere with >60% carbon dioxide is effective in controlling fungal infestation except *Aspergillus* spp. for which 80% CO_2 is required but even 80% CO_2 was unable to provide complete protection. Hocking (1988) reported that even 20% CO_2 was having inhibitory effect on fungal growth. However, he further observed that atmosphere has high concentration of CO_2 which is more effective against fungal growth and mycotoxin production and 20-60% CO_2 prevented or reduced production of some species of *Fusarium*, *Aspergillus* and *Penicillium* and opined that atmospheres with 20% CO_2 generally inhibit mould growth but >80% CO_2 may be required to prevent fungal deterioration of commodities with high moisture content and thus hypercarbia can inhibit mycotoxin production along with reduction in fungal incidence because mycotoxin production is more sensitive than fungal growth to low O_2 and high CO_2 concentrations. Mokbel and Hashinaga (2004) also observed that low concentration of CO_2 (2-20 kPa) showed no significant inhibition of fungal growth, while high CO_2 (40-60%) significantly suppressed the mycelia growth of almost all the thirteen fungi tested.

The results from present study are also corroborated by Qianyu (1984) who observed that 80% CO_2 inhibited the growth of moulds and yeasts. In their study on influence of controlled atmosphere (98.5% CO_2 and 1.5% O_2) on paddy grain quality, it was reported that temperature influenced the quality even when kept under Controlled Atmosphere (CA) and there was no appreciable change in quality in terms of fat acidity, gelatinization temperature and cohesiveness as compared to rice stored in air. It is preferable to combine CA treatment with reduction of temperature below 20°C to obtain the best storage conditions. El Halouat and Debevere (1997) observed that under aerobic conditions at 5% O_2, germination and growth occurred only at a high water activity, while 10 or 20% O_2 combined with either 80 or 60% CO_2, conidial germination and mould growth were only delayed compared with the control (air). Fleurat-Lessard *et al.* (1994) reported that incidence of *Alternaria*, *Fusarium*, *Cladosporium*, *Epicoccum* and *Penicillium* reduced in MA with 60% CO_2 than control or N_2-O_2 mixtures in wheat seeds. At a storage temperature of 30°C, there was reduction in the incidence of *Aspergillus glaucus* in seeds stored under MA with 60% CO_2. The observations of Fleurat-Lessard *et al.* (1994) also confirmed present findings, who stated that partial inhibition of mould growth is associated with residual oxygen concentration inside the storage vessel rather than hypercarbia as complete air-tightness was not evident. In his study, the residual oxygen concentrations ranged from 4.2-16.8% (16.8% O_2 in 20% CO_2; 12.6% O_2 in 40% CO_2; 8.4% O_2 in 60% CO_2 and 4.2% O_2 in 80% CO_2) in his study. Bera *et al.* (2007) were also of the view that the carbon dioxide concentrations of 60 and 80% reduced fungal incidence but none of the carbon dioxide concentrations completely controlled fungal infestation in rice seed.

Rajendran *et al.* (2000) reported storage of basmati rice under carbon dioxide rich atmospheres for 4 months. Storage of both brown and milled rice under CO_2 rich atmospheres (4.1 and 2.5 Kg tonne^{-1}, respectively so that CO_2 concentration above 35% remained for more than 2 weeks) retained the characteristic aroma and grain elongation upon cooking. Free fatty acid development was very less compared to control samples (9.8 and 12.7% vs. 25.4 and 29.9% in brown and milled rice, respectively). There was an increase in grain hardness (by 1.1-2 kg points) as well as milling breakage (by 2 -2.5% points). A study showed that with the increase in average temperature from 15-20.8°C (5.8°C) and 20.8-30.5°C (9.7°C) the toxicity of CO_2 increased by 10.9 and 21.5%, respectively. Increased susceptibility at higher temperature can be attributed to enhance respiratory demand (Mbata and Phillips, 2001).

Tome *et al.* (2000) studied the effect of MA when beans (*Phaseolus vulgaris* cv. *perola*) were exposed to different doses of CO_2 and N_2 and reported that moisture content, water absorption, cooking time and colour index remained unaffected by all the treatments and treatment duration performed. Amanatidou *et al.* (1999) observed that exposure of microorganisms to high O_2 (80 or 90% balanced with N_2) alone did not inhibit the microbial growth strongly, while strong inhibition was observed only when the two gases CO_2 and O_2 were used in combination. Feng *et al.* (1989) reported storage of husked seeds of japonica and indica rice packaged and treated with 40% CO_2. After storage for 14 months the CO_2 treated seeds had higher protein content than the controls and their vigour was greater. It was concluded that 40% CO_2 was safe and reliable for storage of rice seeds. Wilson and Jay (1976) were also of the opinion that storage of peanuts in controlled atmosphere inhibited growth of the *A. flavus* group but did not eliminate the fungi. The other fungi grew on the peanuts and the overall quality was reduced.

Thus, carbon dioxide is effective in reducing fungal flora associated with seeds especially *Aspergillus flavus* responsible for elaboration of highly toxic aflatoxins but only at higher concentrations. Lower CO_2 concentration

upto 40% was ineffective in the control of seed mycoflora and so higher concentrations of carbon dioxide will be needed to control fungi associated with seed. In situations, where both insect and fungi are involved in storage loss, 80% CO_2 should be used, otherwise 20% CO_2 is sufficient to ward off insect damage. The greatest potential use of modified atmosphere storage would be the residual free dual control of aflatoxin production and freedom from insects. Hence, modified atmosphere storage technology will hold the key to safe storage of foodgrains/seed, avoid chemical treatments and thus overcoming problems of their residues in stored products and will also be environmentally safe.

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