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Application of modified atmosphere technique in seed health management

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ABSTRACT

Modified atmosphere with different concentrations of carbon dioxide (CO_2) was studied to protect seed from fungal infestation. Lower CO_2 concentration up to 40% was ineffective in the control of seed mycoflora. 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 the storage fungi,*Curvularia lunata* (Wakker) Boeidijn*, Cladosporium* sp., *Rhizopus stolonifer* (Ehrenb. Vuill.) and *Alternaria alternate* (Fr. Keissl.) on stored paddy seed. But 80% $CO_2$was required to control *Aspergillus flavus* (*Oryza sativa* L.)*,* an aflatoxin producing fungi. Modified atmosphere with oxygen at 5% concentration resulted in higher incidence of storage fungi (52.0%) compared to 48.0% in basmati rice (*Oryza sativa* L.) exposed to modified atmosphere with 2% O_2concentrations and with CO_2 concentrations varying from 0 to 20%.

Paddy seed stored in an atmosphere flushed with cowdung cake (CDC) smoke, the cheapest and easily available source of CO_2, reduced the fungal load on seed effectively. The fungal incidence was 26.6 and 24.7% when CDC smoke was flushed for 30 min or 60 min in earthen pots, respectively as against 43.9% in untreated control. Flushing of CDC smoke for 30 min under air-tight polythene sheet of 700 gauge with an exposure period of 10 days reduced the total mycoflora associated with paddy seed by 88.8%.

Key words: Atmosphere technique, Health, Management Seed

Although India is becoming self-sufficient in food production with its annual harvest of food grains of about 255 million tonne, there are significant post-harvest losses especially during storage estimated at about 10% caused by biotic organisms such as insects, mites, fungi, bacteria, rodents, etc. In India, financial losses in crop production are estimated about ₹ 1,40,000 crores (equivalent to US$ 10 million) annually [*Crop Care* **32** (3&4): 06-07; 2010]. The United Nations –Food and Agriculture Organization estimated that 20 to 30% of food produced globally is lost every year to various biotic and abiotic damage(http://www.fao.org/save-food/en/). Several chemicals have been recommended for control of stored pests. However, the widespread use of synthetic chemicals has affected human health and environment, and is expensive leading to an appreciable escalation of cost of the production of rice and other grains. Development of resistance to chemical pesticides has aggravated this condition. The incidence of *Aspergillus flavus* Link in paddy (*Oryza sativa* L.) must be avoided as it produces aflatoxins which are highly carcinogenic and is also a limiting factor in the export of rice. Therefore, there is an urgent need to develop safe, cost effective and environment friendly pest control methods. In the North-east Region and Eastern Uttar Pradesh in India, and in Sri Lanka, a traditional method of controlling insects/microbes in grains stored at household level is to expose the grains to smoke generated from biomass combustion by storing the grains in sacks above the kitchen fire place (Wijayaratne et al., 2009; Sinha, 2010). However, application of this traditional technology to commercial level storage has not yet been scientifically established. Modified atmosphere (MA) using carbon

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dioxide (CO_2) is one of the methods which have been successfully used to preserve the foodgrains and seeds from deterioration by insect-pests and microbes (Jayas and Jeyamkondan, 2002). It also preserves grain quality and maintains a high level of germination in the stored grain (Banks, 1981; Bera, 2008). Kader (1982) revealed that carbon monoxide (CO) is a fungistatic gas which suppresses fungal growth. Its effectiveness is pathogen-dependant and is greatly enhanced when CO_2 is combined with reduced O_2 atmospheres. Low O_2 and elevated CO_2 atmospheres have been used for many years to control stored product pests in grains (Bell, 2000). It has no toxicological risk and is environmentally clean. Waghray and Reddy (1995) reported detoxification of aflatoxin B_1 in maize and groundnut kernels with cowdung cake fumes. Exposure of maize and groundnut kernels to smoke generated by CDC av. 10, 15 and 20% (w/w) reduced aflatoxin B_1 by 23-28% in artificially infected maize, 16-33% in naturally infected groundnut kernel and 19-37% in artificially infected groundnuts. Cowdung cake smoke is a cheap and easily available source of CO_2. Its use for the control of insect-pests during storage has been deliberated by a number of workers but its use for the control of microbes has been less studied. The influence of $CO_2$and CDC smoke on the growth of storage fungi on paddy grain during storage was evaluated in this study.

MATERIALS AND METHODS

Bio-assay of carbon dioxide (CO_2) gas on the growth of Aspergillus flavus

Aspergillus flavus (strain 1654), an aflatoxin producing fungus was obtained from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi and maintained on potato-dextrose-agar medium (PDA).0.01 ml of the spore suspension prepared from a fresh culture of the fungus was placed in the centre of a petri-plate containing PDA medium and incubated at 25±1ºC for 2 days. The plates were then transferred to 1,250 ml capacity specially designed wide mouth glass containers used as a storage structure. The lid of each container was fitted with one inlet port, one outlet port (made of silicone tube) and one rubber septum and the containers were made air tight using petroleum jelly (Fig.1). CO_2 gas was flushed into these jars at different concentrations (0, 20, 40, 60, 80% v/v) in three replications and then incubated for 5, 10, 15 and 20 days. The concentration of CO_2 inside the container was checked using a gas- chromatograph. The containers were kept under ambient conditions and the size of the fungal colony in different treatments was measured after 5, 10, 15 and 20 days of incubation.

Efficacy of CO_2 on mycoflora associated with paddy seed under laboratory conditions

Seed moisture of paddy cv. Pusa Basmati No.1 seed was equilibrated to 15% as described by Matthew and Powell (1981) with modification and one half of this seed was treated with *Aspergillus flavus*. Eighteen samples (450 g each) of treated and untreated seeds were filled into glass containers which were sealed to make them airtight. CO_2 gas @ 0%, 20% and 40% were flushed in to 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 and examined under stereo binocular microscope. Observations on incidence of seed mycoflora was recorded after 10 and 20 days of incubation under hermetic conditions at room temperature.

Efficacy of modified atmosphere on the moisture and associated mycoflora in basmati rice grains during hermetic storage:

'Basmati' rice grain was treated with a spore solution of *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 placed 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%) and two oxygen (O_2) 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

Fig. 1. Specially designed glass containers used for bioassay

Fig. 2. Rice grains packed under modified atmosphere

Fig. 3. Field evaluation of modified atmosphere

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 under hermetic storage:

One seed lot of paddy, variety Pusa Basmati No. 1, was inoculated with *Aspergillus flavus* and the other lot was left untreated. Both of 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 (CO_2) gas was flushed in each bin by weight at controlled flow rate. The 250g 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 (CO_2) 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, seed germination and vigour.

Fig. 4. CDC Smoke generator

CDC Smoke generation, vacuum creation and seed fumigation: Cowdung cake (CDC) smoke was generated by partial combustion of CDC (2.5 kg) in a simple device. The smoke generator was connected with the inlet port of an earthen pot with a PVC tube (Fig. 1). The outlet port was connected with a pump for creating partial vacuum to reduce inter-granular spaces. The test material, paddy seed was kept inside the earthen pots of 25 kg capacity and pots were made air-tight using clay as sealant for effective flushing and retention of smoke from the container. Before flushing the smoke, vacuuming was conducted for 10 min by using an electric vacuum pump fitted to the outlet in the earthenpots. After treatment**,** pots were kept under ambient conditions in the seed store with an average temperature of 27° to 32°C and 60-65% r.h. After each treatment with smoke, seed germination and vigour were analyzed to ensure that there was no adverse effect of smoke on quality attributes of seeds.

Evaluation of cowdung cake (CDC) smoke on health status of paddy seed:

Paddy seed variety 'Pusa Basmati 1' was collected and divided into four lots of three replications, each weighing 500 g. Two lots of three replications each were placed in two earthen pots; one lot with three replications was placed under a polythene cover of 700 gauge and one lot with three replications served as control. One of the earthen pots was flushed with cowdung cake (CDC) smoke for 30 min and the second

pot was flushed with CDC smoke for 60 min. The CDC smoke was also flushed under the polythene cover for 30 min. After 10 days of treatment the samples were taken out and assessed for seed mycoflora. In another experiment 10 samples of paddy seed were prepared in three replications and one of each replication was placed in ten earthen pots, which were made airtight using a sealant. Five pots were flushed with CDC smoke for 30 min and 5 were flushed with CDC smoke for 60 min. Samples were drawn from one pot of each type after 0, 2, 4, 8 and 16 days and assessed for the associated mycoflora by blotter technique.

Evaluation of cowdung cake smoke in combination with plant derivativeson health status of paddy seed:

Paddy seed variety 'PB-1', was collected and divided into five lots of three replications each. One lot of each replication was placed in 5 earthen pots. The earthen pots were made airtight using the sealant. These earthen pots were flushed with cowdung cake (CDC) + 100 g neem leaves smoke for 30 min. Samples were withdrawn after 0, 2, 4, 8 and 16 days ofexposure and assessed for the associated mycoflora by blotter technique. The same experiment was repeated with addition of 200 g, 300 g or 500 g of neem leaves and 500 g of paddy straw.

RESULTS

Bio-assay of carbon dioxide (CO_2) gas on 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 was statistically similar for CO_2 concentrations at 0, 20, 40 or 60% but it was significantly different

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

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

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Colony size at 0 period storage = 1.9 cm.

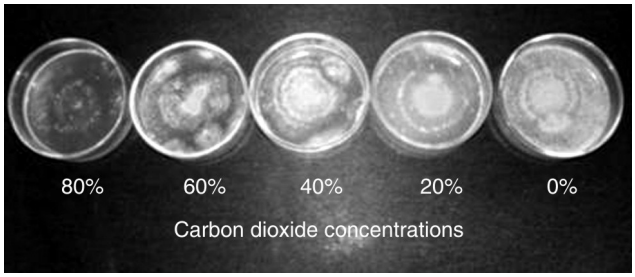

Fig. 5. Growth of *Aspergillus flavus* as affected by CO_2 concentrations

for 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 similar and significantly different from 0 and 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 similar.

The size of the fungal colony was significantly reduced at 80% concentration of CO_2 gas after 20 days of incubation (Fig. 5). The plates, which were not exposed to CO_2 gas, showed maximum growth of the fungus irrespective of incubation intervals.

Thus, the growth of fungus remained unaffected at lower concentrations of CO_2but the fungal growth was reduced at higher concentrations of the gas.The loss of CO_2 gas from the glass containers was minimum at up to 15 days of storage. After 20 days of storage there was slight loss of the gas in the lower concentrations, and the maximum loss found in the containers flushed withCO_2 at 60 and 80%concentrations.

Efficacy of CO_2 on mycoflora associated with paddy seed under laboratory conditions

The results revealed that the moisture content of paddy seeds decreased with an increase in the concentration of CO_2 and also with an increase in the storage period. Seeds treated with *Aspergillus flavus* had comparatively low moisture compared withthe untreated seeds. The effect of CO_2 was not apparent on seed germination and seed vigour (Table 2) though there was a slight decrease in both germination and seed vigour by about 4% and 8%, respectively when compared with control which after 10 and 20 days of storage of seeds treated with *Aspergillus flavus* had a higher seed vigour than untreated ones and the vigour of the seeds also increased with length of storage. Five fungi, *Rhizopus stolonifer*, *Penicillium* spp., *Curvularia lunata*, *Alternaria alternata* and *Aspergillus flavus* were found associated with paddy seed in varying concentrations. Carbon dioxide (CO_2) concentrations up to 40% were ineffective against seed mycoflora and *Aspergillus flavus* in particular

Table 2. Effect of CO_2 concentration and exposure period onseed moisture, seed germination, vigour and total seed mycoflora in paddy cv PB No.1

Sr. No.	CO_2 concentration (%v/v)	Observation after 10 days				Observation after 20 days			
		%MC	%G	Seed vigour	TF (*Af*)	%MC	%G	Seed vigour	TF (Af)
1	0% CO_2	14.8	90	1349	17.7 (0)	14.4	92	1695	17.3 (0)
2	0% CO_2 + *A. flavus**	14.2	87	1656	17.4 (0.4)	13.7	90	1733	23.9 (1.3)
3	20% CO_2	14.5	87	1672	13.3 (0)	13.4	92	1833	24.3 (0)
4	20% CO_2+*A. flavus*	13.9	91	1719	12.7 (0)	13.6	92	2001	25.4 (1.7)
5	40% CO_2	14.3	92	1846	13.7 (0)	13.1	90	1859	26.5 (0.3)
6	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

after 10 and 20 days ofexposure.The total mycoflora on the seeds increased after 10 and 20 days of incubation irrespective of treatments as against zero period storage. Thus, lower concentrations of CO_2 were found ineffective against fungi associated with paddy seed.

Efficacy of modified atmosphere on 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 modified atmosphere (MA) treatments (Table 3). At 0-period storage only *Rhizopus stolonifer* and *Aspergillus flavus* were found associated with rice grains but with an increase in storage duration, other fungi appeared on the grains in varying incidence. With an increase in the storage period there was an 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. Amongst different fungi, *A. flavus* accounted for 44% of the total mycoflora associated with rice grains.

The moisture content of rice grains was almost similar in all the treatments up to 10 days of storage but a slight increase in MC was observed after 15 days of storage.

Field evaluation of different concentrations of CO_2 on mycoflora of paddy seed under hermetic storage:

The results revealed that concentration of CO_2 was 9% in the bin kept as untreated control. In bins where 250 g of CO_2 was flushed, the concentration of CO_2 varied from 30.5 to 34.3% (average 32%). In the bins flushed with 300g of CO_2, the resultant concentration in the bin varied from 35.5 to 37.9% (average 37%).

Though the atmospheric air contains 0.03% CO_2, the higher concentration of gas that was assessed in the control bin was probably because the bins contained insect infested seed material and the seeds and insects were respiring. 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, *Rhizopus* sp., *Penicillium* sp., *Curvularia lunata*, *Aspergillus flavus* and *A. niger* van Tieghem 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 but *Chaetomium* sp. and *Alternaria alternata* were found on the 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, indicating that there was increase in the fungal incidence in untreated seeds under MA conditions (Fig. 6).Thus, it appeared that CO_2 gas up to 37.9%

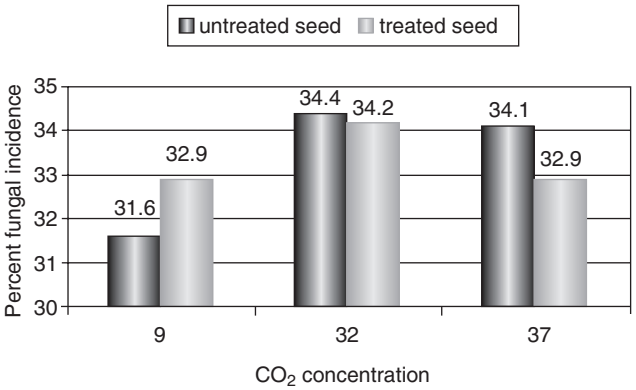

Fig. 6. Effect of CO_2 on seed microflora in paddy (untreated and treated with *Aspergillus flavus* prior to storage) under hermetic storage

Table 3 Effect of MAP on seed moisture and incidence of fungi during storage

MAP Treatment	MC (%)	Per cent incidence of fungi										
		R. stolonifer	*A. flavus*	*A. fumigatus*	*A. niger*	*Penillium* spp.	*Curvularia* spp.	*F. semitectum*	*A. alternata*	*Dreschlera* spp.	*Cladosporium* sp.	*Epicoccum* sp.
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

concentration was not able to effect the growth of different fungi significantly even under hermetic storage conditions both in untreated (uninoculated) and treated (inoculated) paddy seed.

Evaluation of cowdung cake smoke on health status of paddy seed

There was a significant reduction in the incidence of different fungi associated with paddy seed exposed

Table 4 Effect of CDC smoke flushing for different durations on seed mycoflora under different hermetic conditions 10 days after incubation

Fungi	Fungal incidence in control (%)	Fungal incidence (%)			LSD (P = 0.05)
		Earthen pot		Polythene cover	
		Flushed for 30 min	Flushed for 60 min	Flushed for 30 min	
Rhizopus sp.	98.0 a#	61.8 b	60.2 b	2.0 c	20.3
Aspergillus flavus	6.0 a	4.8 ab	3.0 ab	2.0 b	**3**
Curvularia lunata	4.2 a	1.5 b	1.5 b	1.8 b	**1.6**
Alternaria alternata	3.8 a	1.8 ab	0.2 b	0.2 b	**2.2**
Penicillium sp.	4.0 a	2.0 b	2.0 b	1.0 c	**0.7**
Fusarium semitectum	5.0 a	3.5 b	2.5 b	1.0 c	**1.4**
Sterile mycelia	20.0 a	10.0 b	10.0 b	7.5 b	**3.1**
Chaetomium sp.	1.8 a	1.0 ab	0.8 ab	0.5 b	**1**

Means in a column followed by same letter(s) are not significantly different

to cowdung cake smoke as against control. Eight fungi (*Rhizopus* sp., *Fusarium semitectum* (Desm. sacc.), *Aspergillus flavus*, *Curvularia lunata*, *Alternaria alternata*, *Penicillium* sp., *Chaetomium* sp. and sterile mycelia) were found associated with paddy seed with variable frequency (Table 4), maximum incidence was of *Rhizopus* sp. (98.0%) followed by sterile mycelia (20.0%) and *Aspergillus flavus* (6.0%) in the untreated control after 10 days of incubation.

After application of 30 min of CDC smoke to exposed seeds in earthen pots (61.8, 1.5, 2.0, 10.0), 60 min CDC smoke to exposed seed in earthen pots (60.2, 1.5, 2.0, 10.0) and 30 min CDC smoke to exposed seeds under polythene cover (2.0, 1.8, 1.0, 7.5), the incidence of *Rhizopus* sp., *Curvularia lunata*, *Penicillium* sp. and Sterile mycelia was statistically different to untreated seed (98.0, 4.2, 4.0 and 20.0),respectively after 10 days of incubation. The incidence of these fungi was statistically similar to seeds exposed to CDC smoke in earthen pots for 30 min and 60 min but statistically different from seeds exposed under polythene cover for 30 min and untreated seeds. The incidence of *A. flavus* and *Chaetomium* sp. was statistically similar to untreated seeds (6.0% and 1.8%) and seeds exposed to CDC smoke in earthen pots for 30 min (4.8% and 1.0%) or 60 min (3.0% and 0.8%), but was significantly reduced in seeds exposed to CDC smoke under a polythene cover (2.0, 0.5%), respectively.

However, incidence of *Alternaria alternata* was similar in untreated seeds (3.8%) and in seeds exposed to CDC smoke in earthen pots for 30 min (1.8%), but significantly reduced to 0.2% both in seed exposed to CDC smoke for 60 min in earthen pots and for 30 min under a polythene cover.

The incidence of all the associated fungi was

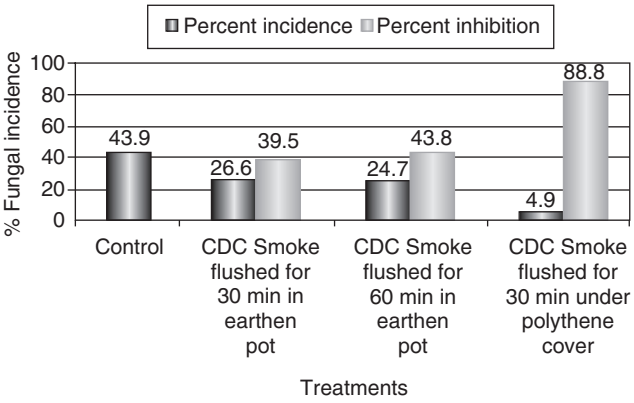

Fig. 7. Effect of CDC Smoke on mycoflora inpaddy seed under different conditions

significantly reduced in seeds exposed to CDC smoke for 30 min under the polythene cover. The fungal incidence on paddy seed exposed to CDC smoke in earthen pots for 30 min, 60 min or under the polythene cover was 26.6, 24.7 and 4.9%, respectively as against 43.9% in control treatment (Fig. 7).There was apparently no difference in the incidence of fungi when CDC smoke was flushed for 30 min or 60 min in earthen pots.

Seven fungi (*Rhizopus* sp., *Fusarium semitectum*, *Curvularia lunata*, *Drechslera oryzae* (Breda de Haan) Subram. & Jain, *Penicillium* sp., *Chaetomium* sp. and sterile mycelia) were found associated with paddy seed exposed to CDC smoke for 30 min and subsequently incubated from 0 to 16 days under the same atmosphere as in earthen pots (Table 5).

Along with the above fungi, *Alternaria alternata* and *Cladosporium* sp. were also found associated with paddy seed exposed to CDC smoke for 60 min and incubated from 0 to 16 days under the same atmosphere (Table 6), but all fungi were not present together in

Table 5. Effect of CDC smoke flushed for 30 min on fungal incidence in paddy seed

Duration (days)	*Rhizopus* sp.	Sterile mycelia	*F. semitectum*	*C. lunata*	*Drechslera oryzae*	*Penicillium* sp.	*Chaetomium* sp.
0	0.00 b	15.00 bc	0.67a	2.32 a	0.00 a	0.00 a	0.00 a
2	1.67 b	24.33 a	0.67a	0.67a	0.00 a	0.67 a	0.00 a
4	8.67 b	13.67 c	1.0a	0.33a	0.00 a	0.00 a	0.33 a
8	0.33 b	20.33 abc	0.33a	1.0 a	0.00 a	0.00 a	0.00 a
16	41.00 a	22.67 ab	0.67a	0.33a	0.67 a	0.00 a	0.00 a
LSD ($P = 0.05$)	8.84	7.75	1.65	1.99	0.97	0.97	0.49

Table 6. Effect of CDC smoke flushed for 60 min on fungal incidence in paddy seed

Duration (days)	Sterile mycelia	*Rhizopus* sp.	*F. semitectum*	*C.lunata*	*D. oryzae*	*Penicillium* sp.	*Chaetomium* sp.	*A. alternata*	*Cladosporium* sp.
0d	35.00 a	7.33 b	1.33a	4.33 a	0.00 a	1.00 a	0.67a	0.00 a	0.00 a
2d	24.00 b	0.33 b	0.67a	0.67 b	0.33 a	1.67 a	0.33a	0.67 a	0.00 a
4d	13.33 b	2.67 b	1.67a	2.67 ab	0.00 a	0.00 a	1.67a	1.33 a	0.33 a
8d	20.33 b	12.67 b	0.67a	3.00 ab	0.00 a	0.67 a	0.33a	0.00 a	0.00 a
16d	35.67 a	33.33 a	0.33a	3.67 a	0.00 a	0.67 a	0.67a	0.00 a	0.00 a
LSD ($P = 0.05$)	10.4	12.52	2.19	2.5	0.49	3.15	2.02	1.75	0.49

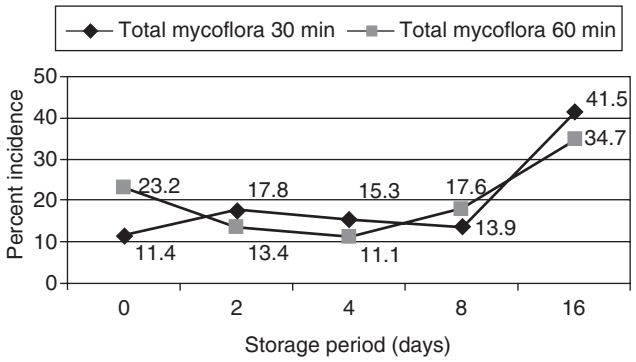

Fig. 8. Effect of CDC Smoke (flushed for 30& 60min) on seed mycoflora in paddy seed stored in earthen pots at different intervals

any treatment at a time.

The effect of CDC smoke flushed for both 30 and 60 min was similar on *Fusarium semitectum*, *Drechslera oryzae*, *Penicillium* sp., *Chaetomium* sp., *Alternaria alternata* and *Cladosporium* sp. when assessed at different durations. But in case of *Rhizopus* sp. and Sterile mycelia the incidence remained low up to 8 days and increased after 16 days of incubation (Fig. 8). However, the fungal flora was reduced initially at up to 4 days of incubation under modified atmosphere (MA) of CDC smoke for 30 min or 60 min and thereafter it increased.

The incidence of all the fungi except *Rhizopus* sp. and Sterile mycelia was statistically similar. The incidence of these two fungi at 0, 2, 4 and 8 days of incubation was statistically similar but different at 16 days of incubation, when it increased (Table 7).

Evaluation of CDC smoke in combination with plant derivatives on health status of paddy seed:

The results revealed that the number of fungi (2-4) and the fungal incidence on paddy seed was low when paddy seed was exposed to CDC smoke supplemented with paddy straw at 0 day (15.0%), after 2 days (15.3%) and 4 days (10.0%) of incubation (Table 8). Thereafter the number of fungi increased to 5 (*Rhizopus* sp., *Fusarium semitectum*, *Curvularia lunata*, *Chaetomium* sp. and sterile mycelia) and the fungal incidence also increased to 21.01% and 64.3% after 8 and 16 days of incubation.

The fungal incidence on paddy seed exposed to cowdung cake smoke supplemented with 100 g, 200

Table 7. Effect of cowdung cake smoke on total mycoflora associated with paddy seed

Incubation Duration (Days)	Total Mycoflora			
	CDC smoke flushed for 30 min		CDC smoke flushed for 60 min	
	Fungal incidence (%)	Total No. of fungi	Fungal incidence (%)	Total No. of fungi
0	17.99	3	49.66	5
2	28.01	5	28.67	8
4	24.00	5	23.67	7
8	21.99	4	37.67	6
16	65.34	5	74.34	6

Table 8. Effect of cowdung cake smoke with supplementations on seed mycoflora in paddy

Incubation duration (days)	Total mycoflora on paddy seed exposed with CDC smoke +									
	100 g Neem leaves		200 g neem leaves		300 g neem leaves		500 g neem leaves		Paddy straw	
	Fungal incidence (%)	No. of fungi	Fungal incidence (%)	No. of fungi	Fungal incidence (%)	No. of fungi	Fungal incidence (%)	No. of fungi	Fungal incidence (%)	No. of fungi
0	56.00	8	30.00	5	36.67	6	21.65	7	15.00	3
2	24.66	5	23.98	5	9.65	6	10.67	5	15.33	2
4	31.00	7	40.00	7	26.98	7	17.67	7	10.00	4
8	68.68	7	42.66	6	26.66	5	22.33	7	21.01	5
16	72.00	7	42.65	6	25.66	7	33.00	7	64.32	5

g, 300 g and 500 g of neem leaves at 0 day exposure was 56.0, 30.0, 36.7 and 21.7 % and after 2 days the fungal incidence reduced to 24.7, 23.9, 9.7 and 10.7 %, respectively in the same treatments. However, after 4 days of exposure and thereafter the number of fungi and the fungal incidence increased irrespective of treatments. Thus, cowdung cake smoke was found effective in the reduction of fungal incidence upto 4 days of incubation and thereafter the incidence and the number of fungi increased.

DISCUSSION

It was evident from the above results that CO_2 rich modified atmosphere influenced the growth and development of mycoflora associated with paddy seed. However, to achieve complete control of *Aspergillus flavus,* an aflatoxin producing fungi, relatively higher concentration of CO_2 were required. The incidence of all the fungi except *Aspergillus* reduced at 60% CO_2. Reduction was sharp when CO_2 concentration reached 80%. The incidence of *Aspergillus flavus* reduced significantly at 80% CO_2. It is evident from the present study that 20-40% CO_2 concentrations were incapable of reducing the fungal incidence on paddy seed/rice grain. These results are in conformity with the findings of Hocking (1988) who observed that atmospheres high in CO_2 were more effective against fungal growth and mycotoxin production. Bera et al. (2007) were also of the view that the CO_2 concentrations of 60 and 80% reduced fungal incidence but none of the concentrations controlled fungal infestation in rice seed completely.

Our results revealed that modified atmosphere 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 @ 5, 10 and 20%, the incidence of *Rhizopus stolonifer* was restricted but there was no effect of modified atmosphere (MA) on the incidence of *A. flavus* which increased with an 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 this 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. These results corroborate with Halouat and Debevere (1997) who reported 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). Gibb and Walsh (1980) reported reduced growth of eight fungi with reduction in atmospheric O_2 concentration. The *Fusarium moniliforme*, *F. solani* and *Rhizopus* sp. grew appreciably at a lower O_2 concentration (0.01% v/v) than other fungi. CO_2, up to 4% (v/v) was generally stimulatory to fungi growing at 0.1% O_2.

The experimental results revealed that treatment with cowdung cake (CDC) smoke significantly reduced the fungal load on paddy seed effectively when flushed for both 30 min and 60 min durations under hermetic conditions. when flushed under hermetic conditions. There was 88.8% inhibition in the total mycoflora associated with paddy seed when CDC smoke was flushed for 30 min under polythene cover of 700 gauge as against 43.8 and 39.5% when seeds were exposed to CDC smoke in earthen pots for 30 and 60 min, respectively under hermetic conditions.The effect was more pronounced when CDC smoke was flushed for 30 min under the polythene cover. There was significant reduction in percentage incidence of all the storage fungi in different treatments as against control.However, there was no apparent difference in the incidence of fungi when CDC smoke was flushed for 30 min or 60 min in the earthen pots. The use of CDC smoke alone was as effective in the control

of associated mycoflora on paddy seed without any supplementation of neem leaves or paddy straw and the CO_2 treatments. Our results revealed that the fungal incidence decreased initially when paddy seed was exposed to CDC smoke supplemented with different amounts of neem leaves or paddy straw. However, under long storage, the fungal growth could not be checked by application of neem leaves at any concentration or by paddy straw in combination with CDC smoke. Hypercarbic atmosphere with >60% carbon dioxide (CO_2) 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.CDC smoke alone was effective in the control of associated mycoflora on paddy seed without any supplement.

The carbon dioxide content in CDC-smoke was found to be around 5% (Sinha et al., 2001). Smoke fromthe mixture of CDC, rice bran and rice husk in the ratio of 4:1:0.5 gave around 13% CO_2 and 9.5% O_2 (Sinha, 2010). Kumar and Shende (2006) reported the typical ultimate analysis of cowdung dry powder (20 g) burnt per minute as- Carbon 31.6%; Hydrogen 05.18%; 0xygen 37.8%; Nitrogen 06.12%; and Ash 19.3%.

The CDC smoke was found to be more effective than pure CO_2 in the control of fungal flora on the seed perhaps because of combination of elements and gases found in the CDC smoke. Also the CDC smoke is readily available and inexpensive. The effective pest management system has become an essential component of good agricultural practices in terms of efficacy, cost-effectiveness, with safety for human health and the environment.

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