

Effects of modified atmosphere on microbial incidence on cauliflower seeds

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ABSTRACT The modified atmosphere (MA) containing 20 percent CO₂ and 2 percent O₂ is capable of controlling the incidence of seed microflora (fungal infestation) in cauliflower seeds. At the same time, low seed moisture contents kept seed borne microflora low, making the modified atmosphere more effective. Hence, it was concluded that hypercarbic atmosphere (20 percent CO₂) compounded with hypoxic condition (2 percent O₂) is highly efficient in controlling fungal infestation in cauliflower seeds during storage. However, an increase in amount of *Aspergillus spp.* as well as *Penicillium spp.* in modified atmospheres was noticed.

Keywords: Cauliflower, modified atmosphere, microbial incidence, seed quality

Storage causes severe damage due to fungal infestation in seeds and reduction in germinability, production of abnormal seedlings, heating and mustiness, discoloration production of toxins and various biochemical changes. The incidence of *Alternaria brassicae* and *A. brassicicola* is associated with production of abnormal seedlings and decrease in germinability. Contamination of mycotoxins like aflatoxin (*Aspergillus flavus*), patulin (*Penicillium*, *Aspergillus*) and fumonisins (*Fusarium moniliforme*, *Alternaria alternata*) in stored grains deserves serious attention. Thus, its prevention needs attention from the view point of seed as well as feed. Cauliflower is cultivated in 2.4 lakh ha in India and around 564 tonnes of its seed is used for sowing annually all over the world. Nearly 240 tonnes of seeds are subject to storage for varying periods in different agro climatic zones in India under biotic and abiotic stress. Various factors like micro organisms cause biological deterioration of seed in the field as well as during storage, leading to lowering of seed quality.

Hence, the present study was undertaken to investigate whether modified atmosphere (MA) with high carbon dioxide in combination with a low concentration of oxygen could protect seeds with low and high moisture content from fungal infestation.

MATERIALS AND METHODS

The seeds of two varieties of cauliflower *viz.*, Pusa Deepali and Pusa Synthetic (IARI, Regional Station, Karnal) were packed in low density polyethylene lined aluminum pouches containing three modified atmospheres with varying levels of carbon dioxide (MA₁= 5 percent CO₂, MA₂= 10 percent CO₂, and MA₃= 20 percent CO₂), a fixed level of O₂ (2 percent) and rest with N₂. The treatments were compared with a control *i.e.*, MA₁= 5 percent CO₂, MA₂= 10 percent CO₂, and MA₃= normal air. The seed samples were divided into two equal halves and dried to moisture levels of 5.5 percent and 7.5 percent. The pathogen incidence was recorded using ISTA deep freeze method, prior to packing of seeds in modified atmosphere at every 3 months interval from storage until completion of 12 months. The

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samples were stored at chosen temperature regimes viz., cold (5°C) and ambient (15-30°C). The observations were recorded on all fungal species, sterile mycelia, bacteria and total mycoflora.

RESULTS AND DISCUSSION

A rapid and steady decrease in mycofloral incidence of *Alternaria* spp., *Fusarium* spp. and *Cladosporium* spp. resulted in drastic fall of total mycoflora incidence on seed surface (Table 1). Among the four species of *Aspergillus* i.e., *A. candidus*, *A. niger*, *A. flavus* and *A. fumigatus*, the population of *A. flavus* however, increased from 1.0 - 5.0 percent during one year of storage. Incidence of *A. fumigatus* was absent initially, rose to a mean of 1.0 percent at the end of one year storage. The incidence of *Rhizopus* remained constant

throughout the storage period. The bacterial count decreased from 40 percent at the time of packing to 12.8 percent at the end of one year storage. A steady increase in incidence of *A. flavus* and *Penicillium* spp. was observed. *Alternaria tenuis* was the most prevalent (28.5 percent) in the beginning, which fell to 11.8 percent at the end of one year storage. The modified atmosphere was found effective in controlling the incidence of pathogen during storage, whereas, total seed mycoflora came down from 83.5 to 70 percent in case of control, it fell to 40 percent in MA₃ at the end of one year storage. A steady decline in total fungi was primarily due to drastic fall in *Alternaria* spp. An increase in *A. flavus* and *Penicillium* spp. with increase in duration of storage period was observed. Some other sterile mycelia were also observed during the study, but no

Table 1. Pathogen incidence (%) on cauliflower seeds during storage in modified atmosphere

Pathogen/ Fungi/ Bacteria	Pusa Deepali									
	Pusa Synthetic									
	Period of storage (months)									
	0	3	6	9	12	0	3	6	9	12
<i>Alternaria tenuis</i>	28.5	25.1	14.4	13.4	11.2	21.5	18.0	11.5	1.7	2.4
<i>A. brassicae</i>	0.5	0.3	0.0	0.0	0.3	0.5	0.1	0.2	0.0	0.0
<i>A. brassicicola</i>	1.5	3.8	0.0	0.1	0.0	2.0	1.2	0.0	0.0	0.0
<i>Aspergillus candidus</i>	1.0	0.0	0.1	0.0	0.1	0.5	0.0	0.0	0.0	0.0
<i>A. flavus</i>	1.0	3.0	3.4	2.0	5.0	1.5	0.8	3.0	1.5	3.6
<i>A. niger</i>	1.0	0.2	1.3	0.9	1.1	1.0	0.1	0.4	0.1	0.1
<i>A. fumigatus</i>	0.0	0.3	1.0	0.6	0.5	0.5	1.0	0.3	0.0	0.0
<i>Cladosporium</i> spp.	2.0	0.6	0.1	0.7	0.6	0.0	1.3	0.0	0.0	0.1
<i>Fusarium</i> spp.	1.5	0.1	0.1	0.9	0.3	0.5	0.3	0.1	0.0	0.0
<i>Rhizopus</i> spp.	5.5	4.2	6.3	4.2	5.3	3.0	0.5	3.1	1.7	2.7
<i>Penicillium</i> spp.	0.0	0.3	3.0	5.1	3.5	0.0	0.4	2.7	2.1	3.7
Fungi	42.5	37.8	29.6	27.8	27.8	31.0	23.8	21.3	7.2	12.6
Sterile mycelia	1.0	1.2	3.6	2.3	3.8	1.0	1.5	2.0	0.7	0.9
Bacteria	40.0	34.4	25.9	23.5	12.8	57.0	40.1	33.3	26.2	12.0
Total	83.5	73.5	59.1	53.6	44.5	89.0	65.4	56.7	34.1	25.5

constant pattern of increase or decrease was recorded.

In Pusa Synthetic, *Alternaria tenuis* was observed to be the most prevalent species of fungus at the start of seed storage (21.5 percent), but it gradually decreased to 2.4 percent at the end of one year storage. Similar to pattern of Pusa Deepali, total seed mycoflora incidence was recorded; it was 89 percent initially, which reduced to a mean of 25.5 percent at the end of one year storage. The fall was mainly due to reduction in bacterial incidence as well as that of *Alternaria tenuis*. The incidence of *Rhizopus spp.* and *Cladosporium spp.* remained constant throughout the storage period. The incidence of *Fusarium spp.*, *Alternaria brassicicola*, *A. brassicae*, *Aspergillus candidus* and *A. fumigatus* fell to zero at the end of six months storage, but a constant increase in the incidence of *A. flavus* and *Penicillium* was noted. Similar trend of some sterile mycelia were also observed during the storage but its increase or decrease was random. The seed mycoflora incidence reduced (89 percent to 70 percent) in control, whereas it fell down (89 percent to 25.5 percent) in case of MA₃. The seed moisture content as well as storage temperature did affect the pathogen incidence; but the effects of cold temperature during storage were more profound in reducing the seed-borne pathogen incidence than low seed moisture.

The present study comprised of 11 fungal species, besides bacteria were associated with cauliflower seeds. In the beginning, only 10 fungal species were detected in both the varieties and *Penicillium* was detected to build-up later on. *Alternaria tenuis* was the most predominant species throughout the storage. A difference in total fungal incidence was observed among the seeds of two varieties under study. The bacterial incidence was higher in Pusa Synthetic due to heavy rains before harvest

(Table 1). At the beginning of storage, the incidence of storage fungi was nominal but an increase in the incidence of these two storage fungi (*Penicillium spp.* and *Aspergillus spp.* viz., *A. candidus*, *A. niger*, *A. flavus* and *A. fumigatus*) was noted at the end of three months storage in both the varieties. Interestingly, the increase was higher in *Aspergillus flavus* and *A. fumigatus* but decrease in count of *A. niger* and *A. candidus* was observed. At the end of one year storage, incidence of fungi (e.g., *Alternaria brassicicola*, *Aspergillus niger* and *Fusarium spp.*) fell to zero in treatment of 5.5 percent seed moisture in Pusa Deepali.

It was observed that the incidence of fungi reduced under modified atmosphere with increased CO₂ concentration in during storage (Fig.1 and 2). The effects were more visible for *Alternaria tenuis*, *Aspergillus flavus*, *Rhizopus spp.*, *Penicillium spp.* and the bacteria. A lesser incidence of fungi as well as bacteria was observed in treatments with low seed moisture contents (Table 2). Similar trend was noticed under low temperature and ambient temperature storage. Although MA₃ reduced the incidence of *Penicillium spp.*, *Aspergillus flavus* and *Aspergillus niger*, but none of the treatments was able to check these fungi completely. However, a combination of MA₃, 5.5 percent moisture content and storage at low temperature resulted in the lowest fungal and bacterial count and *ipso facto* the total pathogen incidence (Table 2). *Rhizopus spp.* and sterile mycelia were present throughout the storage period, irrespective of temperature, moisture content and modified atmosphere. At low moisture content and temperature storage, the reduction in seed-borne mycoflora was faster than those at high moisture content and ambient storage.

A similar trend of 54.33 and 64 percent incidence of *Alternaria brassicae* and *A. brassicicola*, respectively was reported in

Table 2. Effects of seed moisture content and temperature on pathogen incidence (%) of cauliflower seeds under modified atmosphere during storage

TREATMENT	PATHOGEN INCIDENCE (%)	PUSA DEEPALI												PUSA SYNTHETIC											
		PERIOD OF STORAGE (MONTHS)												PERIOD OF STORAGE (MONTHS)											
		0	3	6	9	12	MEAN	0	3	6	9	12	MEAN	0	3	6	9	12	MEAN						
MOISTURE LEVEL I (5.5%)	FUNGI	33.4	20.3	23.9	24.6	25.6	33.4	32.9	17.6	13.0	12.2	18.9	32.9	0.7	3.0	2.4	4.1	2.6	0.7	1.7	2.2	0.9	1.5	1.6	1.7
	STERILE	28.1	29.7	20.2	9.5	21.9	28.1	34.0	31.8	26.9	10.5	25.8	34.0	28.1	29.7	20.2	9.5	21.9	28.1	34.0	31.8	26.9	10.5	25.8	34.0
	MYCELIA	62.2	53.0	46.4	38.1	49.9	62.2	68.5	51.6	40.8	24.2	46.3	68.5	62.2	53.0	46.4	38.1	49.9	62.2	68.5	51.6	40.8	24.2	46.3	68.5
	BACTERIA	38.1	38.9	31.8	31.2	35.0	38.1	27.6	25.0	24.6	13.0	22.5	27.6	38.1	38.9	31.8	31.2	35.0	38.1	27.6	25.0	24.6	13.0	22.5	27.6
MOISTURE LEVEL II (7.5%)	TOTAL	1.8	4.3	2.2	3.6	3.0	1.8	1.4	1.9	2.3	1.4	1.7	1.4	1.8	4.3	2.2	3.6	3.0	1.8	1.4	1.9	2.3	1.4	1.7	1.4
	FUNGI	40.8	22.0	26.8	16.2	26.5	40.8	46.3	34.8	25.4	13.4	30.0	46.3	40.8	22.0	26.8	16.2	26.5	40.8	46.3	34.8	25.4	13.4	30.0	46.3
	STERILE	16.0	24.1	17.9	15.4	18.4	16.0	26.8	15.6	14.4	1.4	17.1	26.8	16.0	24.1	17.9	15.4	18.4	16.0	26.8	15.6	14.4	1.4	17.1	26.8
	MYCELIA	0.3	2.8	1.5	1.2	1.5	0.3	1.2	1.3	1.4	1.8	1.4	1.2	0.3	2.8	1.5	1.2	1.5	0.3	1.2	1.3	1.4	1.8	1.4	1.2
TEMPERATURE LEVEL I (15°C)	BACTERIA	41.5	19.0	18.7	11.6	22.7	41.5	24.0	27.4	19.9	9.3	20.1	24.0	41.5	19.0	18.7	11.6	22.7	41.5	24.0	27.4	19.9	9.3	20.1	24.0
	TOTAL	57.9	45.8	38.1	28.1	42.5	57.9	52.0	44.2	35.7	22.6	38.6	52.0	57.9	45.8	38.1	28.1	42.5	57.9	52.0	44.2	35.7	22.6	38.6	52.0
	FUNGI	55.5	35.2	37.8	40.4	42.2	55.5	33.7	27.0	23.2	13.8	24.4	33.7	55.5	35.2	37.8	40.4	42.2	55.5	33.7	27.0	23.2	13.8	24.4	33.7
	STERILE	2.1	4.5	3.1	6.5	4.1	2.1	1.8	2.8	1.8	1.0	1.9	1.8	2.1	4.5	3.1	6.5	4.1	2.1	1.8	2.8	1.8	1.0	1.9	1.8
TEMPERATURE LEVEL II (15-30°C)	MYCELIA	27.3	32.7	28.3	14.1	25.6	27.3	56.3	39.3	32.4	14.6	35.6	56.3	27.3	32.7	28.3	14.1	25.6	27.3	56.3	39.3	32.4	14.6	35.6	56.3
	BACTERIA	84.9	72.4	69.2	60.9	71.9	84.9	91.8	69.1	57.3	29.4	61.9	91.8	84.9	72.4	69.2	60.9	71.9	84.9	91.8	69.1	57.3	29.4	61.9	91.8
	TOTAL																								

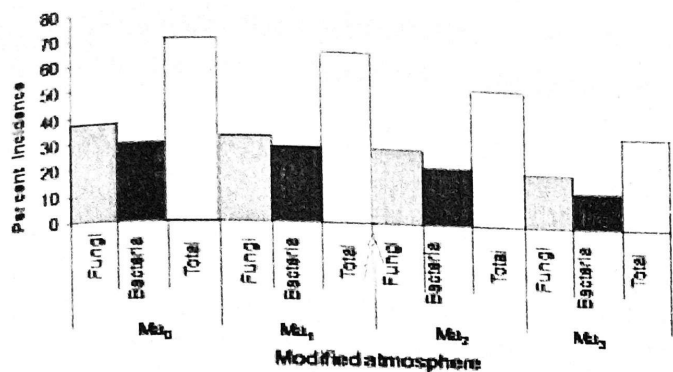


Fig. 1. Effects of modified atmosphere on pathogen incidence on cauliflower var. Pusa Deepali seeds in Storage.

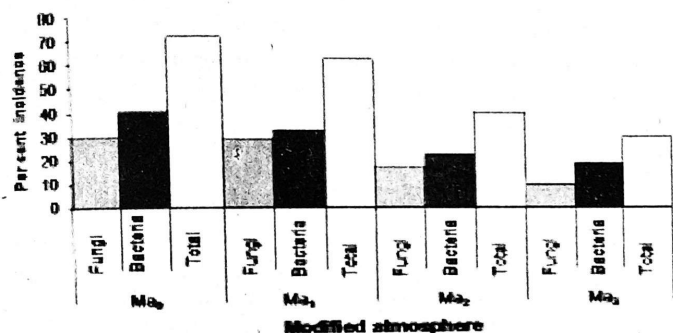


Fig. 2. Effects of modified atmosphere on pathogen incidence on cauliflower var. Pusa Synthetic seeds in storage

untreated seeds of cauliflower [2]. The modified atmosphere treatments did not have detrimental effect on germination of sorghum and soybean seeds (8, 12 and 16 percent moisture content) at the end of 8 months storage with 20, 35 and 90 percent CO₂ concentration. Further, a reduction in fungal count in MA storage was also reported [3-4].

It is evident from the present study that modified atmosphere containing 20 percent

CO₂ and 2 percent O₂ (MA₃) is capable of controlling the incidence of seed microflora. At the same time, it was also clear that low seed moisture content results in lowering of seed-borne microflora, making the modified atmosphere more effective. Therefore, it can be concluded that hypercarbic atmosphere (20 percent CO₂) compounded with hypoxic condition (2 percent O₂) is highly efficient in controlling fungal infestation. However, there was a certain amount of increase in *Aspergillus* spp. as well as *Penicillium* spp. in all investigated modified atmospheres.

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