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Effect of Modified Atmosphere on Seed Mycoflora in Cauliflower Seeds

P.R. KUMAR, S.N. SINHA, S.K. LAL[1], SHIV K. YADAV[1], S.S. SINGH AND ANUJA GUPTA[2]

IARI Regional Station, Pusa, Bihar 848 125
ourpk@gmail.com

ABSTRACT The modified atmosphere (MA) containing 20% CO_2 and 2% O_2 is capable of controlling the incidence of seed mycoflora (fungal infestation) in cauliflower seeds. Low seed moisture contents kept seed-borne mycoflora low, by making the modified atmosphere more effective. Hence, it was concluded that hypercarbic atmosphere (20% CO_2) compounded with hypoxic condition (2% O_2) is highly efficient in controlling fungal infestation. However, an increase in amount of *Aspergillus* spp. as well as *Penicillium* spp. in modified atmospheres was noticed.

Key words: Modified atmosphere, seed storage, mycoflora, cauliflower

Fungal infestation causes sevre damage during seed storage which is manifested as reduced germinability, production of abnormal seedlings, heating and mustiness, discolouration, 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* spp., *Aspergillus* spp.) and fumonisins (*Fusarium moniliforme* and *Alternaria alternata*) in stored grains deserves serious attention from the view point of seed as well as feed.

Cauliflower is cultivated in 2.4 lakh ha area in India and around 564 tonnes of its seed is used for sowing annually all over the world. Nearly 240 tonnes of seeds are subjected to storage for varying periods in different agro-climatic zones in India. Various factors, like microorganisms can 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 (MA_1 = 5% CO_2, MA_2 = 10% CO_2 and MA_3 = 20% CO_2), a fixed level of O_2 (2%) and rest N_2. The treatments were compared with the control, *i.e.* MA_0 = normal air. The seed samples were divided into two equal halves and conditioned to moisture levels of 5.5 and 7.5 per cent, respectively. Each conditioned sub-samples was stored at chosen temperature regimes, *viz.* cold (5°C) and ambient (15°-30°C). Prior to packing of seeds in modified atmosphere and at every 3 months interval from starting of seed storage until completion of 12 months, the pathogen incidence was recorded using ISTA deep freeze method [1]. The observations were

[1]Division of Seed Science and Technology, IARI, New Delhi 110 012
[2]IARI Regional Station, Karnal 132 001

recorded on all fungal species, sterile mycelia, bacteria and total seed 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 seed mycoflora (Table 1). Among the four species of *Aspergillus* (*i.e. A. candidus, A. niger, A. flavus* and *A. fumigates*), the population of *A. flavus* increased (1.0-5.0%) during one year of storage. Incidence of *A. fumigatus*, though absent initially, rose to 1.0 per cent at the end of one-year storage. The incidence of *Rhizopus* spp. remained constant throughout the storage. The bacterial count decreased (40-12.8%) from the time of packing to the end of one-year storage. A steady increase of *A. flavus* and *Penicillium* spp. was observed. *Alternaria tenuis* was the most prevalent (28.5%) in the beginning, but reduced to 11.8 per cent at the end of one-year storage.

The modified atmosphere was effective in controlling the incidence of pathogen during storage. Total seed mycoflora in control, came down (83.5-70%), whereas it reduced to 40 per cent in MA_3 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 was observed. Some other sterile mycelia were observed during the study, but no constant pattern of increase or decrease was recorded for the same.

In Pusa Synthetic, *Alternaria tenuis* was observed to be the most prevalent species of fungus (21.5%) at the starting of seed storage but it gradually decreased (2.4%) at the end of one-year storage. Similar pattern was recorded for Pusa Deepali, for total seed mycoflora incidence, it was 89 per cent initially, which reduced to 25.5 per cent. 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* reduced to zero at the end of six months during storage, but a constant increase in the incidence of *A. flavus* and *Penicillium* spp. was noted. Similar trend of some sterile mycelia were observed during the storage but its increase or decrease was random. The seed mycoflora reduction was (89-70%) in the control, whereas it was (89-25.5%) in MA_3. The seed moisture content and storage temperature both affected the infestation incidence; however, the effects of cold temperature was considerable (in reducing seed-borne infestation) than that of low seed moisture content.

The present study comprised 11 fungal species, besides bacteria that were associated with cauliflower seeds. In the beginning, only 10 fungal species were detected in both the varieties and *Penicillium* spp. was detected later. *Alternaria tenius* was the most predominant species throughout the storage. A difference in total fungal incidence was observed among seeds of two varieties. The bacterial incidence was higher in Pusa Synthetic due to heavy rains before harvesting (Table 1). At the starting of seed storage, the incidence of storage fungi was nominal, but an increase in the incidence of 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 the seeds with 5.5 per cent seed moisture in Pusa Deepali.

It was observed that the incidence of fungi reduced under modified atmosphere increased CO_2 concentration during storage (Figs 1, 2). The effects were more visible for *Alternaria tenius, Aspergillus flavus, Rhizopus* spp., *Penicillium* spp. and bacteria. A lesser incidence of fungi as well as bacteria was observed in treatments with low seed moisture contents. Similar trend was noticed under low temperature and under ambient storage. Although MA_3 reduced the incidence of *Penicillium* spp., *Aspergillus flavus* and *Aspergillus niger*, but none of the treatments was able to

Table 1. Seed mycoflora/bacteria incidence (%) under modified atmosphere during storage in cauliflower seeds

Pathogen/fungi/bacteria	Period of storage (month)									
	Pusa Deepali					Pusa Synthetic				
	0	3	6	9	12	0	3	6	9	12
Alternaria tenuis	28.5	25.1	14.4	13.4	11.2	21.5	18.0	11.5	1.7	2.4
A. brassicae	0.5	0.3	0.0	0.0	0.3	0.5	0.1	0.2	0.0	0.0
A. brassicicola	1.5	3.8	0.0	0.1	0.0	2.0	1.2	0.0	0.0	0.0
Aspergillus candidus	1.0	0.0	0.1	0.0	0.1	0.5	0.0	0.0	0.0	0.0
A. flavus	1.0	3.0	3.4	2.0	5.0	1.5	0.8	3.0	1.5	3.6
A. niger	1.0	0.2	1.3	0.9	1.1	1.0	0.1	0.4	0.1	0.1
A. fumigatus	0.0	0.3	1.0	0.6	0.5	0.5	1.0	0.3	0.0	0.0
Cladosporium spp.	2.0	0.6	0.1	0.7	0.6	0.0	1.3	0.0	0.0	0.1
Fusarium spp.	1.5	0.1	0.1	0.9	0.3	0.5	0.3	0.1	0.0	0.0
Rhizopus spp.	5.5	4.2	6.3	4.2	5.3	3.0	0.5	3.1	1.7	2.7
Penicillium 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

check these fungi completely. However, a combination of MA_3 with 5.5 per cent moisture content of seeds during storage at low temperature resulted in the least fungal and bacterial count. *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 during 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 per cent incidence of *A. brassicae* and *A. brassicicola*, respectively was reported in untreated seeds of cauliflower [2]. The modified atmosphere treatments did not have deterimental effects on germination as observed by subjecting sorghum and soybean seeds (8%, 12% and 16% mc) at the end of 8 month storage with 20, 35 and 90 per cent CO_2 concentration. Rathi *et al.* [3] also reported reduction in fungal count in MA storage.

It is evident from the present study that modified atmosphere containing 20 per cent CO_2 and 2 per cent O_2 (MA_3) is capable of controlling the incidence of seed-mycoflora. At the same time, low seed moisture content results in lowering of seed-borne mycoflora, making the modified atmosphere more effective. Therefore, it can be concluded that hypercarbic atmosphere (20% CO_2) compounded with hypoxic condition (2% O_2) is highly efficient in controlling fungal infestation. However, there was a certain amount

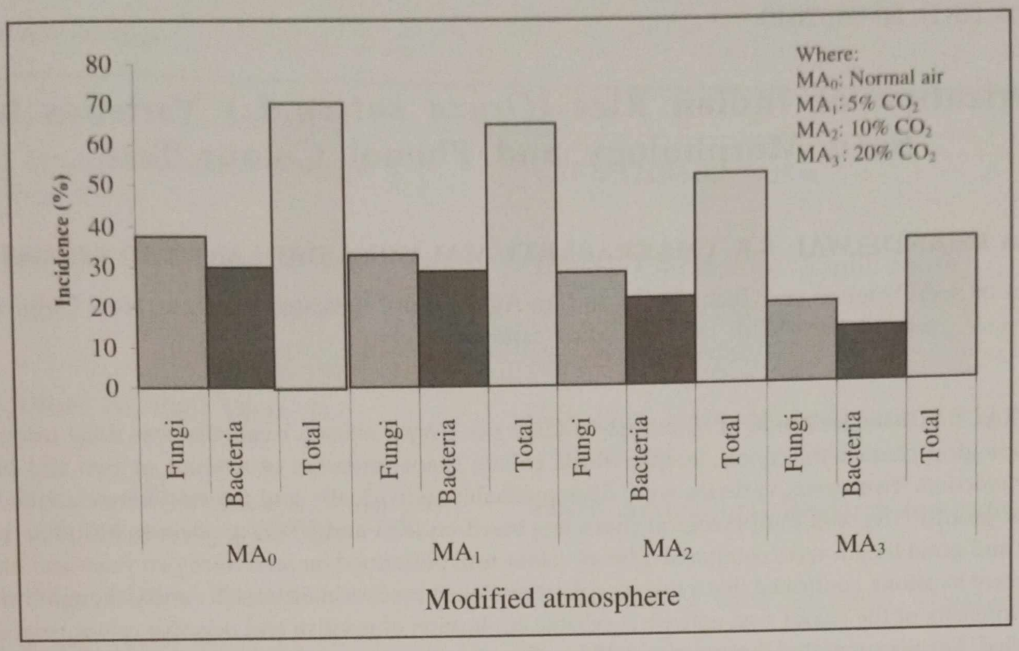

Fig. 1. Effect of modified atmosphere during storage on seed mycofora incidence in cauliflower var. Pusa Deepali

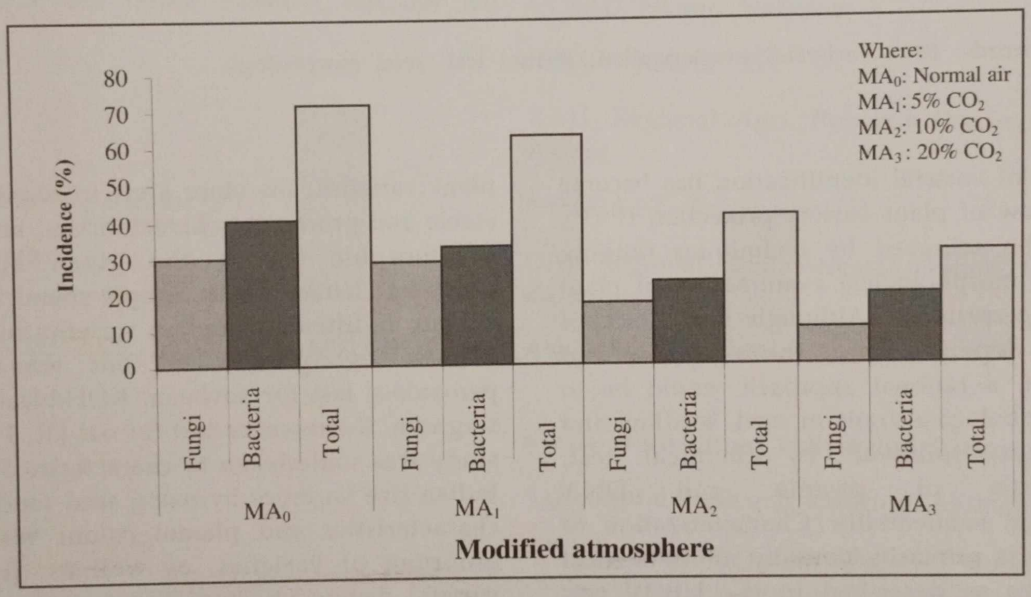

Fig. 2. Effect of modified atmosphere during storage on seed mycofora incidence in cauliflower var. Pusa Synthetic

of increase in *Aspergillus* spp. as well as *Penicillium* spp. in all investigated modified atmospheres.

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