EFFECT OF STORAGE CONTAINERS ON THE PERCENT INCIDENCE OF *ASPERGILLUS* SPECIES

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ABSTRACT:

The aim of present study is carried out to know the effect of storage containers on the percent incidence of *Aspergillus* species. Association of different species of *Aspergillus* with the seeds has been reported to cause decrease in percent germination of seeds, seed discoloration, heating and mustiness, loss of seed weight, seed rotting, biochemical changes, production of mycotoxins, etc. Storage containers play an important role in preventing or multiplying the original seed mycoflora in stored seeds of different crops. In order to confirm the seeds were stored in irrespective containers at room temperature for the period of three months. The seeds were analysed for their load of *Aspergillus* species by using Potato Dextrose Agar plates. Different *Aspergillus* species, isolated from seeds of jowar and groundnut. The seeds of both the crops (jowar and groundnut) showed maximum incidence of *Aspergillus* species that were stored in the tin boxes followed by polythene bags while, the seeds stored in cloth bags and gunny bags yielded less number of *Aspergillus* species.

KEY WORDS: ***Aspergillus species,* Potato Dextrose Agar, stored containers, stored seeds**

INTRODUCTION:

The isolation and identification of species of *Aspergillus* for which seeds of cereals, pulses, oil seeds, etc. were collected both from fields as well as from various market places. In all 14 species of *Aspergillus* with numerous strains were isolated by using both blotter and agar medium as recommended by ISTA (1966). The species like *A. flavus, A. niger, A, ruber, A. fumigatus* were found to be very common on all types of seeds used for isolation. While, species like *A. sulphureus, A. glaucus* and *A. japonicus* exhibited their association only with the seeds of some particular crops. The number of *Aspergillus* species appeared more on storage seeds than on the freshly collected seeds. Many *Aspergillus* species on the seeds showed their association with the other moulds like species of *Alternaria, Dreschleria, Fusarium, Curvularia* and *Cladosporium* while, poor incidence of *Aspergillus* was noted on the seeds which showed of *Rhizoctonia, Syncephalostrum, Penicillum, Trichoderma* and Chaetomium. Seeds of most plant

species may be safely stored for several months by carful control of temperature and relative humidity (Lacerda *et al*., 2003 and Chattha *et al.*, 2012). During storage, seed quality can remain at the initial level or decline to a level that may make the seed unacceptable for planting purpose, what is related to many determinants: environment conditions during seed production, pests, diseases, seed oil content, seed moisture content, mechanical damages of seed in processing, storage longevity, packaging, pesticides, air temperature and relative air humidity in storage, biochemical injury of seed tissue (Al-Yahya, 2001; Šimic *et al*., 2004; Guberac *et al*., 2003; Heatherly and Elmore, 2004). Storage fungi i.e., *Aspergillus* spp. and *Penicillium* can grow in stored grain under bad storage conditions and cause serious losses (Mehrotra, 1983).

It is clear from the literature that *Aspergilli* are mainly storage fungi and the results given in table 8 are in support of this fact. As load of *Aspergilli* was found to be increased with increase in storage period and also with the type of storage containers. The storage containers like tin box and polythene bags supported maximum multiplication of *Aspergillus* population as compared to the same in gunny bags. Hence, it can be conducted that by using a proper storage containers population of *Aspergilli* can be controlled successfully without the aid of chemicals. Similarly, observations regarding use of chemicals as seed dressers for the control of *Aspergilli* are found to be interesting. The present study aimed to determine the effect of storage conditions in combination with packaging materials on the incidence of storage fungi.

MATERIALS AND METHODS:

Isolation of *Aspergilli*

1) Collection of Seed Samples:

The method described by Neergaard (1973) has been adopted for the collection of seed samples. Accordingly seed samples were collected from field, store houses and market places and from farmers. A composite sample was prepared by mixing the individual sample together, preserved in cloth bags at room temperature during the studies.

i) Detection and Identification of Seed Borne Fungi from Stored Plant Seeds by Blotter Plate Method:

A pair of white blotter paper of 8.5 cm diameter was jointly soaked in sterile distilled water, placed in pre-sterilized corning Petriplates of 10 cm diameter. Ten seeds per plate were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25 ± 2^0C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

ii) Detection and Identification of Seed Borne Fungi by Agar Plate Method:

In this method, pre-sterilized corning glass Petri-plates of 10 cm diameter were poured with 25 ml of autoclaved water Agar (WA) medium. On cooling the medium, 10 seeds per plate were equispaced aseptically. One hundred seeds were tested for each treatment. The plates were incubated at 25 ± 2^0C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus*. Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

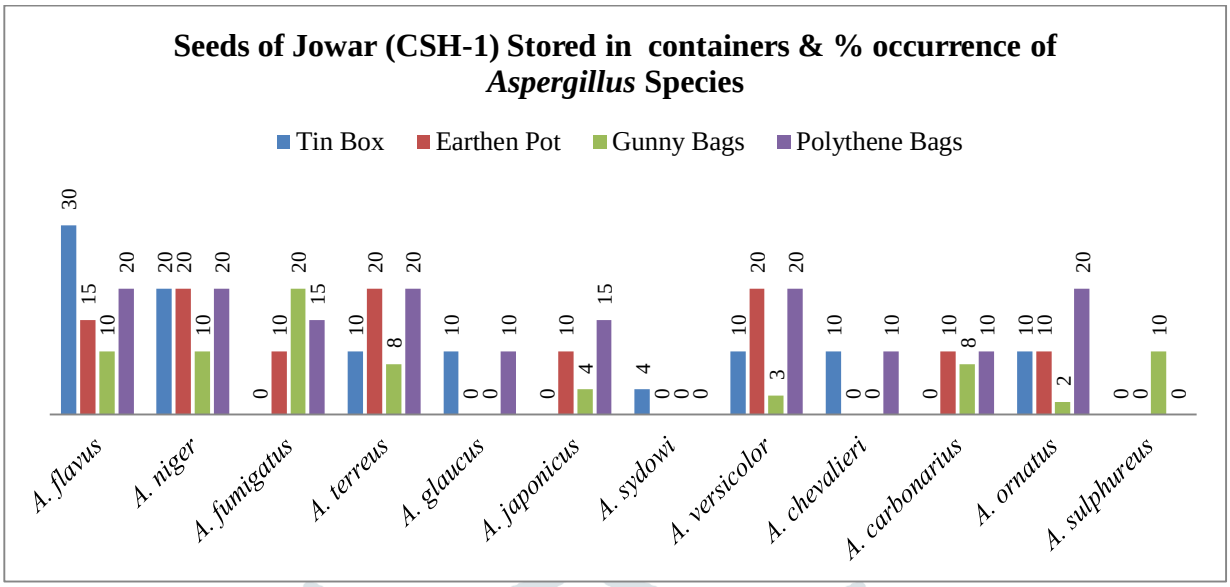
In order to isolate only internal seed mycoflora, seeds were pre-sterilized with 0.1% solution of mercuric chloride for 1 minute. Subsequently, thoroughly washed twice with sterile distilled water and placed on agar plates, blotter plate and water agar plates. Seeds without any such pre-treatments were employed for the total seed mycoflora (control). For blotter test, agar plate and water agar plate methods was followed as described by International Seed Testing Association, ISTA (1966) De Tempe (1970), Neergaard (1973) and Agarwal (1976).

RESULTS AND DISCUSSIONS:

Table 1: Effect of Storage Containers on the Percent Incidence of *Aspergillus* Species after 3 Months

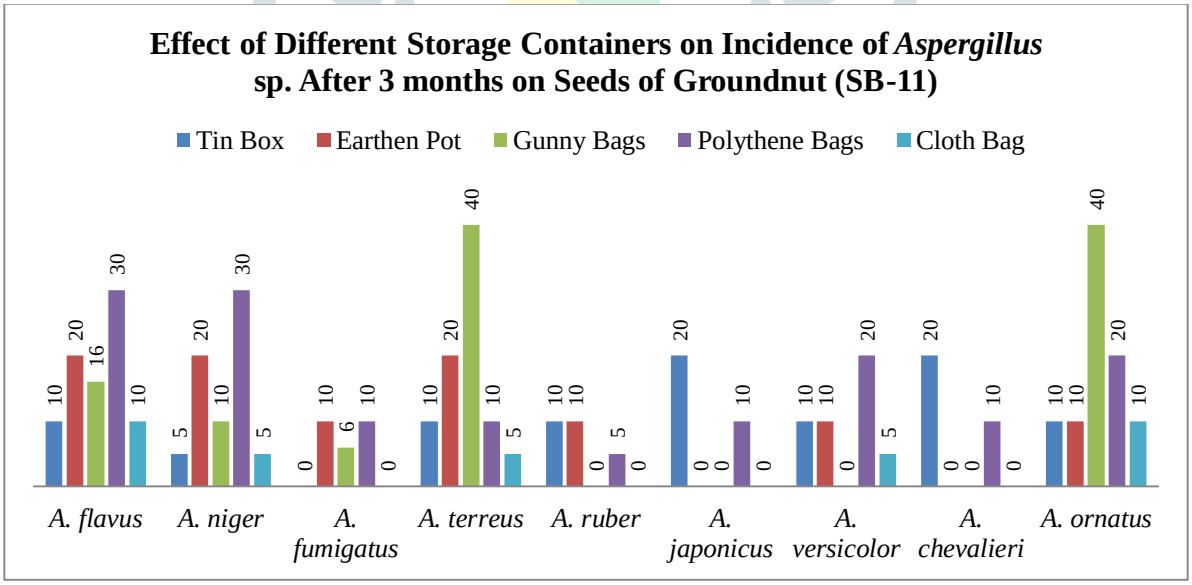
Sr. No.	*Aspergillus* Species Isolated	Seeds of Jowar (CSH-1) Stored in containers & % occurrence of *Aspergillus* Species			
		Tin Box	Earthen Pot	Gunny Bags	Polythene Bags
1	*A. flavus*	30	15	10	20
2	*A. niger*	20	20	10	20
3	*A. fumigatus*	-	10	20	15
4	*A. terreus*	10	20	08	20
5	*A. glaucus*	10	-	-	10
6	*A. japonicus*	-	10	04	15
7	*A. sydowi*	04	-	-	-
8	*A. versicolor*	10	20	03	20
9	*A. chevalieri*	10	-	-	10
10	*A. carbonarius*	-	10	08	10
11	*A. ornatus*	10	10	02	20
12	*A. sulphureus*	-	-	10	-

Graph No 1: Effect of Different Storage Containers on Incidence of *Aspergillus* sp. After 3 months on Seeds of Jowar (CSH-1)


Seeds of Jowar (CSH-1) Stored in containers & % occurrence of Aspergillus Species
Tin Box
Earthen Pot
Gunny Bags
Polythene Bags
30 15 10 20
20 20 10 20
0 10 20 15
10 20 8 20
10 0 0 10
0 10 4 15
4 0 0 0
10 20 3 20
10 0 0 10
0 10 8 10
10 10 2 20
0 0 10 0
A. flavus
A. niger
A. fumigatus
A. terreus
A. glaucus
A. japonicus
A. sydowi
A. versicolor
A. chevalieri
A. carbonarius
A. ornatus
A. sulphureus

Sr. No.	*Aspergillus* Species Isolated	Seeds of Groundnut (SB-11) Stored in				
		Tin Box	Earthen Pot	Gunny Bags	Polythene Bags	Cloth Bag
1	*A. flavus*	10	20	16	30	10
2	*A. niger*	05	20	10	30	05
3	*A. fumigatus*	-	10	06	10	-
4	*A. terreus*	10	20	40	10	05
5	*A. ruber*	10	10	-	05	-
6	*A. japonicus*	20	-	-	10	-
7	*A. versicolor*	10	10	-	20	05
8	*A. chevalieri*	20	-	-	10	-
9	*A. ornatus*	10	10	40	20	10

Graph- 2: Effect of Different Storage Containers on Incidence of *Aspergillus* sp. After 3 months on Seeds of Groundnut (SB-11)



It is clear from the results summarised in **table 1 and graph 1 & 2** that the seeds of both the crops (jowar and groundnut) showed maximum incidence of *Aspergillus* species that were stored in the tin boxes followed by polythene bags while, the seeds stored in cloth bags

and gunny bags yielded less number of *Aspergillus* species. It is known from the literature that storage containers have their role in preventing or multiplying the original seed mycoflora in many cases. In order to confirm the same in case of different *Aspergillus* species, harvested mustard seeds of jowar and groundnut. The seeds were stored in irrespective containers at room temperature for the period of three months. After that the seeds were analysed for their load of *Aspergillus* species by using Potato Dextrose Agar plates.

A. flavus shows maximum incidence on seeds of jowar stored in tin box followed by polythene bags and whereas it was recorded that lowest percent incidence in gunny bags by *A. ruber*. A. sydowi was reported very low percent of incidence and only in tin box. No other containers supported growth of this species. A. sulphureus shows presence in lowest quantity on seeds of stored seeds of groundnut in gunny bags. The seeds stored in tin boxes and polythene bags favoured multiplication of *Aspergilli* more than the seeds stored in gunny bags.

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