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EFFECT OF INCUBATION TEMPERATUREANDSTORAGE CONTAINERS ON SEED MYCOFLORA FOR HERBAL DRUGS

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ABSTRACT

A preliminary survey of seed mycoflora was undertaken at four locations during the years 2018-2019. Seeds are used as medicine. These seeds are found to be frequently contaminated by fungi (Roy *et al*. 1988, Mamatha *et al*., 2000). Chaurasia (1990) investigated that almost all medicinal seed samples were associated with a large number of fungi. Some of these had heavy contamination of toxigenic Aspergillusflavus strains. The drug manufacturers without examining the raw drug samples from microbial association manufacture the finished herbal drugs. Therefore, it is essential to pay adequate attention to the effect of weather on medicinal seed mycoflora.

The storage methodology of seeds is found to be different at various places such as market, godowns, laboratories etc. They may be stored in gunny bags, tin box, glass bottles etc.

Keywords: Seed mycoflora, medicinal seeds

INTRODUCTION

Seeds of medicinal plants, like those of agricultural and horticultural crops, carry a wide variety of micro-organisms like fungi, bacteria and even some viruses. Seeds may be attacked by the microbes while still borne on the trees in the field, during storage and subsequent handling before use. Therefore, the study of effect of weather on mycoflora of medicinal seeds is essential. The number of fungi was reduced when the seeds were stored in cotton bags, tin boxes and plastic bottles in

descending order. Storing seeds in plastic bottle was found to eliminate maximum storage fungi from all the seed samples.

MATERIAL AND METHOD

In this method, pre-sterilized glass petriplates of 5 and 20 cm diameter were poured with 15 ml sterilized Potato Dextrose Agar (PDA) medium. On cooling the medium, seed per petriplate were placed at equal distance aseptically. Incubation conditions and other details were the same as described for blotter paper method.In order to distinguish internal seed borne mycoflora with that of external one, the seeds were pretreated with 0.1 % solution of $HgCl_2$ for 2 minutes and thoroughly washed twice with sterile distilled water and then placed on agar plates. Seeds without any such pre-treatment were employed for the study of seed mycoflora.

OBSERVATIONS:

When the seed mycoflora of medicinal plant was studied, it was found that the mycoflora increased in the seeds stored in gunny bags for 6 months **(Table 1)** than that of freshly collected seed. Therefore, an experiment was designed to store the seeds in different containers, for a period of 6 months. The seeds of medicinal plant were stored in cotton bags, polypropylene bags, polythene bags, tin box and plastic bottles, in addition to gunny bags which served on control. The bags used were of the size 30 x30 cm. The bags and tin boxes were used after sterilizing in an autoclave for this purpose. After putting the seeds in these containers, they were tied with cotton threads and stored at room temperature for 6 months. The seed mycoflora was assessed after 6 months storage and the results obtained are presented in **Table 1**.

The experiment on storage of medicinal seeds in different types of containers showed promising results. In case of *Azadirachta indica,* the number of fungi on the seeds was found to be reduced than control, when they were stored in different types of containers **(Table 1)**. When cotton bag was used for storage, two fungi viz., *Aspergillus parasiticus* and *Pythium indigoferae* were eliminated. In case of polypropelene bags *A. parasiticus, Necosmospora africana* and *Pythium indigoferae* were eliminated. When polythene bags were used, 5 fungi were eliminated, while 6 fungi were eliminated when tin box was used for storage. The most efficient container observed was plastic bottle, where 7 fungi were eliminated and only 2 fungi appeared as seed mycoflora; compared to 9 fungi in control (Gunny bags).

Table 1: Effect of different storage containers on the incidence of seed mycoflora of *Azadirachta indica*

Sr. No.	Fungi	Container					
		1	2	3	4	5	6
1	*Aspergillus carbonarius*	+	+	+	+	+	-
2	*A. niger*	+	+	+	+	+	+
3	*A. parasiticus*	+	-	-	-	-	-
4	*Cladosporium cladosporioides*	+	+	+	+	-	-
5	*Fusarium oxysporum*	+	+	+	+	-	-
6	*F. solani*	+	+	+	+	-	-
7	*Neocosmospora Africana*	+	+	-	-	-	-
8	*Pythium indigoferae*	+	-	-	-	-	-
9	*Rhizopus oryzae*	+	+	+	+	+	+
Total no. of Fungi		9	7	6	4	3	2

1-Gunny bags (control), 2- Cotton bag, 3- Polypropylene bag, 4- Polythene bag, 5-Tin box, 6- Plastic bottle

Effect of incubation temperature

In order to study the effect of incubation temperature on the occurrence of mycoflora, the seed of medicinal plant was tested at different incubation temperatures *viz.,* 10, 20, 25, 30 and 40°C the results obtained are presented in **Table 2**. It observed from table 2 that 3 fungi appeared at incubation temperature at 10°C on the seeds of *Azadirachta indica* 7 fungi were recorded at temperatures 20, 25, 30 and 40°C. The percent incidence of seed borne fungi was found to be maximum at 25°C, followed by 30°C. At 20° and 40°C less occurrence of seed mycoflora was noted. Therefore, it can be inferred that temperature range of 25°C to 30°C is optimum for the development of seed mycoflora and maximum appearance of fungi was at 25°C. Maximum percent incidence of seed mycoflora was of *Aspergillus niger* and *Rhizopus oryzae,* while minimum was of *Sporotrichum carnis.*

Table: 2 Effect of incubation temperature on percent incidence of seed borne fungi of *Azadirachta indica*

Sr. No.	Fungi	Temperature in °C				
		10	20	25	30	40
1	*Aspergilluscabonarius*	10	10	50	20	10
2	*A. nigers*	10	20	60	30	10
3	*A. parasiticus*	_	10	50	50	10
4	*Cladosporiumcladosporioides*	_	10	40	20	10
5	*Neocosmosporaafricana*	_	10	40	30	20
6	*Rhizopusoryzae*	10	20	60	30	10
7	*Sporotrichumcarnis*	_	10	30	20	10

Source: Field Survey

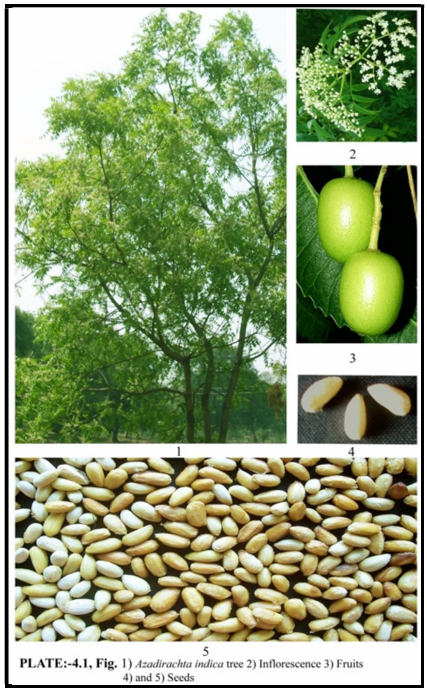

Fig. 1: ***Azadirachta indica***

RESULT

The plastic bottles served as best containers for the elimination of seed mycoflora, followed by tin box. Two fungi *viz., Pythium indigoferae* and *P. intermedium* were found to be eliminated in all the containers, compared to control. However, *Aspergillus flavus, A. niger* and *Rhizopusoryzae* were the storage fungi which were found to persist in all the containers, even after 6 months storage.

The numbers of fungi were reduced when the seeds stored in cotton bags, polypropylene bags, polythene bags, tin boxes and plastic bottles in descending order. Storing seeds in plastic bottles was found to eliminate maximum storage fungi on the seeds of all the plants tested. *Aspergillus niger* and *Rhizopus oryzae* were found to persist on all the medicinal seeds tested, even after storage for 6 months. It is interesting to note form the observations that different fungi have been totally eliminated in different medicinal plants, irrespective of the containers used. It is inferred from the data presented that incubation temperature of 25°C supported the isolation of maximum number of fungi on solid media, from the medicinal seeds studied.

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