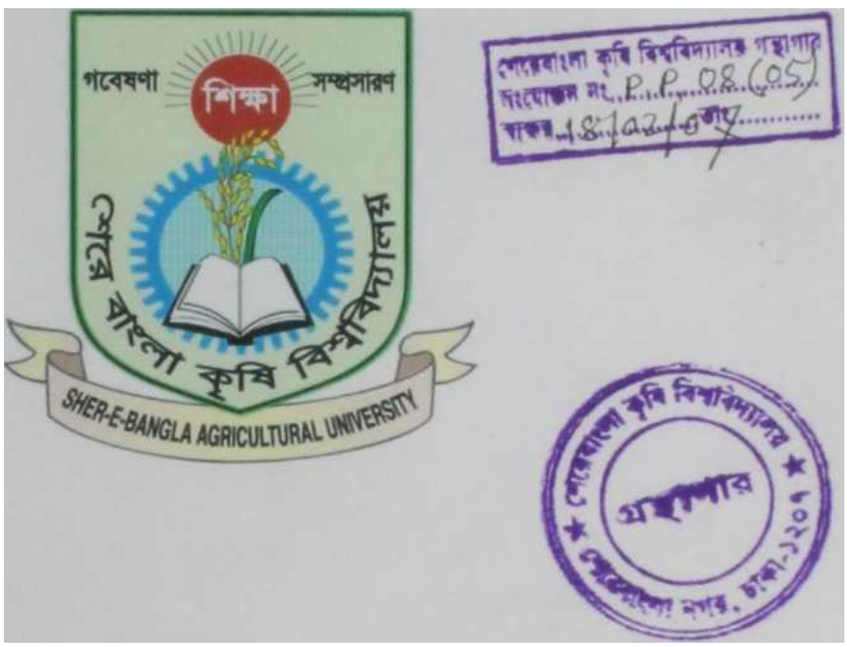
DETECTION OF SEED BORNE FUNGI OF SOME SELECTED MEDICINAL PLANTS

PRADIP KUMAR BISWAS



DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY,

DHAKA-1207

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DETECTION OF SEED BORNE FUNGI OF SOME SELECTED MEDICINAL PLANTS

By

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REGISTRATION NO. 00367

A Thesis

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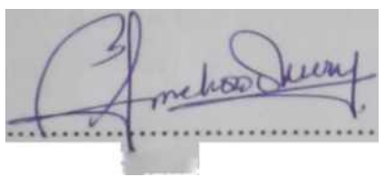

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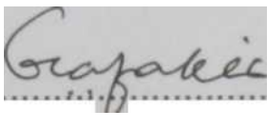

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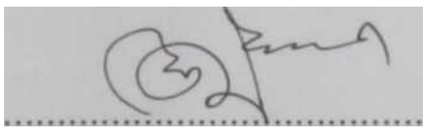

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This is to certify that the thesis entitled, *"DETECTION OF SEED BORNE FUNGE OF SOME SELECTED PLANTS"* submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in *PLANT PATHOLOGY,* embodies the result of a piece of *bona fide* research work carried out **by PEADIP KUMAR BISWAS**, Roll No. *00367* Registration No. *00367* under **my** supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by him.

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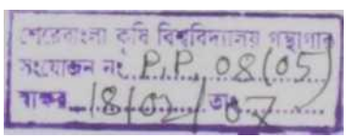

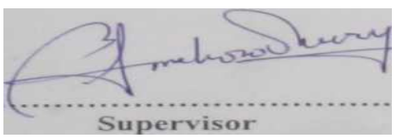

(Assist. Prof. M. Salahuddin M. Chowdhury)

Dedicated to
My Friend
Late Parvez Rana

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The Author

DETECTION OF SEED BORNE FUNGI OF SOME SELECTED MEDICINAL PLANTS

ABSTRACT

Occurrence of fungi in seeds of ten selected medicinal plants viz. Andrographis, Angle's trumpet, Asparagus, Butterfly pea, Changeable rose, Devil's cotton, Indigo, Jatropha, Licorice and Margosa tree were detected. Inspection of dry seeds revealed that the seed samples contained apparently healthy seeds, discolored seeds and shriveled and malformed seeds. The germination percentage was recorded higher in Changeable rose (88.50%) and lower in Margosa tree (41.00%) in blotter method. Germination percentage was recorded higher in blotter method than pot soil test. Fungi were detected by blotter method both with and without pre-treatment of seeds. $Hgcl_2$ (0.01%) solution was used for pre-treatment. Seven fungal species viz. *Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Penicillium sp and Rhizopus stolonifer* were recorded from without pre-treated seeds while only three species of fungi viz. *Alternaria tenuis, Curvularia lunata* and *Fusarium oxysporum*, were recorded from pretreated seeds. All these fungi are new records for seeds of medicinal plants in Bangladesh.

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Chapter 1

Introduction

INTRODUCTION

Plant provides us not only food, cloth and shelter but also supply many other requirements such as medicines, flavoring agents etc. For long time plant was the only source of our medicines for treating different diseases. Even today, hilly tribes of Bangladesh are dependent primarily on plants for medicines. Plants possess medicinal properties as they store secondary metabolites such as alkaloids and terpenoids, which have the ability to correct our health disorders. In the course of advancement in the field of modem medicines, which are mostly synthetic organic compounds, plant based medicines have been ignored. But in many cases synthetic medicines have been proved to have side effect, which plant based medicines do not possess. Plant based medicines are therefore gaining popularity in both developed and developing countries. Many foreign countries such as Thailand, China, and India are exploring their medicinal plant resources for their own use and also for earning foreign currency.

Bangladesh with a rich medicinal flora in its bounds has a scope of exploiting medicinal plants. It is pertinent to mention here that only 50 years back people were dependent to a great extent on herbal medicines, Kabiraji Ayurvedy and Unani method of treatment which were very common among the village people. Brahmmi shak was a common item at lunch which was considered to be effective for improving nervous system. Recently photochemical analysis revealed that the extract of this plant has chemicals, which can improve

nervous system and brain function. Unfortunately this plant has now become rare in Bangladesh.

Medicinal plants constitute an important natural wealth of our country. They play a significant role in providing primary health care services to rural people. They serve as important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines. Substantial amounts of foreign exchange can be saved and earn commercial production of medicinal plants.

Recently, the Government of Bangladesh has launched campaign to plant medicinal plant as it is a safe, side effectless, low cost and very effective sources of disease prevention and cure. Millions of village people can cultivate medicinal plants in their homestead garden which will give them benefit of chief medication as well as green plantation of agro-social forestry.

Since the medicinal plants grow mostly in natural forest and fallow lands many of them have become threatened. On the various factors for extinction of medicinal plants diseases so to say seed-borne diseases might be play an important role. Seeds of Agricultural, Horticultural and forest crops are vulnerable to attack by many seed-borne pathogens (Fakir, 2003 and Richardson, 1990). For successful plantation of medicinal plants, availability of disease free seeds is the key factor. But limited works has been done on diseases of medicinal plants particularly seed-borne diseases. Richardson (1990) listed some medicinal plants which attacked by a number of seed-borne

fungi, bacteria and viruses. He listed 30 fungi associated with seeds of different medicinal plants. No works has been done on seed-borne diseases especially fungi associated with the seeds of medicinal plants in the country.

In view of the above facts, the present study has been undertaken to detect fungi associated with the seeds of some selected medicinal plants grown in Bangladesh.

Chapter 2

Review of literature

REVIEW OF LITERATURE

Ten medicinal plants viz. *Abroma augusta Andrographis paniculata, Asparagus racemosus, Clitoria ternatea, Datura metel, Glycyrrhiza glabra, Hibiscus mutabilis, Indigofera tinctoria, Jatropha pandurifolia, Melia azadirachta* which have high medicinal value were selected for studying the prevalence of seed-borne fungi. Limited works have been conducted on the detection of seed borne fungi of these medicinal plants. However, few workers worked on detection of seed borne fungi of Calendula, Coriander, Cumin seed, fenugreek, etc which known to have some medicinal value. Some related literature have been cited in this section chronologically:

Kirkpatrick and Bazzaz (1979) found certain seed-borne fungi on Angl's Trumpet seeds and their influence on the colonizing herb *Datura stramonium.*

Gilberston and Manning (1980) isolated *F. moniliforme, F. oxysporum, F. solani* and *F. tricinctum* from both surface sterilized and unsterilized seeds of Asparagus. They also stated that *Fusarium moniliforme* and *Fusarium oxysporum* were pathogenic.

Richardson (1990) revealed that the seeds of *Datura metel* var. *muricata* was infected by *Alternaria crassa* and seedling with chlorotic cotyledons that reduces seed quality and causes pre-and post-emergence blight. He listed different fungi, bacteria and viruses associated with the seeds of some medicinal plants. They are as follows:

i mangold L ***iula)***	*aienauia ojjicinans*	**2.** *Fusarium sp* **3.** *Botrytis cinerea* **4.** *Curvularia pallescens* **5.** *Drechslera hawaiiensis*
hemp ;)	*Cannabis sativa*	**1.** *Botrytis infestans* **2.** *Fusarium spp* **3.** *Pseudomonas syringae pv. Cannabina* **4. Cannabis streak virus** **5. Virus (unidentified^**
foot 1 **saag)**	*Chenopodium album*	*1. Ascochyta hyalospora* *2.* **Viruses**
i seed)	*Cuminum cyminum*	**1.** *Alternaria burnsii* **2.** *Fusarium burnsii* **3.** *Pseudomonas cumini*
uda grass a)	*Cynadon dactylon*	*1. Claviceps cynodontis* *2. Cochliobolus spicifer* *3. Setosphaeria* *4. Ustilago spp*
imom hi)	*Elettaria cardamomum*	**1. Cardamom mosaic virus**

1. **Siinzr nur IU. [JKJI > ***

Sl. No.	***Common name***	***Scientific name***	***Seed-borne pathogen***
1.	*African mangold (Calendu*	Calendula officinalis	1.Alternaria porri 2.Fusarium sp *3.Botrytis cinerea* *4.Curvularia pallescens* *5.Drechslera hawaiiensis*
2.	Indian hemp (bhang)	Cannabis sativa	1.Botrytis infestans 2.Fusarium spp 3.Pseudomonas syringae pv. Cannabina 4.Cannabis streak virus 5.Virus (unidentified
3.	Goose foot (Bethu saag	Chenopodium album	*1.Ascochyta hyalospora* 2.Viruses
4.	Cumin seed (Jeera)	Cuminum cyminum	*1.Alternaria burnsii* *2.Fusarium burnsii* *3.Psdomonas cumini*
5.	Bermuda grass (durva	Cynadon dactylo	1.Claviceps cynodontis 2.Cochliobolus spicifer *3.Setosphaeria* 4.Ustilago spp
6.	Cardamom(Elachi)	*Elettaria* cardamomum	1.Cardamom mosaic virus

Sl. No.	***Common name***	***Scientific name***	***Seed-borne pathogen***
7.	Black cumin (Kaligira)	Nigella sativa	1.Aspergillus niger 2.Aspergillus flavus 3.Fusarium sp
8.	Sweet/Common basil (Babuitulsi)	Ocimum basilacum	*1.Botrytis cinerea*
9.	Poppy (Aphim)	Papaver somniferur	*1.Alternaria spp* *2.Peronospora arborescens*
10.	Ispaghul/Spogel seed (Isabgul)	Plantago ovata	*1.Peronospora plantaginis* *2.Arabis mosaic virus* *3.Tobacco mosaic virus* *4.Ditylenchus dipsaci* *5.Pythium ultimum*
11.	Red sandal wood (Raktqachanchan)	Pterocarpus santati	*1.Fusarium moniliforme* *2.Fusarium pallidoroseum* *3.Fusarium solani*
12.	Castor oil plant	Ricinus communis	1.Alternaria alternata 2.Alternaria compacta 3.Alternaria ricini 4.Cercospora ricinella 5.Fusarium spp 6.Sclerotinia ricini 7.Xanthomonas campestris pv. ric

Sl. No.	***Common name***	***Scientific name***	***Seed-borne pathogen***
13.	Fenugreek	*Trigonella* foenumgraecum	1. Cercospora traversiana 2. Cochliobolus hawaiiensis *3. Rhizoctonia solani* *4.Fusarium oxysporum* 5.Corynespora cassiicola

Chapter 3

Materials and Methods

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experimental period

The experiment was conducted during the period from January, 2006 to June, 2006.

3.3 Medicinal plant species included

The species of medicinal plants included for the present investigation were as follows:

No.	Local Name	English Name	Scientific Name
1	Kalomegh	Andrographis	*Andrographis paniculata*
2	Dutura	Angle's Trumpet	*Datura metel*
3	Shatamuli	Asparagus	*Asparagus racemosus*
4	Apragita	Butterfly pea	*Clitoria ternatea*
5	Sthalpadma	Changeable rose	*Hibiscus mutabilis*
6	Ulatkamble	Devil's cotton	*Abroma august a*
7	Nill	Indigo	*Indigofera tinctoria*
8	**Joy** tun	Jatropha	*Jatropha pandurifolia*
9	Jasthimudhu	Licorice	*Glycyrrhiza glabra*
10	**Neem**	Margosa tree	*Melia azadirachta*

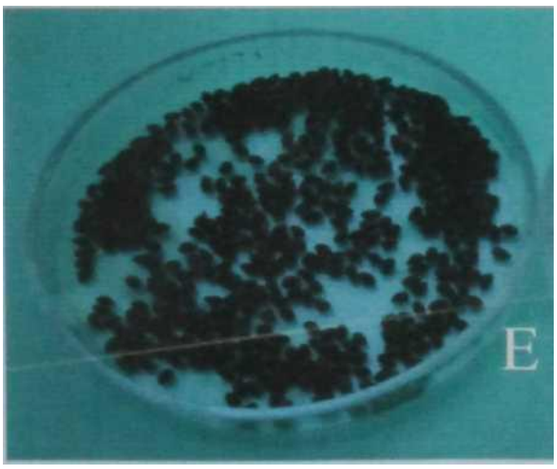
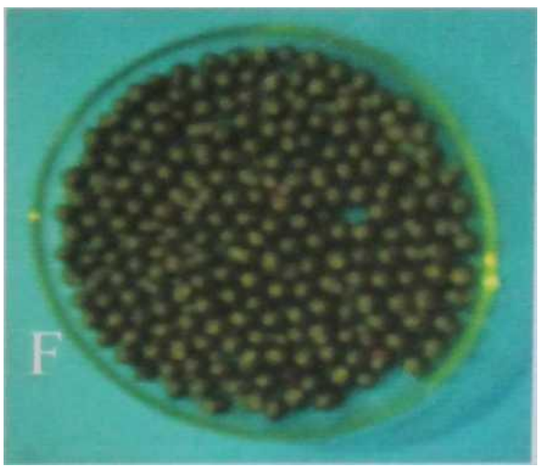


Plate 1. Seeds of different medicinal plants: A) Asparagus seeds, B) Jatropha seeds, C) Margosa Tree seeds, D) Butterfly seeds, E) Devil's cotton seeds and F) Licorice seeds



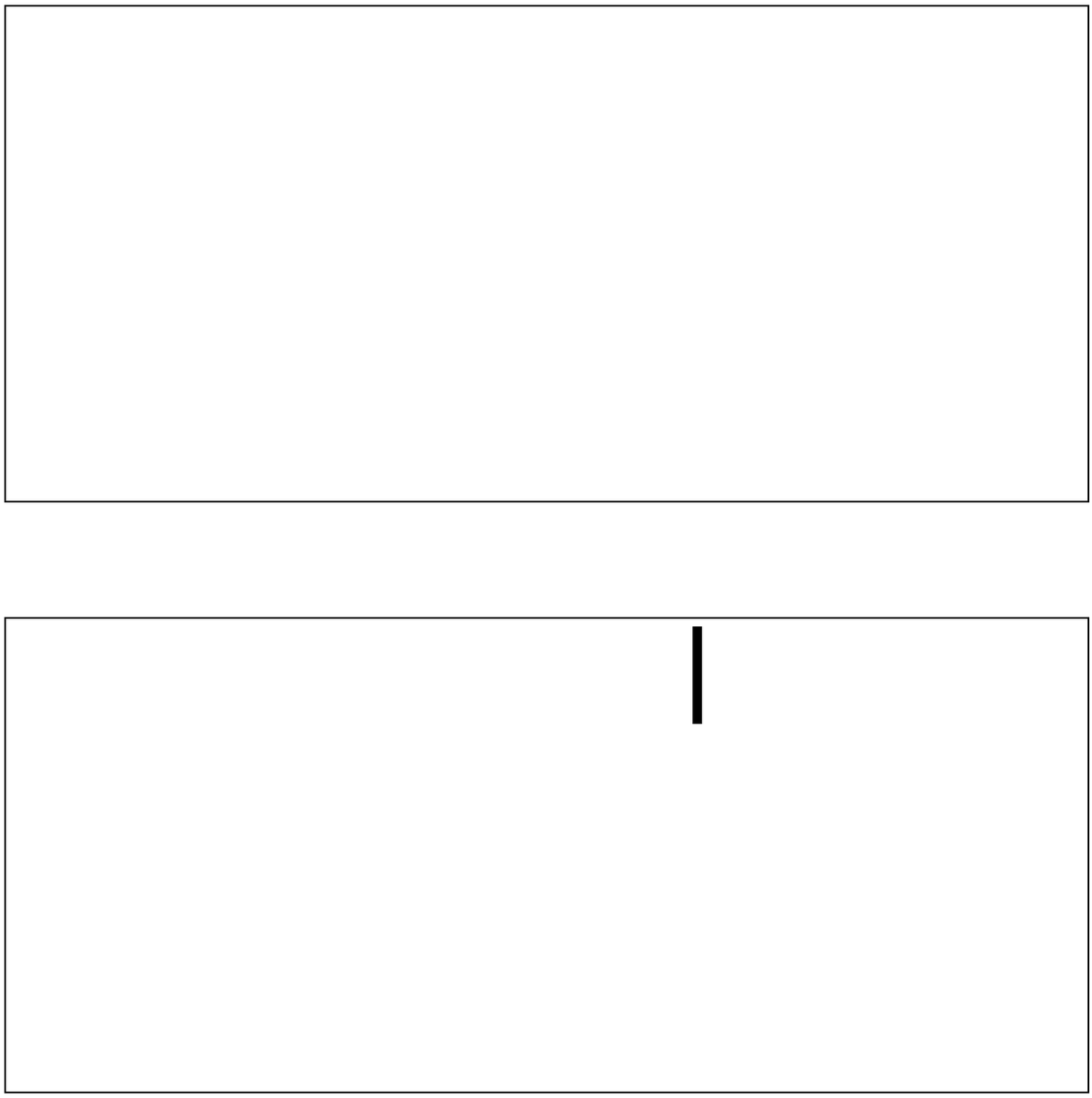


Plate 2. Seeds of different medicinal plants: G) Indigo seeds. H) Changeable seeds. I) Andrographis seeds and J) Angle's Trumpet seeds.

3.4 Collection of seed samples

The seeds of the ten selected medicinal plants were collected from the Agroforestry garden, Sher-e-Bangla Agricultural University, Dhaka-1207 (Plate 1 and 2). For each medicinal plant four hundred seeds were collected randomly. All the seeds were collected immediately after harvest of the crop planted in the Agroforestry garden, SAU, Dhaka. After collection, the seeds were kept in separate paper bags and stored in the refrigerator at 4±2°C in the Seed Health Laboratory.

3.5 Inspection of dry seeds

Inspection of dry seeds was done according to the International Rules of ISTA (1999). In this method, randomly 400 seeds were taken from each material and visually inspected and graded into three categories; i) Apparently healthy seeds; ii) Discolored seeds and iii) Shrivelled and malformed seeds. The seeds under each category were counted and calculated the percentages of each group. After recording the data of dry inspection, all the materials were kept to use in blotter test.

3.6. Detection of fungi

3.6.1 Blotter method

Blotter method test was done in petridishes following the International Rules of ISTA (1976). Two hundred seeds were taken randomly from each sample and were placed in petridishes (25 Or 10 seeds per petridish according to the size of the seeds). Both without pre-treated and pre-treated seeds were used in this

method. $HgCl_2$ solution (0.01%) was used as sterilizing agent in which seeds were dipped about one minute and then washed with sterile water and kept 5 minutes to dry up. Then both pre-treatcd and without pre-treated seeds were placed in petridishes. Before plating seeds, three pieces of Whateman no. 1 filter paper dipped in sterile water and then placed on each dish. The petridishes with seeds were then incubated in the incubator at 22 ± 2° C under an alternate cycle of 12 hours light and darkness in the Seed Health Laboratory, Department of Plant Pathology for 7 days. During incubation water was supplied as required. Seeds were examined after 7 days of incubation to detect the fungi. The fungi were identified by observing their growth characters on the incubated seeds in blotter method under stereomicroscope at 25 X magnification following the keys of Nath *et al.* (1970), Chidambaram *et al.* (1973), Ellis (1971), Benoit and Mathur (1970), Mathur and Cunfer (1993) and Mathur and Kongsdal (2000).

3.7 Germination test

Germination was recorded on blotter method prior to detection of seed-borne fungi. Germination percentage was also determined by pot soil test. Pot soil test has been done in the following procedures

3.7.1 Pot preparation

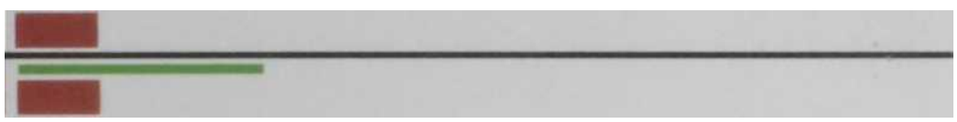
For this experiment soil was sterilized by 0.01% Formaldehyde for five days. After completion of the sterilization, the pots were filled with soil and placed in the net house of Sher-e-Bangla Agricultural University, Dhaka.

3.7.2 Sowing of seeds in pots

The seeds of 10 selected medicinal plants were sown in pots prepared by filling with sterilized soil. 25 seeds of each plant species were sown in each pot and for each species three replications were maintained. After 28 days of seed sowing, the germination percentage of ten selected medicinal plants was recorded.

Chapter 4

Results



RESULTS

4.1 Dry Inspection

The results of dry inspection of seeds of ten medicinal plants are presented in Table 1 and Plate 3-8. It was observed that three categories of seeds viz. i) Apparently healthy seeds, ii) Discolored seeds, and ii) Shrivelled and malformed seeds ranged from 91.00%- 97.50%, 1.50%-5.00% and 2.50%- 4.50%, respectively. The percentage of apparently healthy seeds were recorded in Andrographis (97.00%), Angle's trumpet (96.5%), Asparagus (96.50%), Butterfly pea (92.50%), Changeable Rose (97.50%), Devil's cotton (96.50%), Indigo (91.00%), Jatropha (95.50%), Licorice (97.00%) and Margosa tree (93.00%). All the seed samples of different plant species were found to have above 90% apparently healthy seeds.

Table 1. Dry inspection of the seed samples of ten selected medicinal plants SI.

No.	**Medicinal plant**	**Percent seed showing**		
		Apparently healthy	**Discolored**	**Shrivelled and malformed**
1	Andrographis	97.00	3.00	0.00
2	Angle's Trumpet	96.50	3.50	0.00
3	Asparagus	96.50	0.00	3.50
4	Butterfly pea	92.50	3.00	4.50
5	Changeable rose	97.50	0.00	2.50
6	Devil's cotton	96.50	0.00	3.50
7	Indigo	91.00	5.00	4.00
8	Jatropha	95.50	1.50	3.00
9	Licorice	97.00	0.00	3.00
10	Margosa tree	93.00	3.50	3.50

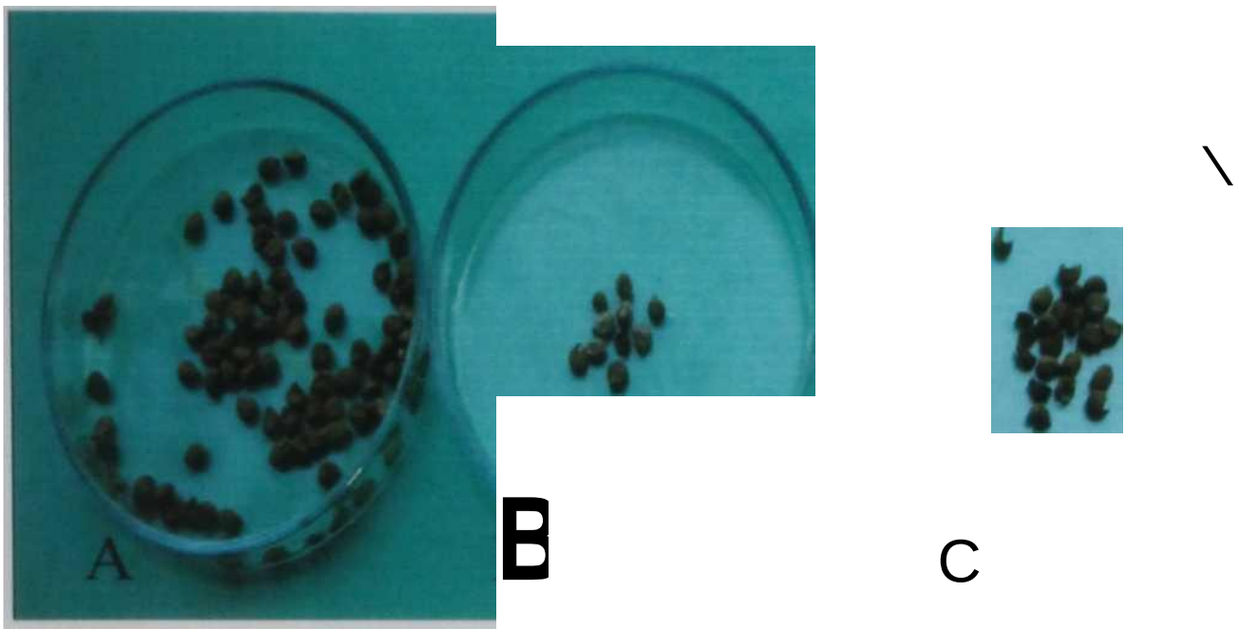


Plate 3. Morphological features of three categories of Jatropha seeds (L-R): A) Apparently healthy seeds. B) Discolored seeds and C) Shrivelled and malformed seeds.

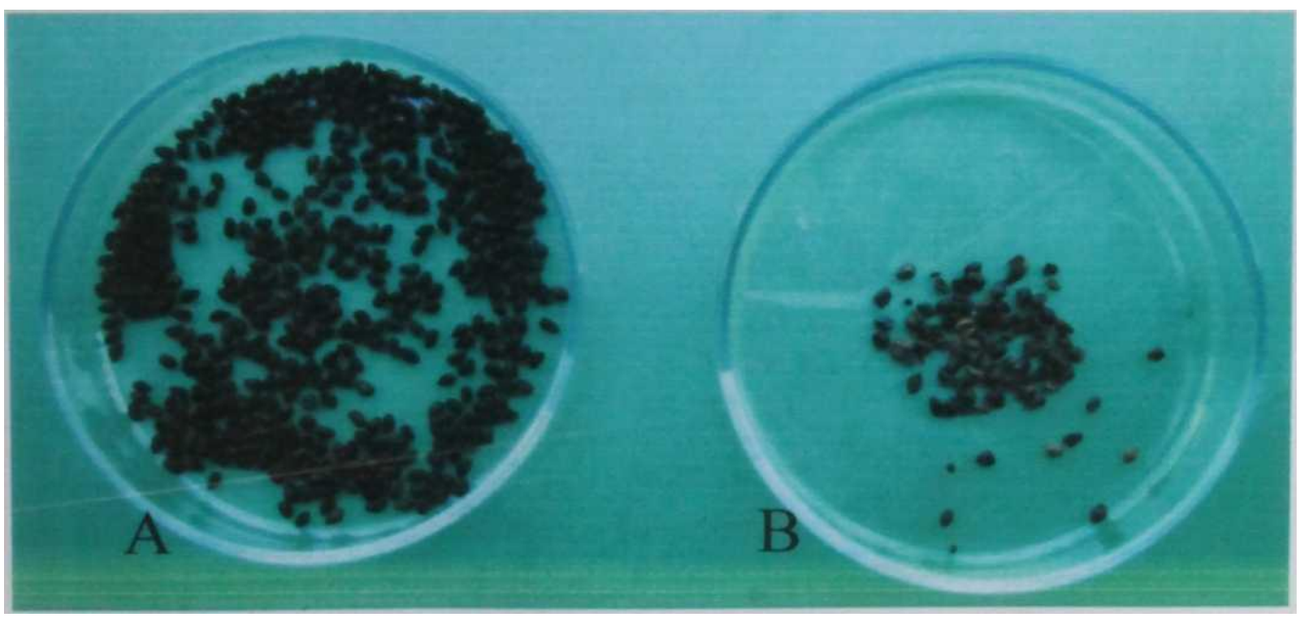


Plate 4. Morphological features of two categories of Devil's cotton seeds (L-R): A) Apparently healthy seeds. B) Shrivelled and malformed seeds.

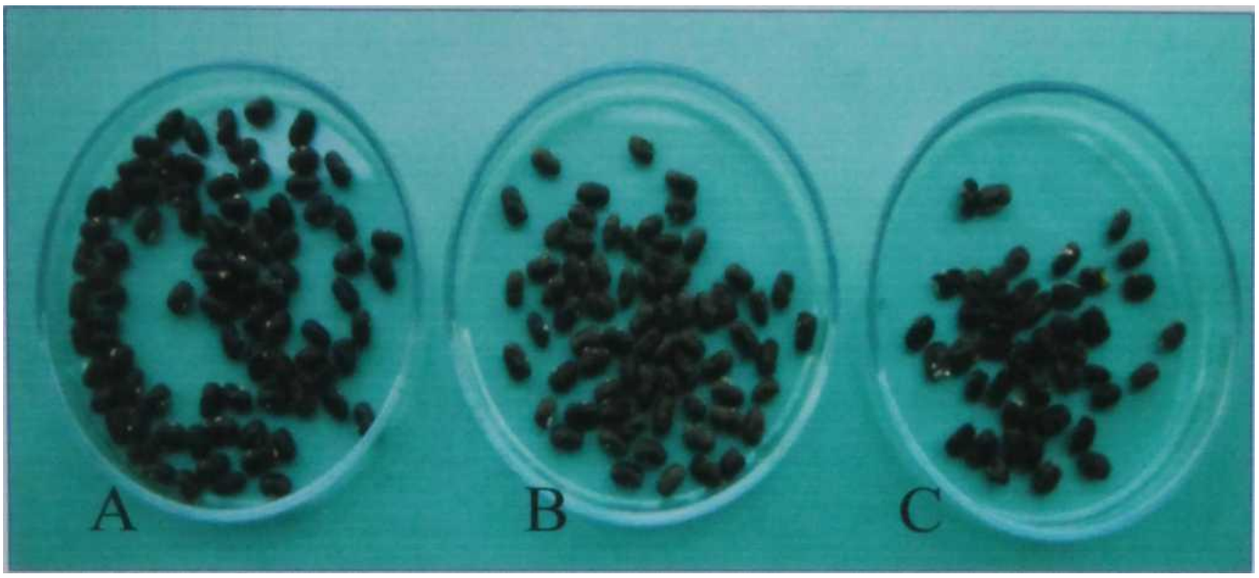


Plate 5. Morphological features of three categories of Butterfly seeds (L-R): A) Apparently healthy seeds. B) Discolored seeds and C) Shrivelled and malformed seeds.

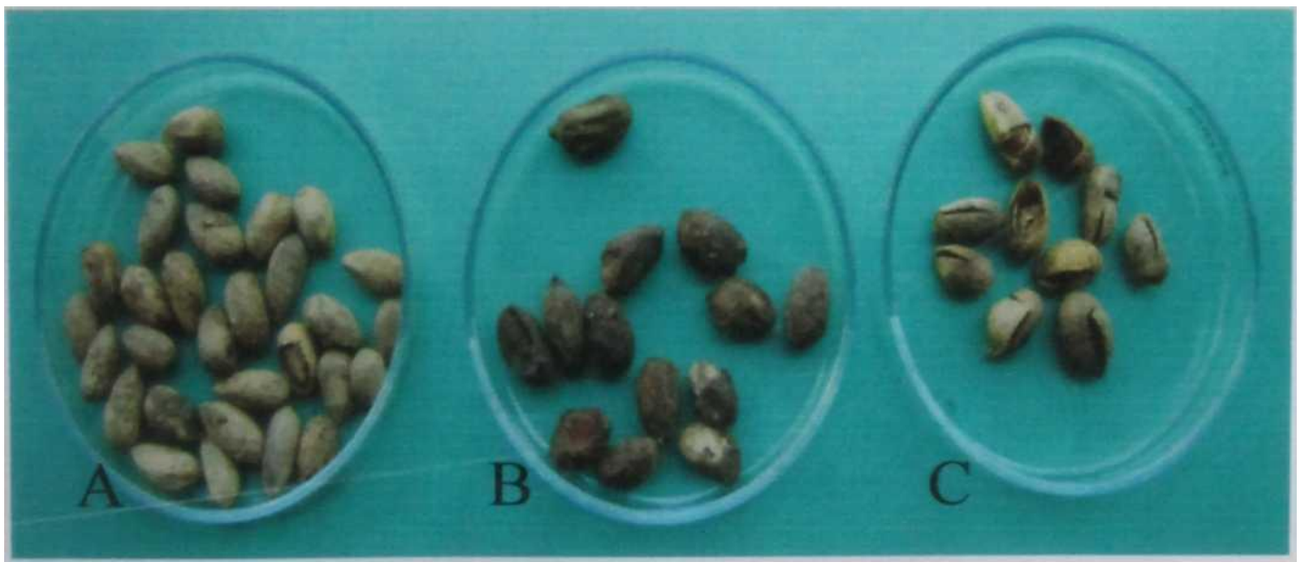


Plate 7. Morphological features of two categories of Asparagus seeds (L-R): A) Apparently

Plate 6. Morphological features of three categories of Margosa I ree seeds (L-R): Apparently healthy seeds, B) Discolored seeds and C) Shrivelled and malformed seeds.

healthy seeds. B)Shriveiled and malformed seeds

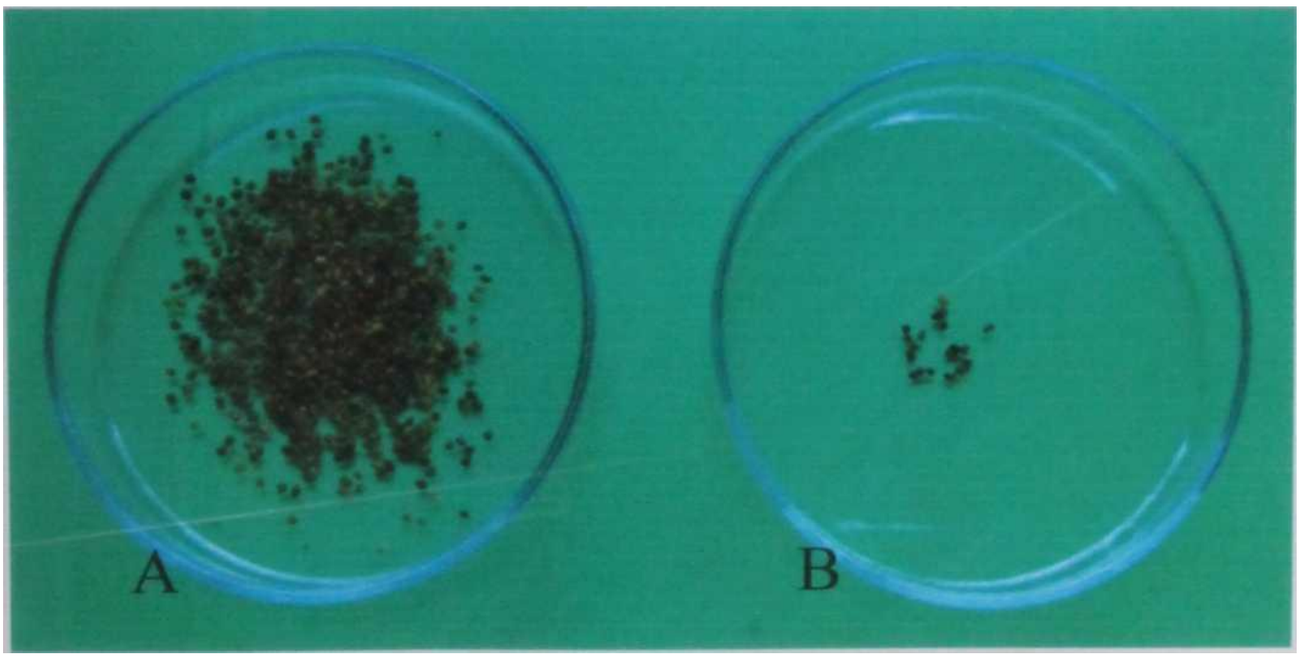


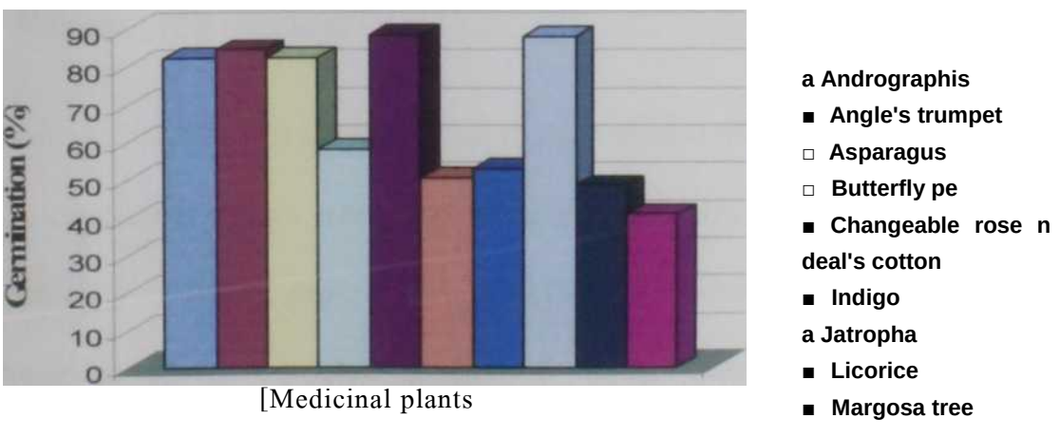
Plate 8. Morphological features of two categories of Andrographis seeds (L-R):

A) Apparently healthy seeds, B) Discolored seeds

The percentage of discolored seeds was found in seeds of six species viz. Angle's trumpet (3.50%), Butterfly pea (3.00%), Indigo (5.00%), Jatropha (1.50%), Andrographis (3.00%) and Margosa tree (3.50%), The percentage of shrivelled and malformed seeds was found in seeds of eight species viz. Asparagus (3.50%), Butterfly pea (4.50%), Changeable Rose (2.50%), Devil's cotton (3.50%), Indigo (4.00%), Jatropha (3.00%), Licorice (3.00%) and Margosa tree (3.50%).

4.2 Germination

The results of germination test carried out by blotter method and pot soil test of ten seeds of medicinal plants are shown in Fig.l and fig. 2 (Appendix 1). In blotter method the percentage of germination in blotter method of Andrographis, Angle's trumpet, Asparagus, Butterfly pea. Changeable rose. Devil's cotton, Indigo, Jatropha, Licorice, Margosa tree seeds recorded were 82.50%, 84.50%, 82.50%. 58.00%, 88.50%, 50.50%, 53.00%. 88.00%, 48.50%, 41.00%, respectively (Figure 1).



[Medicinal plants

Figure 1. Seed germination of ten selected medicinal plants observed in blotter method

In pot soil test the percentages of germination of Andrographis, Angle's trumpet. Asparagus, Butterfly pea, Changeable rose, Devil's cotton. Indigo, Licorice, Jatropha and Margosa tree seed recorded were 66.00%, 60.00%, 75.00%. 53.50%, 71.50%, 48.00%, 47.00%, 40.50%, 69.00% and 30.50%, respectively (Figure 2).

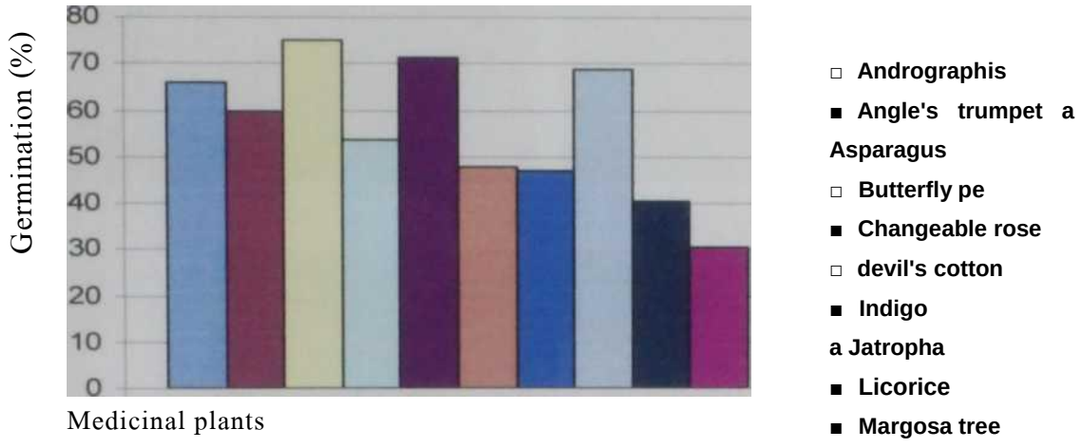


Figure 2. Seed germination of the ten selected medicinal plants observed in pot soil test

4.3 Fungi detected

4.3.1 Without pre-treatment

The results of blotter test (without pre-treated seeds) of the seed samples often selected medicinal plants were presented in Appendix 2. Seven fungi species, representing six fungal genera were detected by blotter method test from the seed samples of ten selected medicinal plants. The fungi were *Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Penicillium sp and Rhizopus stolonifer.* Fungi growing on incubated seeds in this method are shown in plate 9.

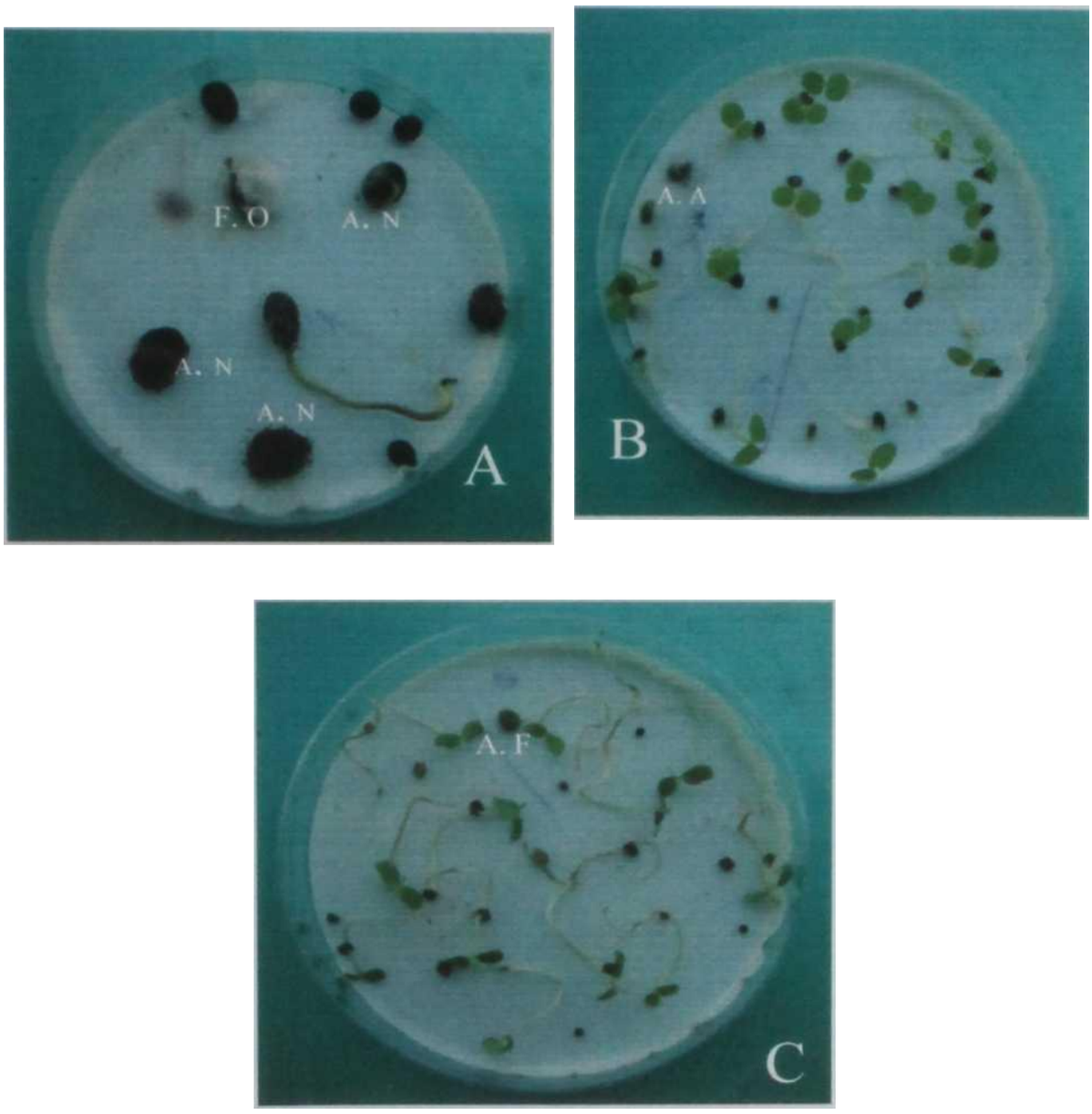


Plate 9. Fungi growing on incubated seeds in the blotter test:

A) *Aspergillus niger* (A. N) and *Fusarium oxysporum* (F. O) on Jasthimadhu

B) *Alternaria tenuis* (A. T) on Changeable Rose

C) *Aspergillus flavus* (A. F) on Indigo

Four tungi viz. Aspergillus flavus, Aspergillus niger, Penicillium sp and Rhizopus stolonifer were recorded from Andrographis seed. The incidence of Aspergillus flavus, Aspergillus niger, Penicillium sp and Rhizopus stolonifer were recorded 18.50%, 17.50%, 12% and 15.00%, respectively (Figure 3).

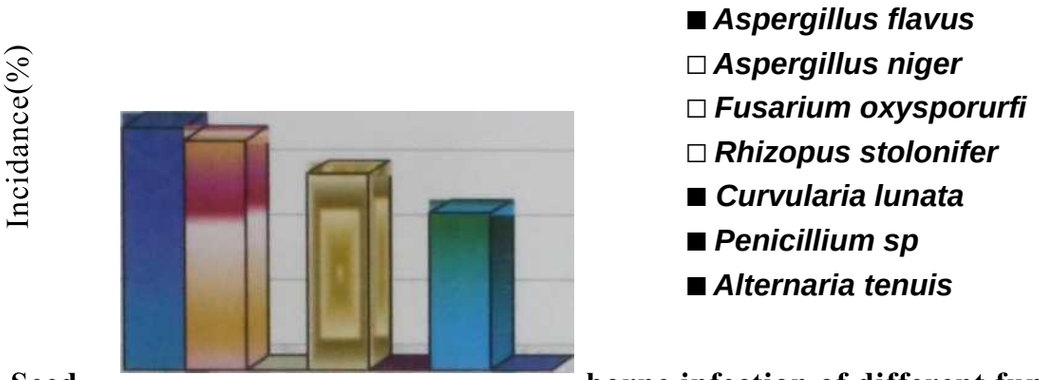


Figure 3. Seed borne infection of different fungi recorded in Andrographis seed by blotter method

Five fungi were recorded in Angle's trumpet seed. The fungi were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer, Penicillium sp and Alternaria alternata. The percentage of Alternaria tenuis. Aspergillus flavus, Aspergillus niger, Penicillium sp, and Rhizopus stolonifer* were recorded 9.5%, 14.50%, 17.00%, 12.00% and 19.00%, respectively (Figure 4).

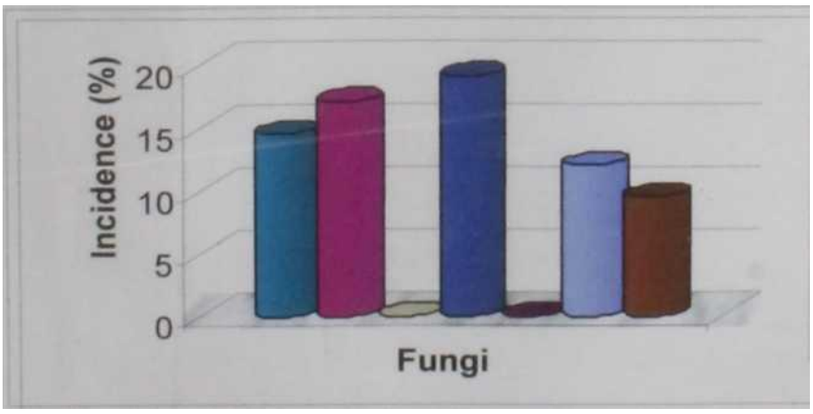


Figure 4. Seed borne infection of different fungi recorded in Angle's trumpet seed by blotter method

In Asparagus seeds three fungi viz. *Aspergillus flavus, Curvularia lunata and Fusarium oxysporum* were found. The percentage of *Aspergillus flavus, Curvularia lunata and Fusarium oxysporum* were recorded as 46.50o%, 30.00%. 46.50%, respectively (Figure 5)

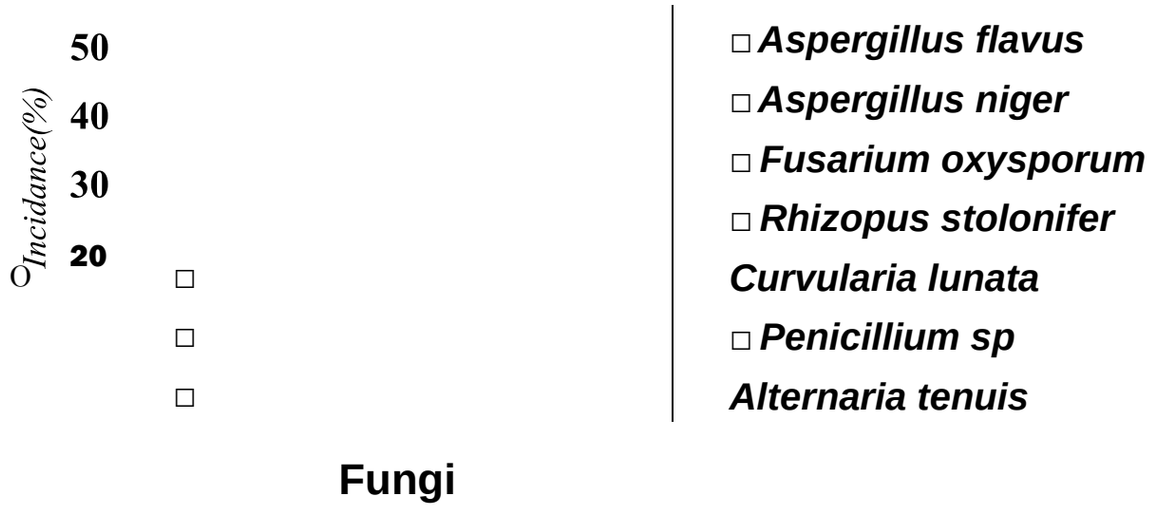


Figure 5. Seed orne infection of different fungi recorded in Asparagus seed by blotter method

Four fungi were recorded from Butterfly pea seed. The fungi were *Aspergillus niger, Curvularia lunata, Fusarium oxysporum and Penicillium sp* and the incidence of *these species were* 28.00%, 16.50%, 21.50% and 28.00%, respectively (Figure 6).

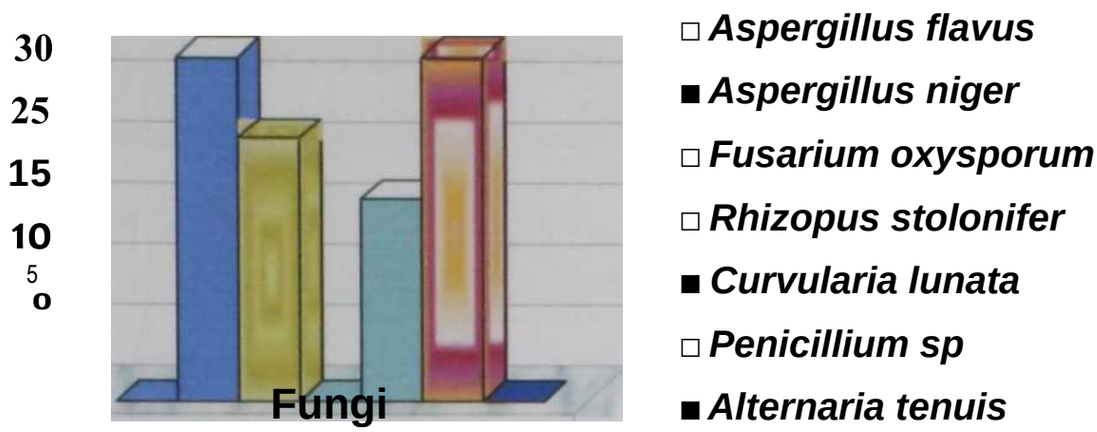


Figure 6. Seed borne infection of different fungi recorded in Butterfly pea seed by blotter method

Three fungi viz. *Alternaria tenuis, Curvularia lunata and Fusarium oxysporum* were found from Changeable rose and the incidence of these fungi were 31.50%, 28.00% and 15.00%. respectively (Figure 7).

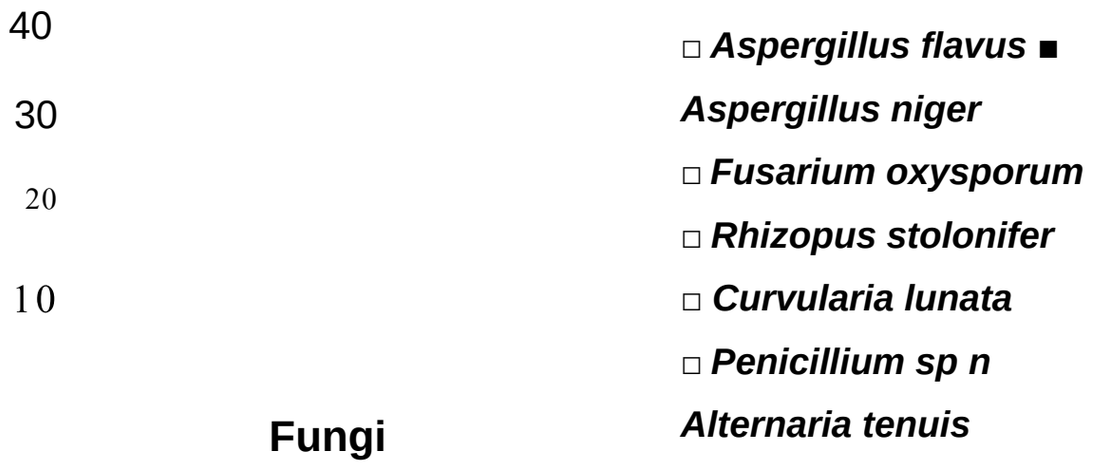


Figure 7. Seed borne infection of different fungi recorded in Changeable rose seed by blotter method

In Devil s cotton seeds three fungi were recorded viz. *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum.* T he percentage of *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* were recorded 27.50%, 17.50% and 14.50% respectively (Figure 8).

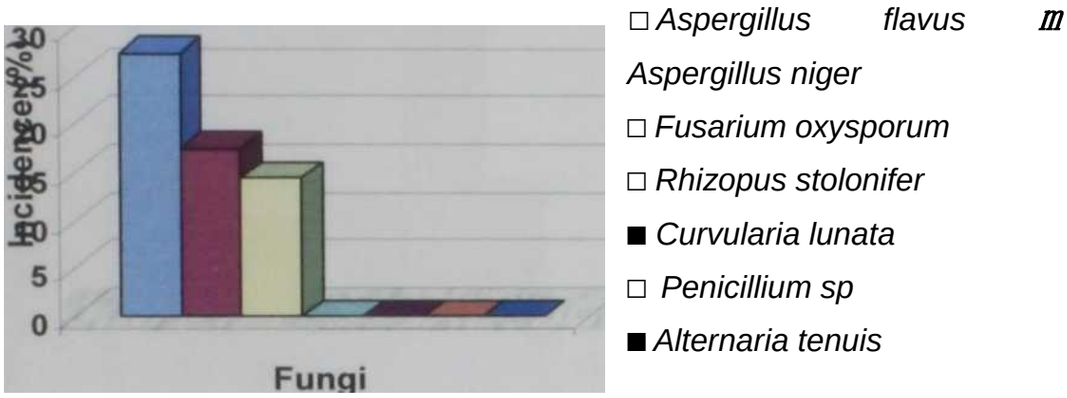


Figure 8. Seed borne infection of different fungi recorded in Devil's cotton seed by blotter method

In indigo seed Five fungi were found to be associated. The fungi were *Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum and Rhizopus stolonifer* and the incidence of the Five fungi were 22.50%., 25.00%, 30.5%, 1 1.50% and 13.00% respectively (Figure 9).

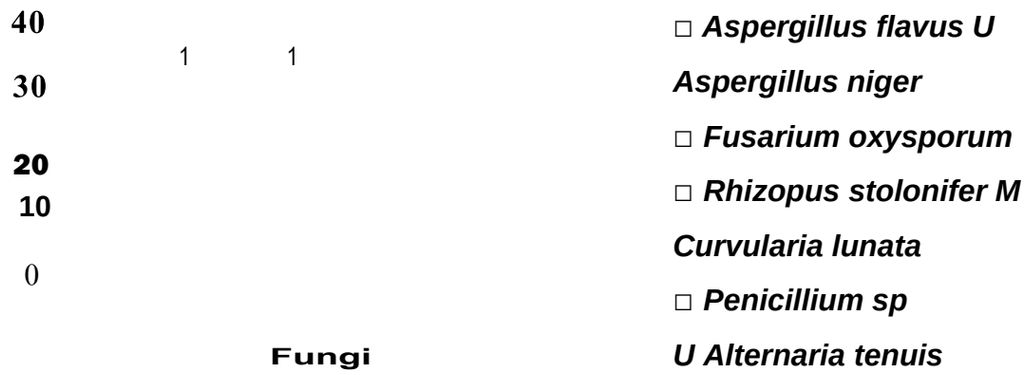


Figure 9. **Seed borne infection of different fungi recorded in indigo seed by blotter method**

In Jatropha seed five fungi viz. *Alternaria tenuis, Aspergillus flavus, Curvularia lunata. Fusarium oxysporum and Penicillium sp* were found and the incidence of these fungi was recorded 13.00%, 20.00%, 11.5% , 9.00% and 30.00%. respectively (Figure 10).

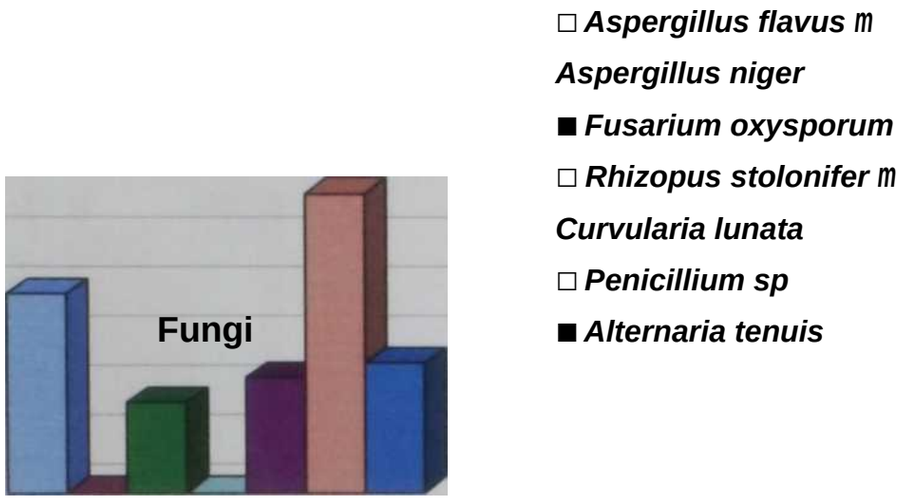


Figure 10. Seed borne infection of different fungi recorded in Jatropha seed by blotter method

In Licorice seed four fungi were found. The fungi were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum and Penicillium sp* and their incidence was 28.00%, 25.00%, 30.00% and 37.5%. respectively (Figure 1 1).

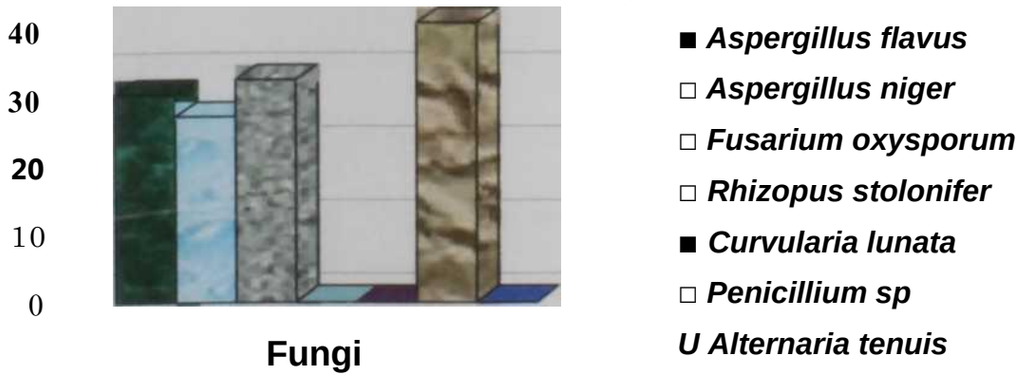


Figure **11. Seed borne infection of different fungi recorded in Asparagus seed by blotter method**

In Margosa tree seed four fungi were recorded. The fungi were *Aspergillus niger, Aspergillus flavus. Fusarium oxysporum and Rhizopus stolonifer* and their incidence was 18.00%. 20.00% 15.50% and 20.00%, respectively (Figure 12**).**

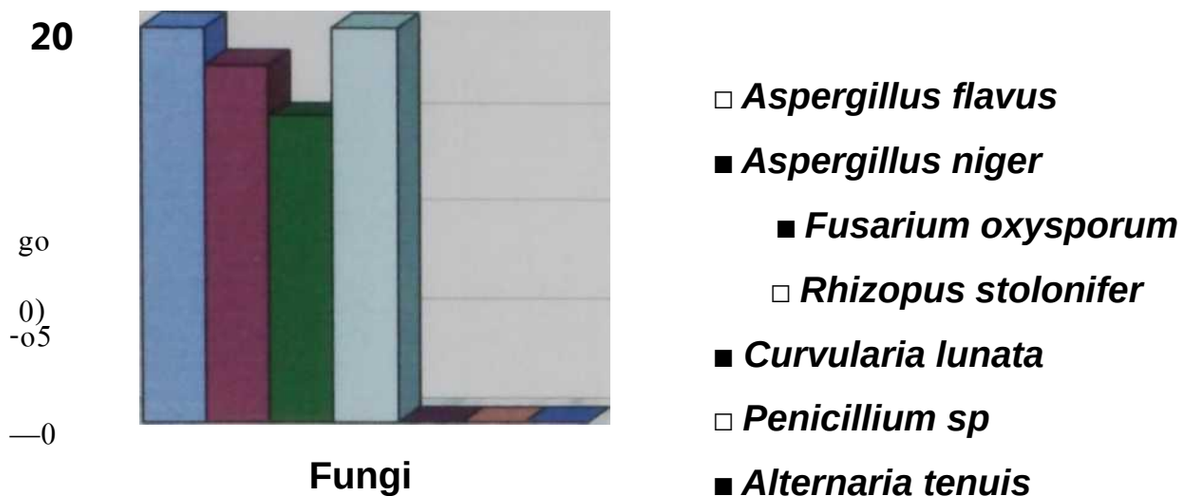


Figure 12. Seed borne infection of different fungi recorded in Margosa seed by blotter method

4.3.2 With pre-treatment

rhe results of blotter method test (Pre-treated with 0.01% $Hgcl_2$) of the seed samples of ten selected medicinal plants were presented in Table 2. After pretreatment of seeds of the ten selected medicinal plants, nine seeds yielded only by three fungal species. The fungi were *Alternaria tenuis, Curvularia lunata and Fusarium oxysporum.* There was no incidence of fungus on Andrographis seeds after pre-treatment.

Table 4. Seed borne infection of different fungi recorded in the selected medicinal plants by blotter method (After pre-treatment of seeds)

SI. No.	Medicinal plant	Percent infection by						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium oxysponitn*	*Rhizopus stolonifer*	*Curvularia lunata*	*Penicillium sp*	*Alternaria tenuis*
1	Andrographis	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Angle's Trumpet	0.00	0.00	0.00	0.00	0.00	0.00	8.00
3	Asparagus	0.00	0.00	26.00	0.00	40.5.0	0.00	0.00
4	Butterfly pea	0.00	0.00	20.50	0.00	14.00	0.00	0.00
5	Changeable rose	0.00	0.00	0.00	0.00	24.00	0.00	28.00
6	Devil's cotton	0.00	0.00	12.00	0.00	0.00	0.00	0.00
7	Indigo	0.00	0.00	11.00	0.00	27.50	0.00	0.00
8	Joytun	0.00	0.00	8.00	0.00	9.00	0.00	9.50
9	Licorice	0.00	0.00	28.50	0.00	0.00	0.00	0.00
10	Margosa tree	0.00	0.00	13.00	0.00	0.00	0.00	0.00

Alternaria tenuis was recorded from Angle's trumpet seed. The incidence of this fungus was recorded 8.00%.

In Asparagus two fungi viz. *Curvularia lunata* and *Fusarium oxysporum* were found. The percentage of *Curvularia lunata and Fusarium oxysporum* were recorded 26.00% and 40.50%, respectively.

From butterfly Pea seed two fungi were recorded. The fungi were *Curvularia lunata* and *Fusarium oxysporum.* The incidence of *Fusarium oxysporum and Curvularia lunata* were encountered 14.00% and 20.50%, respectively.

Two fungi viz. *Alternaria tenuis* and *Curvularia lunata* was found from Changeable rose and the incidence of these fungi were recorded 28.00% and 24.00%, respectively.

In Devil's cotton seed only *Fusarium oxysporum* was found. The incidence of this fungus was 12.00%.

In Indigo two fungi were found to be associated. The fungi were *Curvularia lunata* and *Fusarium oxysporum.* The incidence of *Curvularia lunata* and *Fusarium oxysporum* were recorded 27.50% and 11.00%, respectively.

In Jatropha seed three fungi viz. *Alternaria tenuis, Curvularia lunata* and *Fusarium oxysporum* were found and the percentage of these fungi were recorded 9.50%, 9.00%, and 8.00%, respectively.

In Licorice seed only one fungus was found. The fungus was *Fusarium oxysporum* and the incidence was 28.50%.

In Margosa tree seed only one fungus was found. The fungus was *Fusarium oxysporum* and the incidcncc was recorded 13.00%.

4.4 The characteristics of identified fungi

After seven days of incubation of seeds on wet blotting paper the yielded fungi were detected and identified by the standard method. Seven fungal species were detected from seeds of ten selected medicinal plants. The fungi were *Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Penicillium sp and Rhizopus stolonifer.* The characterisrics of identified fungi have been described below:

4.4.1 *Alternaria tenuis*

Brown to black growth consisting of conidia in long chains is the characteristics of the species. The chains are usually simple but can also be branched. Conidial beaks can sometimes be seen at higher magnifications under stereomicroscope. In most cases, growth of the fungus covers the entire seed surface. Conidia polymorphus, sort of septation (Transverse, longitudinal and oblique) under compound microscope.

4.4.2 *Curvularia lunata*

Growth of the fungus varies. It may consist of black, shiny conidia, dark conidiophore, short to long, bearing clusters of black, shiny conidia at the tips. The growth may be extensive covering part of seed or whole seed. The

conidiophores may he arising from one point as a compact tuft where only few conidiophores bear 1 to 2 conidia (Plate 10). Conidia of the fungus are smooth - walled three septate mostly curved but some straight. Third cell from the base is the largest and darkest, end cell sub hyaline or pale, tip cell rounded, basal cell usually with a scar.

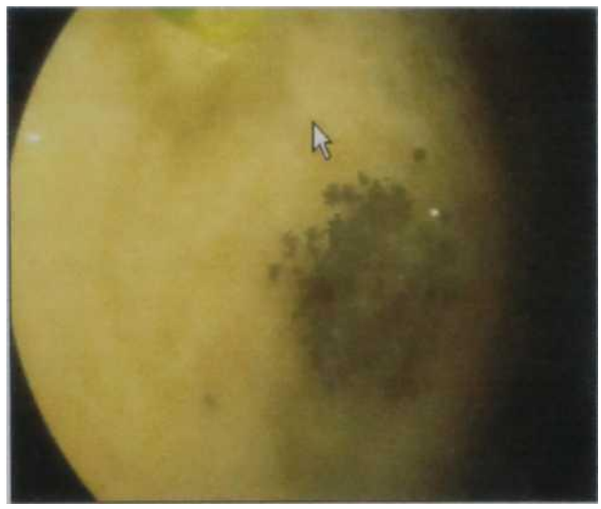


Plate 10. Growth of *Curvularia lunata* on incubated Butterfly Pea seen under stereomicroscope (X 40)

4.4.3 *Fusarium oxysporum*

Presence of white, floccose and septate; sclerotia are generally green but occasionally pink - red. conidiophores are verticillately branched and usually from sporodochia or reduce pionnotes. Sporodochia are salmon or flesh colored. Chlamydospores are intercalary or terminal. The micro-conidia are hyline, mostly three septate and shickle shaped. Microconidia being unicellular and ellipsoidal (Plate 1 1).

Plate 11. Growing of *Fusarium oxysporum* on incubated Licorice seed seen under stereomicroscope (X 40)

4.4.4 *Aspergillus flavus*

In case of *Aspergillus flavus,* growth of the fungus on seed is characterized by immature, white heads and mature heads in shades ranging from yellowish cream to green. Conidiophores bearing the heads are clearly seen when the growth is light.

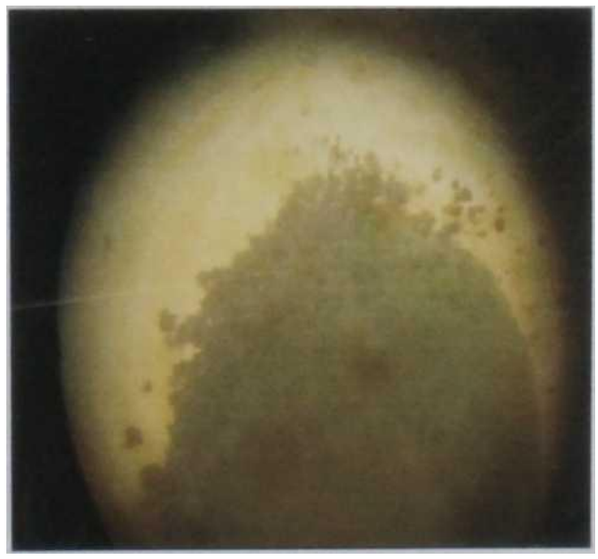


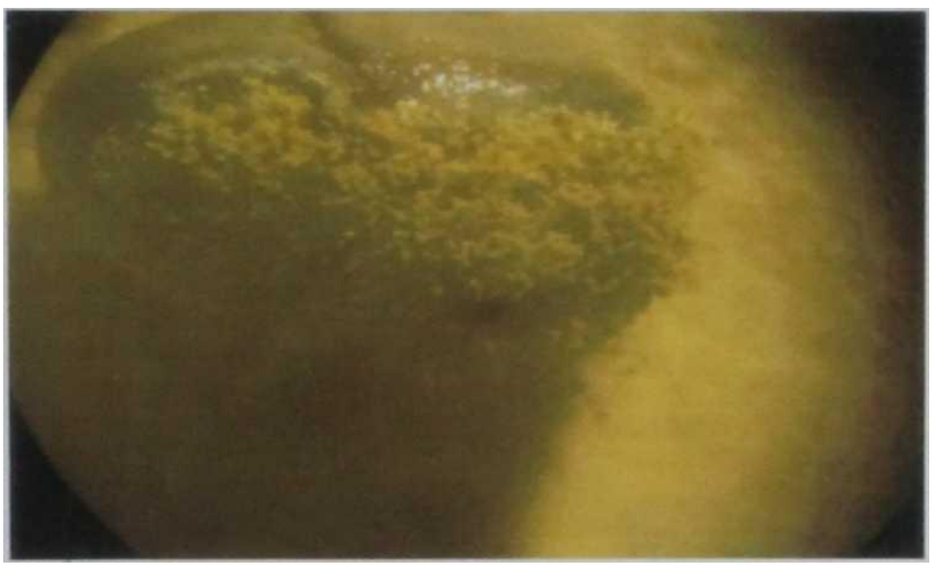
Plate 12. Growth of *Aspergillus niger* on incubated Licorice seed seen under stereomicroscope (X 40)

12).

ium sp

rows readily on general fungal media. Colonies are usually shades n. and white. Free spores are indistinguishable from *Aspergillus* mera with small round to oval colorless or slightly pigmented

13).



irowth of *Penicillum sp* on incubated Margosa seed een under stereomicroscope (X 40) *us stolonifer*

overs the whole seeds and extend to blotter for its fast growing. The igiosphores are long, solitary and arise in twos. The rhizoide at the base phores. Sporangiosphores are seen cottony white at first then darkens to depending on the amount of sporulation

They are long and hyaline terminating is bulbous heads. Conidia globose to sub-globose, usually rough, yellowish to green or black to brownish *(A. niger),* 3-5|im (Plate 12).

4.4.5 *Penicillium sp*

Penicillium grows readily on general fungal media. Colonies are usually shades of blue, green, and white. Free spores are indistinguishable from *Aspergillus* and other genera with small round to oval colorless or slightly pigmented spores (Plate 13).

Plate 13. Growth of *Penicillum sp* on incubated Margosa seed seen under stereomicroscope (X 40)

4.4.6 *Rhizopus stolonifer*

The fungus covers the whole seeds and extend to blotter for its fast growing. The brown sporangiosphores are long, solitary and arise in twos. The rhizoide at the base of sporangiosphores. Sporangiosphores are seen cottony white at first then darkens to gray to black depending on the amount of sporulation

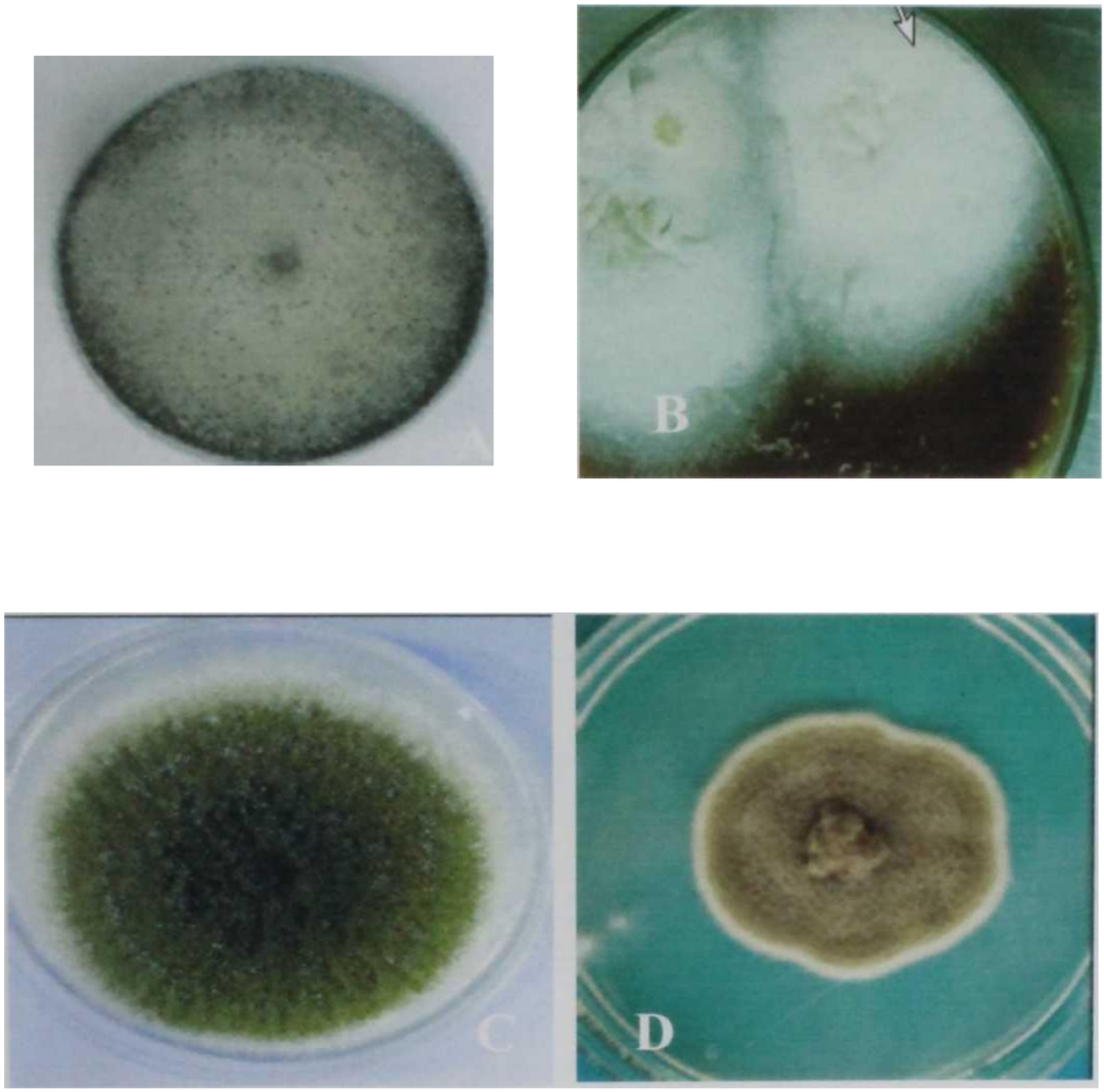


Plate 14. Pure culture of four different fungi detected on medicinal plants: A) *Alternaria tenuis*, B) *Fusarium oxysporum*, C) *Aspergillus flavus* and D) *Curvularia lunata*

Chapter 5
Discussion

DISCUSSION

The present research work was undertaken to study the seed borne fungi on ten selected medicinal plants. In inspection of dry seeds it was observed that there were three categories of seeds viz. i) Apparently healthy seeds, ii) Discolored seeds and iii) Shrivelled and malformed seeds. The highest percentage of apparently healthy seeds was observed in Changeable rose (97.50%) and the lowest percentage was in Indigo (91.00%). The highest percentage of discolored seeds was observed in Indigo (5%) and the lowest percentage was in Jatropha (1.5%). The highest percentage of shriveled and malformed seeds was observed in Butterfly (4.50%) and the lowest percentage was in Changeable rose (2.50%). Out of ten seeds studied, four seeds viz. Devil's cotton, Asparagus, Jsthimadu and Changeable rose were free from discolored seeds and two seeds namely, Algle's trumpet and Andrographis were free from shrivelled and malformed seeds. Discolored and shriveled seeds as observed in medicinal plants have been also encountered in seeds of various agricultural crops reported by Harun-or-Rashid (2003), Khair (2001), Yum *et al.* (1988), Agarwal *et al.* (1990) and Ploper an Raut (1994). In general, more than 90% seeds of the medicinal plants studied were apparently healthy seeds and this data indicates that in comparison to other crops the seed health condition of medicinal plants are relatively better.

Regarding the germination of seeds blotter method and pot soil test were used. In blotter method, the highest percentage of seed germination was recorded in

Changeable rose (88.50%) and the lowest in Margosa tree (41.00%). In pot soil test, the highest percentage of germination was observed in Asparagus (75.00%) and the lowest pcrccntage was found in Margosa tree (30.50%). From dry inspection test and germination test it may be concluded that comparatively higher number of healthy seeds resulted higher number of germination in blotter method. This finding was consistent with Garcia *et al.* (1980) and Skripka *et al. (*1989).

For detecting seed borne fungi of the ten selected medicinal plants blotter method was used. Both pre-treated (0.01% $HgCl_2$) and untreated seeds were tested for detecting fungi in this method. Seven fungi, representing six genera viz. *Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Curvularia lunata, Fusarium oxysporum, , Penicillium sp and Rhizopus stolonifer* were recorded in seeds of ten medicinal plants. All these seven species of fungi were recorded from untreated seeds, while only three species viz. *Alternaria tenuis, Curvularia lunata and Fusarium oxysporum* were recorded in pre-treated seeds. Occurrence of lower number of fungi in pre-treated seeds was due to the effect of 0.01% $Hgcl_2$. Such reduction of seed-borne fungal inocula was observed by Mathur *et al.* (1967) and Nath *et al.* (1970a). Mathur *et al.* (1967) found that *F. oxysporum* and *F. moniliforme* were less in pre-treated sorghum seeds. Similarly, Nath *et al.* (1970a) worked for the detection of seed borne fungi of mungbean (*Vigna radita)* and showed that pre-treated seeds with 0.01% $Hgcl_2$ reduced the counts of *Colletotrichum truncatum, Curvularia*

lunata, Fusarium spp and *Macrophomina phaseolina.* On the other hand, *Fusarium oxysporum* along with other species viz. *Fusarium moniliforme, Fusarium solani* and *Fusarium* tricinctum were isolated from both pre-treated and untreated seeds of Asparagus by Gilberston and Manning (1980).

In the present study seven fungi were detected in seeds of ten medicinal plants. None of the fungi have been found associated with the seeds of these ten plant species. Thus, the seven fungi appear to be new records of seed-borne fungi of medicinal plants in Bangladesh.

Chapter 6
Summary and conclusion

SUMMARY AND CONCLUSION

Seeds of ten medicinal plants were collected from Agroforcstry field laboratory SAU, Dhaka and the seeds were tested for detection of seed-borne fungi during the month of January, 2006 to June, 2006.

Seed samples were categorized by inspection as apparently health seeds, discolored seeds and shriveled and malformed seeds. The highest percentage of healthy seeds were found in Changeable Rose (97.50%) seeds, discolored seeds in Indigo (5.00%) and shrivelled and malformed seeds in Butterfly Pea (4.50%). All seeds of ten selected medicinal plants are found to have above 90% apparently healthy seeds.

Germination was determined by blotter method and pot soil test. The highest percentage of germination was found in Changeable Rose (80.00%) and lowest in Margosa tree (41.00%) in blotter method and in pot soil test the highest percentage of germination was recorded in Asparagus (71.00%) and lowest in Margosa tree (30.50%).

For detection of seed-borne fungi blotter method was used. Both untreated and pre-treated (Treated with 0.01% $Hgcl_2$) seeds were used. Seven fungal species viz. *Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Curvularia lunata, Fusarium oxysporum, Penicillium sp* and *Rhozopus stolonifer* were recorded from of the ten selected medicinal plants while only three fungal species viz.

Alternaria tenuis, Curvularia lunata and Fusarium oxysporum were recorded from pre-treated seeds.

It may be concluded that seeds of medicinal plants attacked by different fungi where *Aspergillus spp, Rhizopus stolonifer and Penicillium sp* are saprophytic fungi. *Alternaria tenuis, Fusarium oxysporum* and *Curvularia lunata* are saphrophytic, but may be pathogenic. These are the new records for Bangladesh. Considering the limitation of this research works, it may be mentioned that present studies need to be repeated including more testing methods and seeds from more representing localities to find out pathogenic nature of fungi on medicinal plants before stating acceptable recommendation.

References

REFERENCES

Agarwal, P. C., Majumdar, A. Ramnath, U. D. and Khetarpal, R. K. (1990). Seed-borne of quarantine in exotic germination of soybean (glycine max). Indian Journal of Agricultural Science. 60 (5): 361-363.

Benoit, M. A. and Mathur, S. B. (1970). Identification of species of *Curvularia* on rice seed. Proceeding of the International Seed Testing Association. 35: 99-119.

Chidambaram, P., Mathur, S. B. and Neergaard, P. (1973). Identification of Seed-borne *Drechslera* species. Friesia. 10: 165-207.

Ellis, M. B. (1971). Dematicious Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey, England.p.608.

Fakir, G. A. (2003). An Annotated list of Seed Borne Diseases of Important Crops in Bangladesh. SPC. BAU, Mymeningh.

Garcia, J. L., Diaz Carrasco H., Gonjales, L. A and Vidal, N. 0980). Incidence of some soybean *(Glycine max)* disease in three sowing seasons. Ciencias de la Agriculture. 6: 3-12.

Gilberston and Manning (1980). Isolation of different fungi from *Asparagus officinale* seeds. Phytopathology. 70: 462pp In: Richardson (1990). An annotated list of seed-borne diseases. Fourth Fdition. The International Seed Testing Association, Zurich, Switzerland.

Harun-or-Rashid (2003). Study on seed borne fungi of soybean and their control by plant extracts. M. S. thesis, Bangladesh Agricultural University, Mymensingh

ISTA (International Seed Testing Association) (1976). Handbook of Seed Health Testing. Proc. Int.Seed.Assoc, p. 180.

ISTA (International Seed Testing Association) (1999). International R ules of Seed Testing Association). Proc. Int . Seed. Assoc, p. 180.

Khair, S. A. (2001). Detection of seed-borne fungi and bacteria in soybean. M.S. thesis, Bangladesh Agricultural University, Mymensingh.

(1990). An annotated list of seed-borne disease. Fourth Fdition. The International Seed Testing Association, Zurich, Switzerland.

Mathur, S. B. and Cunfer B. M. (1993). Seed-borne and seed health testing of wheat. Danish Government Institute of Seed Pathology for Developing countries, Denmark; Jordbrugsforlaget, Fredriksberg, Denmark, p. 168.

Mathur, S. B. and Kongsdal, O. (2000). Common laboratory seed health testing methods for detecting fungi. Danish Government Institute of Seed Pathology for Developing countries. Kandrups Bogtrykkeri, Copenhagen, Denmark.

Mathur, S. B., Sharma, R. and Joshi, L. M. (1967). Mouldy head disease of sorghum.: Isolation of fungi on blotter and agar. Proc Int. Seed. Test. Assoc.32: 639-645.

Nath, R., Mathur, S. B and Neergaard, P. (1970). Seed-borne fungi of mungbean *(Phaseolum aureus* Roxbe.) from India and their significance. Proc. Int. Seed. Test. Assoc. 35: 255-242.

Nath, R., Neergaard P. and Mathur, S. B. (1970a). Identification of *Fusarium* species on seeds as they occur in blotter test. Proceeding of the International Seed Testing Association. 35: 121-144.

Ploper, V. C. and Raut, J. G. (1994). Fungal associated with soybean seed in Vidarbha. PKV Research Joumel. 21: 99-100.

Richardson, M. J. (1990). An Annotated list of seed-borne diseases. ISTA (International Seed Testing Association). Fourth Edition. The International Seed Testing Association, Zurich, Switzerland.

Skripka, O. V., Sizova, T. P. and Bab'eva, E. N. (1989). Fungi on soybean seeds in Abkhazia. Mikologiya i Fitopathologiya. 20 (4): 306-308.

Yum, K. J. and Park E. M. (1988). Occurance and distribution of soybean seed-borne fungi. Korea Journal of Pathology. 20 (2): 113-120.

Appendices

APPENDICES

Appendix 1. Germination of the ten selected medicinal plants observed in Blotter method and pot soil test

Sl. No.	Seeds	Germination (%) Recorded in	
		Blotter method	Pot soil test
1	Andrographis	82.50	66.00
2	Angle's Trumpet	84.50	60.00
3	Asparagus	82.50	75.00
4	Butterfly pea	58.00	53.50
5	Changeable rose	88.50	71.50
6	Devil's cotton	50.50	48.00
7	Indigo	53.00	47.00
8	Jatropha	88.00	69.00
9	licorice	48.50	40.50
10	Margosa tree	41.00	30.50

Appendix 2. Seed borne infection of different fungi recorded in the selected medicinal plants by blotter method (Without pre-treated seeds)

SI. No.	Medicinal plant	Infection (%)						
		Aspergillus flavus	***Aspergillus niger***	***Fusarium oxysporum***	***Rhizopus stolonifer***	***Curvularia lunata***	***Penicillium sp***	***Alternaria tenuis***
1	Andrographis	18.50	17.50	0.00	15.00	0.00	12.00	0.00
2	Angle's Trumpet	14.50	17.00	0.00	19.00	0.00	12.00	9.50
3	Asparagus	46.50	0.00	30.00	0.00	46.50	0.00	0.00
4	Butterfly pea	0.00	28.00	21.50	0.00	16.50	28.00	0.00
5	Changeable	0.00	0.00	15.00	0.00	28.00	0.00	31.50
6	Devil's cotton	27.50	17.50	14.50	0.00	0.00	0.00	0.00
7	Indigo	22.50	25.00	11.50	13.00	30.50	00.00	0.00
8	Joytun	20.00	0.00	9.00	0.00	11.50	30.00	13.00
9	Licorice	28.00	25.00	30.00	0.00	0.00	37.50	0.00
10	Margosa tree	20.00	18.08	15.50	20.00	0.00	0.00	0.00