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SEED BORNE MYCOFLORA OF LEGUME SEEDS

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ABSTRACTS
The seed mycoflora of different varieties of Legumes was screened by standard blotter paper, agar plate and seed washates methods. Among the three methods, the agar paper method was found to be suitable as in less incubation; there was higher percent incidence of seed mycoflora. The different cultivars of Legumes screened for seed mycoflora were TLG – 45, LGN -1-1, BDN – 9 – 4, N – 59, BSMR - 853, BSMR – 736, BPMR-145, S-8, S-1-1 and T – 9. The Cv. TLG- 45 shows higher percent seed mycoflora with sixteen fungi as compared to other cultivars.

Key words – Legume varieties, Standard blotter paper, Agar plate and Seed washates methods.

INTRODUCTION -
In a world facing problem of malnutrition, protein rich crops assume special significance. Obtaining maximum production through all available avenues and protecting adequately what is produced would certainly alleviate the problem. In the Indian context, where Legumes have been a part of daily diet, maximizing production and enriching nutrition through legumes is a better and acceptable alternative. India stands first in production and area under legumes in the world. The major crops used include Groundnut, Gram, Pigeon pea, Green gram and Black gram.

Seeds are generally associated with certain saprophytic or parasitic micro-organisms which perpetuate in the seed lots on the advent of favorable conditions. Seeds are associated with pathogens like fungi, bacteria, nematodes etc. Pathogens present in almost any seed lot of economically important crop which may be disastrous if introduced into disease free areas. Therefore, seed must be “Substantially free” from inoculum with high level of germination and purity before sowing. The reports of Nath et al. (1970) [3], Deo and Gupta (1980) [1] and Nakkeeran and Devi (1997) [2] confine to the mycoflora of Legume seeds.

Materials and Metrhods -
1. Detection of Seed Mycoflora
The seed mycoflora was isolated by using different methods such as Standard blotter paper method, Agar plate method, Rolled paper towel method and Seed washates as recommended by International Seed Testing Association ISTA (1966) [4] and Agarwal (1976)[5]. Observations were recorded in percent incidence of seed borne fungi associated with unsterilized seeds. The fungi which appeared on seed were isolated in pure culture for identification and for further study. Three different methods of isolation techniques for assessment of seed mycoflora were used.

2. Standard blotter paper method
This is the very simple, most convenient and efficient of all the incubation methods. Doyer (1938) [6] and De Temp (1953) [7] was first to adopt blotter paper method in seed health management. A pair of sterile white blotter papers of 8.5 cm diameter were soaked in sterile distilled water and were placed in pre-sterilized petriplates of 90 mm diameter. Ten seeds of test sample per petriplate were then placed at equal distance on moist blotter. 400 seeds were used in each experiment. The plates were incubated at $28^0 \pm 2^0$C under diurnal conditions. On seventh day of incubation, seeds were first examined under stereoscopic microscope for determining the various fungal growth. The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

3. Agar plate method

In Northern Ireland, Musket (1948) [8] first used this method for seed health management. In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described for blotter test method.

4. Seed washates method

100 seeds were taken in flask with sterile distilled water for their soaking. The flasks were subjected to mechanical shaker for 5 – 10 minutes. 1 mL of seed washing, thus obtained was placed on PDA medium for growth of individual spore of fungus. The PDA medium is very easily prepared with few contents and useful in diluting and spreading the seed washing. The seed washing contains spores of the fungi. The plates were incubated at room temperature for development of colonies and observations were made. Fungi developed within 3 days. These colonies were immediately transferred to PDA/ GNA slants for further study. The various moulds appeared on seeds in blotter tast, agar plates and seed washates were isolated and maintained on PDA/ GNA.

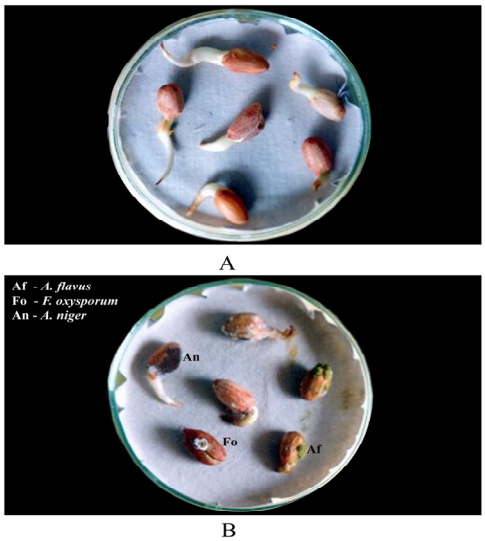

Plate I : Mycoflora of Groundnut (*Arachis hypogea* L.) seeds.
A : Blotter paper with healthy seeds.
B : Blotter paper with infected seeds associated with *A. flavus, A. niger* and *F. oxysporum.*

Experimental Results –

Sr. No.	Name of Fungi	Percent (%) incidence of Mycoflora		
		Std. blotter paper	Agar plate	Seed washates
1	***Aspergillus flavus***	70	72	60
2	***Aspergillus niger***	65	68	50
3	***Aspergillus ustus***	32	35	19
4	***Aspergillus fumigatus***	25	27	22
5	***Aspergillus nidulans***	30	37	27
6	***Aspergillus terrus***	30	32	28
7	***Alternaria tenuis***	40	42	35
8	***Fusarium oxysporum***	50	55	48
9	***Fusarium semitectum***	18	20	15
10	***Macrophomina phaseolina***	12	15	10
11	***Penicillium citrinum***	10	12	8
12	***Sclerotium rolfsii***	10	10	0
13	***Cephalosporum acromonium***	2	5	0
14	***Rhizoctonia solani***	6	7	5
15	***Rhizoctonia batatiocola***	5	5	0
16	***Rhizopus nigricans***	5	6	0
	S.E ±	5.2	5.38	4.72
	C.D. at 5%	11.07	11.45	10.05

Table1: Fungi associated with seeds of Legume Groundnut (*Arachis hypogea* L.) Cv. TLG-45

Fungi associated with seeds of Legume Groundnut:
(*Arachis hypogea* L.). Cv. TLG – 45:

Table 1, indicate that Legume Cv. TLG – 45 was associated with sixteen fungi such as ***Aspergillus*** *flavus, Aspergillus niger, Aspergillus ustus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus terrus, Alternaria tenuis, Fusarium oxysporum, Fusarium semitectum, Macrophomina phaseolina, Penicillium citrinum, Sclerotium rolfsii, Cephalosporium acromonium, Rhizoctonia solani, Rhizoctonia batatiocola and Rhizopus nigricans.*

In case of standard blotter paper, the percent incidence of *Aspergillus flavus* (70%) was highest followed by *Aspergillus niger* (65%), *Fusarium oxysporum* (50%) and *Alternaria tenuis* (40%). *Aspergillus ustus* (32%) *Aspergillus nidulans* (30%), *Aspergillus terrus* (30%), *Aspergillus*

fumigatus (25%), *Fusarium semitectum* (18%), and *Macrophomina phaseolina* (12%) were intermediates within the range of 12-32%. *Penicillium citrinum, Sclerotium rolfsii, Rhizoctonia batatiocola, Rhizopus nigricans* and *Cephalosporium acromonium* were found to be least.

In agar plate, *Aspergillus flavus* (72%) gave highest percent incidence followed by *Aspergillus niger* (68%) and *Fusarium oxysporium* (55%). *Alternaria tenuis* (42%), *Aspergillus nidulans* (37%), *Aspergillus terrus* (32%), *Aspergillus ustus* (35%), *Aspergillus fumigatus* (27%), *Fusarium semitectum* (20%), *Macrophomina phaseolina* (15%) and *Penicillium citrinum* (12%) were found to be intermediate within range of 12 – 42 % . *Sclerotium rolfsi, Rhizoctonia solani, Rhizoctonia nigricans, Cephalosporium acromonium* and *Rhizoctonia batatiocola* were found to be least.

In seed washates *Aspergillus flavus* (60%) gave highest percent incidence followed by *Aspergillus niger* (50%) and *Fusarium oxysporum* (48%) . *Alternaria tenuis* (35%), *Aspergillus nidulans* (30%), *Aspergillus terrus* (28%), *Aspergillus fumigatus* (22%), *Aspergillus ustus* (19%) and *Fusarium semitectum* (15%) were found to be intermediate within the range of 15 – 35 . *Macrophomina phaseolina, Penicillium citrinum, Rhizoctonia solani* and were found to be least. *Rhizoctonia batatiocola, Sclerotium rolfsii, Cephalosporium acromonium* and *Rhizopus nigricans* were not reported. It is clear that among three methods agar plate favours the growth of fungi and gives highest percent incidence due to potato dextrose agar contents.

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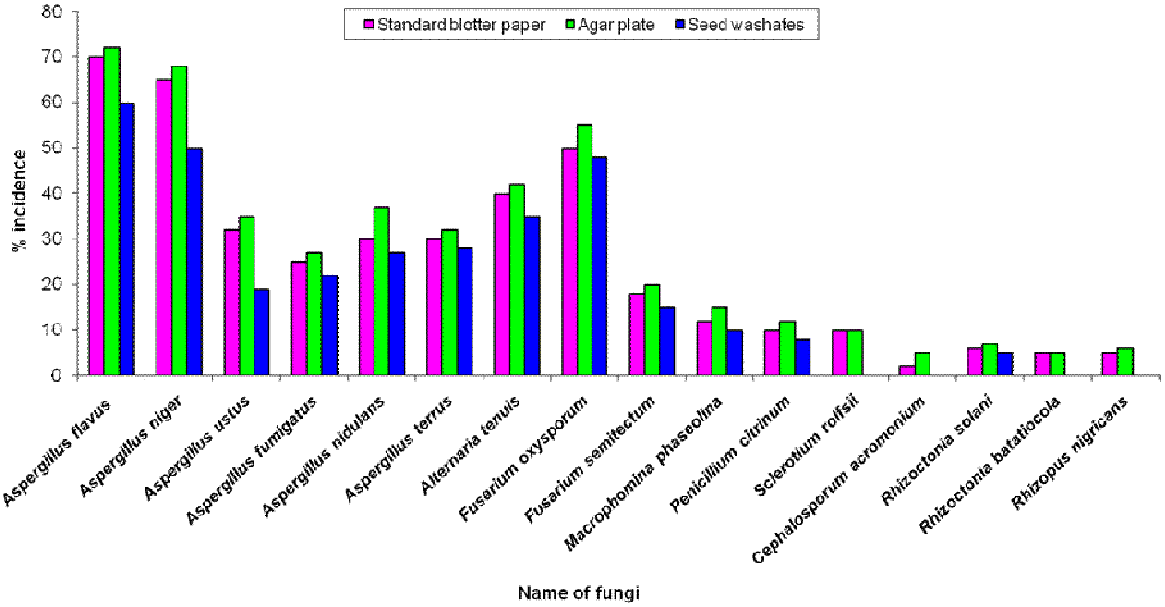

Fig.1. Fungi associated with seeds of Groundnut (*Arachis hypogea* L.) Cv. TLG-45