

## Biodiversity of *Aspergillus* spp. on Groundnut seeds

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### Abstract

Groundnut seeds are generally associated with certain saprophytic or parasitic micro-organisms which perpetuate in the seed lots on the advent of favorable conditions. Several species of fungi belonging to 5 genera were recorded from seeds of cultivar of Groundnut such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus ustus*, *Aspergillus fumigates*. Among these fungi *Aspergillus flavus*, *Aspergillus niger* were found to dominant and causing seed rot and seedling blight Groundnut. Here we analyzed the biodiversity of *Aspergillus* species occurring on two important Groundnut varieties viz. LGN -1 and LGN-2.

**Keywords:** Groundnut varieties (LGN-1, LGN-2) *Aspergillus* sp., Blotter paper and Agar plate methods.

### INTRODUCTION

*Aspergillus*, which is also called as "Eurotium". *Aspergillus* is widely distributed genus. They live mostly as saprophyte on almost all the dead organic material like decaying vegetables damp fruits, fatty substrata like butter, ghee, starchy materials like bread, rice, syrups, jams, jellies and wood and leather goods. Some species of *Aspergillus*, *A. flavus*, *Aspergillus fumigatus* and *Aspergillus niger*, cause diseases are known as Aspergilloses. Some species *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* are responsible for causing the disease of human ear. This disease is called as Otomycosis. *Aspergillus* species are responsible for several disorders in various plant and plant products. The most common species are *A. niger* and *A. flavus*, followed by *Aspergillus candidus*, *Aspergillus ustus*, *Aspergillus fumigates*, *Aspergillus nidulance*. Various mycotoxins have been produced by *Aspergillus* species, the most important are the aflatoxins and ochratoxin A (Varga *et al.*, 2004). Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> are the most toxic and carcinogenic

naturally occurring mycotoxins. The extensive research has been carried out on the natural occurrence, identification, characterisation, biosynthesis, and genetic regulation of aflatoxins (Bennett and Klich, 2003; Yu et al., 2004).

## MATERIALS AND METHODS

### Collection of Seed Sample:

Seed samples of Groundnut seeds were collected from Oil research center, Latur. The following Groundnut cultivar – LGN-1 & LGN-2 were used in the study. The externally and internally seed borne fungi are identified or detected by two important method which are commonly used in laboratory and research institute. These methods are known as fallows. a. Blotter paper method. b. Agar plate method.

### Blotter paper method

Doyer (1938) and De Temp (1953) were first to adopted blotter paper method in seed health management. This is the very simple, most convenient and efficient of all the incubation methods. Pair of sterile white blotter papers of 8.5 cm diameter was 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  $28^{\circ} \pm 2^{\circ}\text{C}$  under diurnal conditions. On seventh day of incubation, seeds were first examined under stereoscopic microscope for determining the various fungal growths. The identification and

further confirmation of seed borne fungi was made by preparing slides of the fungi.

### Agar plate method

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.

## RESULTS

In case of blotter paper methods (Table 2 and Plate I) the seeds of groundnut were associated with fungi such as *A. flavus*, *A. niger*, *A. candidus* and *A. ustus*. The % incidence of *A. flavus* was high (35) followed by *A. candidus* (15) and *A. niger* (10). In case of Agar plate methods (Table 3 and plate II) reveals that untreated seeds of groundnut were associated with fungi such as *A. flavus*, *A. niger*, *A. candidus* and *A. ustus*. and *A. fumigatus*. The % incidence of *A. niger* was high (40) followed by *A. flavus* (15) and % of low incidence were *A. ustus* and *A. fumigatus* (5). In untreated seeds of groundnut there was high % incidence of mycoflora and low % of seed germination. There was low % incidence of mycoflora but high % seed germination. *A. flavus* and *A. niger* caused discoloration of seeds and loosening of seed coat. There is also reduction in seed germination, length of radicle and length of plumule. In LGN - 2 of groundnut *A. flavus* and *A. ustus* disappear.

**Table 1: Groundnut seeds associated with fungi on blotter paper (Untreated)**

Sr. No.	Seed Sample	Variety	% incidence of mycoflora	% of seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi Associated
1	Groundnut	LGN-1	100	20	10.0	5.0	<i>A. flavus</i> , <i>A. niger</i> and <i>A. candidus</i>
2	Groundnut	LGN-2	60	60	12.5	6.0	<i>A. niger</i> , <i>A. flavus</i> , <i>A.candidus</i> <i>A. ustus</i>

**Table 2: Effect of fungi on incidence and damage done to the groundnut seeds on blotter paper (Untreated)**

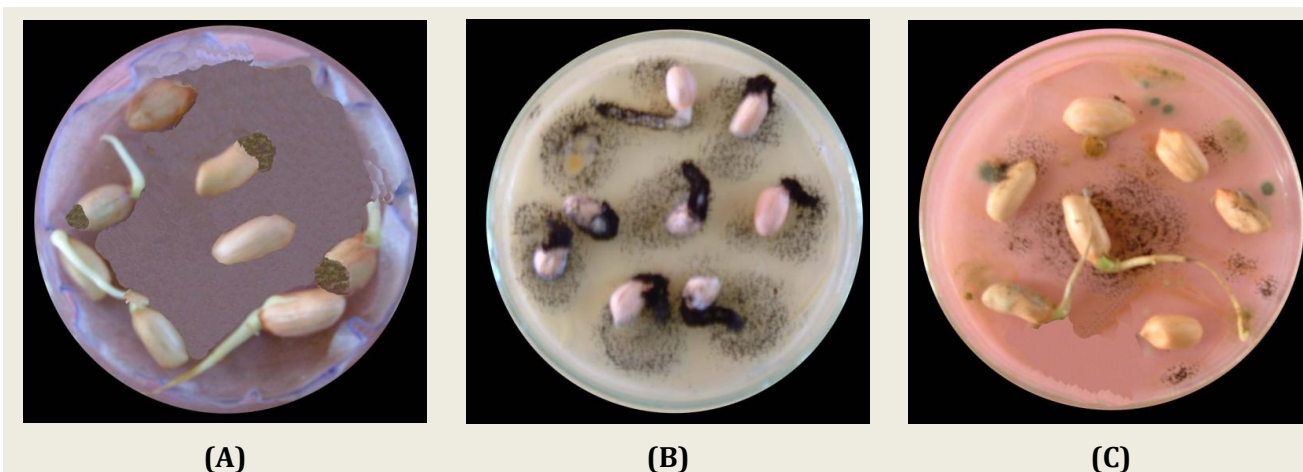
Sr. No.	Fungi Associated	% incidence of mycoflora on cultivars		Nature of damage done to the seed
		LGN-1	LGN-2	
1	<i>A. flavus</i>	30	35	Discoloration of seeds and loosening of seed coat. Germination of seed is reduced.
2	<i>A. niger</i>	10	10	
3	<i>A. candidus</i>	5	15	

**Table 3: Groundnut seeds associated with fungi on Agar plate (Untreated)**

Sr. No.	Seed Sample	Variety	% incidence of mycoflora	% of seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi Associated
1	Groundnut	LGN-1	90	10	8.0	4.0	<i>A. flavus</i> , <i>A. ustus</i> and <i>A. fumigatus</i>
2	Groundnut	LGN-2	80	10	9.0	4.5	<i>A. niger</i> and <i>A. flavus</i>

**Table 4: Effect of fungi on incidence and damage done to the groundnut seeds on Agar plate (Untreated)**

Sr. No.	Fungi Associated	% incidence of mycoflora on cultivars		Nature of damage done to the seed
		LGN-1	LGN-2	
1	<i>A. flavus</i>	25	-	Discoloration of seeds, inhibition of seed growth, loosening of seed coat and reduction in seed germination.
2	<i>A. niger</i>	25	40	
3	<i>A. ustus</i>	5	-	
4	<i>A. fumigatus</i>	5	5	



**Fig. 1: Showing *Aspergillus* species on Groundnut seeds.**  
**(A)** Blotter paper with infected seeds associated with *A. flavus*;  
**(B)** Agar Plate with infected seeds associated with *A. niger*  
**(C)** Agar Plate with infected seeds associated with *A. fumigatus* and *A. flavus*.

**DISCUSSION**

In Groundnut varieties like LGN-1 and LGN-2 the fungi associated are *A.flavus*, *A. niger*, *A. candidus*, *A. ustus* and *A. fumigates*. The most dominant fungi in these varieties are *A. flavus* and *A. niger*. Gupta and Chohan (1976) observed seed borne fungi and seed health testing in relation to seedling disease of groundnut kernels and pod shells during storage. Mixonet et al. (1984) studied effect of *Aspergillus flavus* and Aflatoxin contamination of peanut seed. Dange and Saradava (1985) screened of selected groundnut cultivars against seed rot and collar rot caused by *Aspergillus niger*. Bansal and Sobti (1988) observed the *Aspergillus flavus* associated with groundnut seeds. Magnoli et al. (2003). studied mycoflora and ochratoxin producing strains of *Aspergillus*. Varga et al. (2004). reported molecular diversity of agriculturally important *Aspergillus* species.

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