

## RESEARCH ARTICLE

# Study of Associated and Non-associated Seed Mycoflora of Groundnut

Deshmukh RS

Department of Botany, B. Raghunath Arts, Commerce & Science College, Parbhani

Email: [rsdeshmukh19@gmail.com](mailto:rsdeshmukh19@gmail.com)

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**ABSTRACT**

Fungi alone and in association can produce various types of toxic substances which can reduce the nutritional value of seeds and make them unsuitable for consumption. It is understood from the isolation studies on seed mycoflora of Groundnut varieties (SB XI & TAG 24) that some fungi were always found to be associated with a particular group of fungi and at the same time some fungi grew on the seeds individually without showing any association with any other type of fungi.

- i) *Aspergillus flavus* showed its association with most of the fungi including *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus nigricans*.
- ii) *Aspergillus niger* showed its association with *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Rhizopus nigricans*.
- iii) *Alternaria alternata* showed its association only with *Aspergillus flavus* and *Rhizopus nigricans*.
- iv) *Macrophomina phaseolina* was found associated with only *Rhizopus nigricans*.

**Key words:** Mycoflora, SB XI, TAG-24, Association.

**INTRODUCTION**

The degree of susceptibility of a particular variety can be predicted on the intensity of its seed mycoflora. Variation in the composition of seed mycoflora in different varieties of various crops was studied by many scientists which gives an idea about the degree of varietal resistance against the incidence of seed borne fungi.

It is evident from the literature that among seed mycoflora of Groundnut varieties, some fungi were always found to be associated with a particular group of fungi and at the same time some fungi grew on the seeds individually without showing any association with any other type of fungi. Taking this into consideration, the present investigation was undertaken to study associated and non-associated seed mycoflora of Groundnut.

## MATERIAL AND METHODS

### I) Collection of seed samples

The method described by Paul Neergaard (1977) has been adopted for the collection of seed samples. Accordingly, three random samples of seeds (half Kg each) were collected from oil mills, market place and Oil Seed Research Station (ORS), Latur. Groundnut cultivars used in the present study are TAG-24 and SB-XI.

### II) Detection of seed mycoflora

The seed mycoflora was detected by using standard moist blotter paper method and agar plate methods as recommended by International Seed Testing Association (ISTA, 2003), Neergaard, (1977) and Agarwal, (1981).

### III) Identification of test fungi

The fungus was identified by observing microscopic characteristics. Identification was confirmed with the help of latest manuals, Subramanian, (1971), Neergaard and Mathur, (1980), Jha, (1993) and Mukadam, (1997) and with the help of Information Bulletin of International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Patancheru, Andhra Pradesh (India). Pure culture of the identified fungi was prepared and maintained on PDA (Potato Dextrose Agar) slants.

### IV) Associated and non-associated seed mycoflora

For this the seeds of Groundnut variety TAG-24 and SB-XI were plated on moist blotter plate and incubated for

seven days at room temperature. After incubation, the fungal association and non- association was studied with the help of microscope.

## RESULT AND DISCUSSION

### I. Seed mycoflora of different varieties of Groundnut

In order to study varietal variations in the seed mycoflora, seeds of different varieties of Groundnut i.e. LGN-169, LGN-189, VG-9816, TAG-24 and SB-XI were plated separately on moist blotters and agar plates and incubated for seven days at room temperature. The percent incidence of seed-borne fungi was recorded and presented in table-1 and table-2.

From the results presented in table-1 and table-2, it was observed that among the seeds of five different varieties of Groundnut, there was variation in the degree of incidence of mycoflora in both moist blotter plate method and agar plate method. In all agar plate method yielded more per cent incidence of seed borne fungi as compared to moist blotter plate method.

### II. Identification of seed mycoflora of Groundnut

The fungi were identified by observing colony morphology and microscopic characteristic. Pure cultures of the isolated fungi were transferred to PDA slants and kept in refrigerator at 4°C for further use. The results are presented in table-3.

**Table 1:** Per cent incidence of seed mycoflora of different varieties of Groundnut by moist blotter plate method.

Sr. No.	Seed borne Fungi	Per cent incidence of seed-borne fungi on Groundnut varieties				
		LGN-169	LGN-189	VG-9816	TAG-24	SB-XI
1	<i>Alternaria alternate</i> (Fr.) Keissler	10	00	00	10	00
2	<i>Aspergillus candidus</i> Link	00	10	00	10	00
3	<i>Aspergillus flavus</i> Link ex Fr.	05	15	10	20	00
4	<i>Aspergillus fumigatus</i> Fresenius	15	00	00	10	00
5	<i>Aspergillus niger</i> van Tieghem	25	20	30	40	25
6	<i>Aspergillus terreus</i> Thom.	00	05	00	10	00
7	<i>Fusarium oxysporum</i> Schlechtend emend Sny. & Hans.	00	05	00	10	00
8	<i>Macrophomina phaseolina</i> (Tassi) Goldanich	00	00	10	10	00
9	<i>Rhizopus nigricans</i> Ehrenb.	40	00	45	20	00
10	<i>Sclerotium rolfsii</i> Sacc.	00	05	00	05	00
	S.E. $\pm$	1.47	0.64	1.69	2.62	0.91
	C.D. at 0.05%	4.36	1.90	5.01	7.77	2.71

**Table 2:** Per cent incidence of seed mycoflora of different varieties of Groundnut by agar plate method.

Sr. No.	Seed borne Fungi	Per cent incidence of seed-borne fungi on Groundnut varieties				
		LGN-169	LGN-189	VG-9816	TAG-24	SB-XI
1	<i>Alternaria alternata</i> (Fr.) Keissler	15	00	00	20	00
2	<i>Aspergillus candidus</i> Link	05	10	00	15	00
3	<i>Aspergillus flavus</i> Link ex Fr.	10	15	10	30	20
4	<i>Aspergillus fumigatus</i> Fresenius	20	00	00	10	00
5	<i>Aspergillus niger</i> van Tieghem	25	20	30	50	25
6	<i>Aspergillus terreus</i> Thom.	05	05	00	20	00
7	<i>Fusarium oxysporum</i> Schlechtend emend Sny. & Hans.	05	05	00	20	00
8	<i>Macrophomina phaseolina</i> (Tassi) Goldanich	05	00	10	10	00
9	<i>Rhizopus nigricans</i> Ehrenb.	55	30	50	60	20
10	<i>Rhizopus stolonifer</i>	20	00	00	25	00
11	<i>Sclerotium rolfsii</i> Sacc.	05	10	00	00	00
	S.E. $\pm$	1.56	2.40	1.80	2.88	1.56
	C.D. at 0.05%	4.59	7.07	5.32	8.50	4.59

**Table 3:** Microscopic characteristics used for the identification of Seed borne fungi of Groundnut.

Sr. No.	Name of Fungi	Microscopic characteristics
1	<i>Alternaria alternata</i> (Fr.) Keissler	The mycelium is profusely branched, brownish and septate. Conidiophores: Developed singly or in small groups, branched or unbranched. Conidia: In long chains (often branched), oval to ellipsoidal, with 2-7 transverse and 1-4 longitudinal or oblique septae, tapering end to form a short beak at the apex. The number of conidia in a chain varied from 2-8.
2	<i>Aspergillus candidus</i> Link.	Colony pale yellow, the fungus produces white as well as globose conidia-producing bodies which produce globose and subglobose conidia. These conidia were smooth, thin-walled and revealed to be about 2.5–3.5 $\mu\text{m}$ in diameter. Vesicles spherical to subspherical which were entirely covered with metulae.
3	<i>Aspergillus flavus</i> Link ex Fr.	The mycelium is found to be submerged in the seed coat and forms a white to grey, tough mass. Conidiophores erect, simple, unbranched, hyaline, transparent and smooth. The apex of the conidiophores was inflated into a vesicle upon which radiating phialides are formed. Conidial heads were biseriate, globose to radiate often columnar, very light to deep yellow green, olive brown often brown. Conidia were found to be hyaline, single celled and produced in chains. They were globose to subglobose, often elliptical to pyriform and conspicuously echinulate.
4	<i>Aspergillus fumigatus</i> Fresenius	The mycelium produced blue-dull green colony on PDA medium, conidial heads being light green to dull blue green, vesicles uniseriate, pyriform, conidial head columnar, compact, densely crowded, Conidia globose to subglobose, green in mass 2-3 $\mu\text{m}$ in diameter.
5	<i>Aspergillus niger</i> van Tieghem	Mycelium was found to be often scanty, hyaline to white or light yellow. Conidiophores were found to be developed directly from the seed coat They were hyaline to light brown, long, thin, unbranched, erect, brittle and terminating in to an inflated apex. Conidial heads appeared globose, but subsequently split into a few to several irregular or well-defined divergent columns of conidial chains. They were black, globose or radiate. Conidia found to be in chains on the sterigmata. They were single celled, pale to dark brown, more or less globose, with low to prominent ridges surfaces.

Table 3: Continued...

Sr. No.	Name of Fungi	Microscopic characteristics
6	<i>Aspergillus terreus</i> Thom.	Mycelium found to be rapidly growing with variable colony appearance ranging from heavily sporulating colonies to fluffy, poorly sporulating colonies. The conidiophores were long, columnar, hyaline and smooth giving rise to sub-spherical biseriate vesicles. Conidia were found to be smooth walled, globose to slightly elliptical and striate.
7	<i>Fusarium oxysporum</i> Schlechtend emend Sny. & Hans.	The mycelium found to be white to light pink, aerial, unbranched or branched, very short monophialides bearing microconidia on false heads. The microconidia produced on microconidiophores were abundant, hyaline, single celled, oval or elliptical. Macroconidia were found to be produced on the pale orange sporodochia. Macroconidia produced on macroconidiophores were hyaline, often 3 to 5 septate, falcate to almost straight, thin walled, with a curved apical cell and slightly foot-shaped basal cell. Chlamydospores were found to be terminal and intercalary, irregular in shape, thick walled with smooth surfaces.
8	<i>Macrophomina phaseolina</i> (Tassi) Goldanich	The mycelium found to be with thick hyphae. They were gray to brown or dark brown to black or dull white to light brown. Pycnidia were larger than the sclerotia, dark brown to black and scattered throughout the surface. They were found to be separate or confluent, rough, globose or irregular, beaked and ostiolate. Mature pycnidia were found to be dehisced and ooze conidia in a dull white, gelatinous mass. Conidia were aseptate, hyaline, ellipsoid to obovoid. Sclerotia were black, shiny, irregularly shaped.
9	<i>Rhizopus nigricans</i> Ehrenb.	The colonies were found to be whitish, with aerial mycelium and black spots of sporangia and dark sporangiophores. Rhizoids were well developed. Sporangiohores (on stolons) were found to be brown, in groups of 1-3. Sporangia were blackish, powdery in appearance. Columellae were conical and mouse-grey. Sporangiospores angular-globose-ellipsoidal and distinctly striate.
10	<i>Sclerotium rolfsii</i> Sacc.	The fungus was found to be produced white, dense, radiating mycelial growth on potato dextrose agar medium. In the early stages, the fungus was found to be produced white mycelium and gradually lost its luster and became somewhat dull in appearance. Aerial hyphae were not uniformly distributed. Initiation of sclerotial bodies were observed from fifth day onwards after inoculation. In the beginning, the sclerotial bodies were white which gradually turned to buffbrown colour and then to chocolate brown at maturity. The fully matured sclerotia were spherical, ellipsoidal.

Table 4: Associated and non-associated seed mycoflora of Groundnut variety TAG-24 (Moist Blotter Plate Method).

Common seed borne fungi of Groundnut var. TAG-24	Other seed borne fungi of Groundnut var. TAG-24										
	ALA	ASC	ASF	ASFU	ASN	AST	FUO	FUS	MAP	RHN	SCR
<i>Alternaria alternata</i> (Fr.) Keissler	+	-	+	-	-	-	-	-	-	+	-
<i>Aspergillus flavus</i> Link ex Fr.	+	+	+	+	+	+	-	-	-	+	-
<i>Aspergillus niger</i> van Tieghem	+	+	+	+	+	+	-	-	-	+	-
<i>Fusarium oxysporum</i> Schlechtend emend Sny. & Hans.	-	-	-	-	-	-	+	+	-	+	-
<i>Macrophomina phaseolina</i> (Tassi) Goldanich	-	-	-	-	-	-	-	-	+	+	-

ALA = *Alternaria alternata*ASN = *Aspergillus niger*FUS = *Fusarium semitectum*RHN = *Rhizopus nigricans*ASC = *Aspergillus candidus*AST = *Aspergillus terreus*MAP = *Macrophomina phaseolina*SCR = *Sclerotium rolfsii*ASF = *Aspergillus flavus*CLH = *Cladosporium herbarum*PES = *Penicillium sp.*ASFU = *Aspergillus fumigatus*FUO = *Fusarium oxysporum*RHB = *Rhizoctonia bataticola*

+ = Associated fungi

- = Non Associated fungi

**Table 5: Associated and non-associated seed mycoflora of Groundnut variety SB-XI (Moist Blotter Plate Method).**

Common seed borne fungi of Groundnut var. SB-XI	Other seed borne fungi of groundnut var. SB-XI										
	ALA	ASC	ASF	ASFU	ASN	AST	FUO	FUS	MAP	RHN	SCR
<i>Alternaria alternata</i> (Fr.) Keissler	+	-	+	-	-	-	-	-	-	+	-
<i>Aspergillus flavus</i> Link ex Fr.	-	-	+	+	+	+	-	-	-	+	-
<i>Aspergillus niger</i> van Tieghem	+	+	+	+	+	+	-	-	-	+	-
<i>Fusarium oxysporum</i> Schlechtend emend Sny. & Hans.	-	-	-	-	-	-	-	-	-	+	-
<i>Macrophomina phaseolina</i> (Tassi) Goldanich	-	-	-	-	+	-	-	-	+	+	-

ALA = *Alternaria alternata*ASN = *Aspergillus niger*FUS = *Fusarium semitectum*RHN = *Rhizopus nigricans*ASC = *Aspergillus candidus*AST = *Aspergillus terreus*MAP = *Macrophomina phaseolina*SCR = *Sclerotium rolfsii*ASF = *Aspergillus flavus*CLH = *Cladosporium herbarum*PES = *Penicillium sp.*ASFU = *Aspergillus fumigatus*FUO = *Fusarium oxysporum*RHB = *Rhizoctonia bataticola*

+ = Associated fungi

- = Non Associated fungi

### III. Associated and non-associated seed mycoflora

To study associated and non-associated seed mycoflora, two different varieties of groundnut (SB XI & TAG-24) were selected. For this five common seed borne fungi were selected and studied for their association with other seed borne fungi on the test Groundnut seeds, such observations are represented collectively in the table 4 and 5.

From the results presented in table 4, it was noticed that in case of Groundnut variety TAG-24, *Alternaria alternata* showed its association only with *Aspergillus flavus* and *Rhizopus nigricans* while it was not found to be associated with the fungi like *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium oxysporum*, *Fusarium semitectum*, *Macrophomina phaseolina* and *Sclerotium rolfsii*.

*Aspergillus flavus* was found associated with most of the fungi including *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus nigricans*.

*Aspergillus niger* was found associated with *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Rhizopus nigricans*.

*Fusarium oxysporum* was found to be associated with *Fusarium semitectum* and *Rhizopus nigricans*. *Macrophomina phaseolina* was found to be associated with only *Rhizopus nigricans*.

From the results presented in table-5, it was noticed that in Groundnut variety SB-XI, *Aspergillus niger* was found to be associated with most of the fungi including *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Rhizopus nigricans*.

*Fusarium oxysporum* was found to be associated with only *Rhizopus nigricans* and *Alternaria alternata* was found associated with *Aspergillus flavus*. *Rhizopus nigricans* was also found associated with all the test seed borne fungi.

### CONCLUSION

Fungi alone and in association can produce various types of toxic substances which can reduce the nutritional value of seeds and make them unsuitable for consumption.

Isolation studies on seed mycoflora of Groundnut varieties showed that some fungi were always found to be associated with a particular group of fungi and at the same time some fungi grew on the seeds individually without showing any association with any other type of fungi.

Similar types of results were recorded by Umatale, (1995) during his studies on fungal enzymes and toxins in biodeterioration of oil seeds.

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