

Original Research Article

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Mycoflora Associated with Groundnut Seeds Collected from Selected Groundnut Growing Districts of Telangana State, India

G. Adithya^{1*}, B. Rajeshwari¹, K. Keshavulu², H. Sudini³ and Y. Swathi¹¹PJTSAU, Telangana, India²TSSCA, Telangana, India³ICRISAT, Patancheru, India**Corresponding author*

ABSTRACT

Groundnut is an important food legume in several developing and developed countries. In India, Telangana state is one of the major groundnut growing states. A survey was conducted to collect a total of 72 groundnut pod samples at the time of harvesting from farmers and local markets in four districts, namely; Karimnagar, Warangal, Nizamabad, Mahabubnagar during 2015-16. These samples studied for detection of seed mycoflora by Agar plate method as recommended by ISTA. The major mycoflora associated with seeds belongs to five fungal genera such as *Aspergillus*, *Fusarium*, *Alternaria*, *Macrophomina*, *Penicillium* and total incidence was ranged from 0.67 % - 47.11 %. Samples collected from farmers were highly infected with different mycoflora compared to the samples collected from market. *A. niger* was predominant (47.11 %) while, the least was *Penicillium* (0.44 %). Out of four districts surveyed, the total incidence of mycoflora was high in Mahabubnagar and low in Nizamabad. The fungal species *A. niger* was found associated with all the collected samples of four districts at maximum incidence when compared to other fungal species. Current results imply the urgent need for application of management measures against different seed borne fungi to maintain the quality of

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop in India. It contains oil to an extent of 48 - 51 %. In India, the crop is grown to an extent of 5.53 M ha with a production of 9.67 M t and productivity of 1750 kg ha⁻¹ (FAOSTAT, 2013 - 14).

In Telangana state, the crop is grown to an extent of 0.21 M ha with a production of 0.355 M t and productivity of 1320 kg ha⁻¹ (Statistical year book of Telangana, 2015). Groundnut being an oil seed, it contains lesser amount of carbohydrate than cereals but more amount of oil and protein and are likely to be

breakdown into simple sugars and amino acids which is essential for germinating seed as an energy source. Ramamoorthy and Karivaratharaju (1989) noticed that progressive decrease in oil and protein content and an increase in free fatty acids in the stored kernels than in the pods because of the invasion of storage fungi to kernels.

Increasing the storage period of groundnut seeds upto nine months decreases the viability, while pathogen activity, moisture and sugar content in seeds increase gradually.

Groundnut seeds are rich in mono unsaturated fats, the type of fat that is emphasized in the healthy heart mediterranean diet.

Seeds are generally associated with certain saprophytic or parasitic micro-organisms which perpetuate in the seed lots on the advent of favourable conditions. Groundnut seeds are highly susceptible to fungi such as, *Rhizopus*, *Aspergillus flavus*, *Penicillium*, *Fusarium*. Infection of groundnut by species of *Aspergillus* occurs both at pre-harvest and post-harvest stages (Dharmaputra *et al.*, 2003, Craufurd *et al.*, 2006 and Goncales *et al.*, 2008).

Groundnut plants are highly susceptible to fungal contamination, including toxin producing fungi. However, toxigenic fungal pathogens are important constraints to the production of the crop, affecting the quality of the seeds during storage.

Generally there are several toxigenic fungi types, but the predominant fungi *Aspergillus*, *Fusarium* and *Alternaria*. Post - harvest conditions favour infestation during storage which may lead to production of different mycotoxins.

Mould fungi are also known to produce mycotoxin (Rodricks, 1976). Many workers have detected different mold fungi and their toxin production ability in stored grains, which deteriorate seed products (Afjal *et al.*, 1979, Vedahayagam *et al.*, 1989).

The objective of the present study was designed to examine the predominant fungal species which causes storage losses. This knowledge of the distribution of contamination by fungal species in the selected regions is helpful to follow reduction strategy in future (Bankole and Adebajo, 2003).

Materials and Methods

Collection and processing of seed samples

Groundnut seeds were collected from farmers and local markets of selected districts (Karimnagar, Nizamabad, Mahabubnagar and Warangal) of Telangana state and the mycoflora associated with seeds were detected by following agar plate (Musket, 1948; Agarwal, 1976; ISTA, 1966) method. In each district, three farmers and three markets were chosen based on the groundnut growing locations. Three samples were collected from each farmer and market and a total of 18 samples were collected per each district. A total of 72 samples were collected from the four districts and assessed for seed mycoflora. Approximately 1 kg of pods were collected from each, brought to the laboratory and dried in paper bags. Pods were then hand shelled and divided into sub samples. These samples were used for examination of seed mycoflora.

Detection of seed mycoflora

Agar plate method (ISTA, 1966)

Potato Dextrose Agar (PDA) was prepared and sterilized in an autoclave. About 20 ml of the medium was distributed to each of the sterile Petri plate under aseptic conditions. Groundnut seeds were transferred to the plates containing PDA medium. Ten seeds per plate were placed at equidistance in a circular fashion. Four hundred seeds from each sample were placed in the plates in four replications. The Petri plates were incubated at $25 \pm 2^{\circ}\text{C}$ in the incubator for seven days and observed every day for the growth of fungi. Per cent infection was assessed as suggested by Jha (1995) and the per cent incidence of each species was calculated as follows.

No. of seeds colonized in each plate by a particular fungal species
Total fungal
Colonies (%) = $\frac{\text{Total fungal Colonies}}{\text{Total number of seeds in each plate}} \times 100$

Potato Dextrose Agar (PDA) medium

Peeled potato pieces were boiled in 500 ml of distilled water in a 1000 ml beaker till the pieces got softened and the extract were collected in a beaker by sieving through a double layered muslin cloth. Agar - agar was melted in another 500 ml of distilled water in 1000 ml beaker into which 20g dextrose was added. The final volume of the medium was made up to 1000 ml by adding sterile distilled water. The pH of the medium was adjusted to 6.8 with 0.1 N NaOH or 0.1 N HCl as the case may be with the pH meter. The medium was sterilized in an autoclave at 15 psi for 15 minutes.

Frequency of groundnut seed contamination

After keeping the seeds for incubation data were recorded on the number of infected and non-infected kernels. The frequency of different mycoflora associated was determined by proportion of kernels contaminated by each fungal species to the total number of kernels plated.

Isolation and identification of fungal species

Fungi identification was carried out based on macro morphological characteristics like surface coloration of colonies and colony texture and micro morphological characteristics like conidial head, conidia shape and shape of vesicle. Isolates were observed and identified based on morphological feature used by Cotty, 1994, Eghel *et al.*, 1994, Kurtzman *et al.*, 1997,

Okuda *et al.*, 2000. Fungal colonies that grew rapidly and produced white, yellow, yellow brown, brown to black or shades of green, mostly consisting of adence felt of erect conidiophores were classified as *Aspergillus* species. Medium, creamy white or white colonies with puffed growth sometimes. The colony color may vary creamy white/white to light orange colonies, dense mycelial/hyphal growth are categorized as *Fusarium* species. Grey white/grey colour to black color suppressed colonies. In some isolates aerial mycelium also observed. Microconidia also observed and after sclerotial formation the entire colony turn into black color were classified as *Macrophomina* species. On the other hand, grey to green color suppressed colonies were present. Initial creamy white to light green color colonies with fast growing were classified as *Penicillium*. Initially grey/white/black color colonies, dense hyphal colonies with supplemental growth were categorized as *Alternaria* species.

Results and Discussion

The incidence of seed borne fungi associated with groundnut kernels varied among different districts surveyed. The seeds are the basic input in agriculture and it plays vital role in establishment of a healthy crop. Seed mycoflora greatly influences the germination and establishment of crop stand. In our study, the results indicate that a total of six fungal species viz., *A. flavus*, *A. niger*, *Fusarium*, *Alternaria*, *Macrophomina*, *Penicillium* were found to be associated with the seeds of groundnut. The total incidence was ranged from 0.67 % - 47.11 %. Samples collected from farmers were highly infected with different mycoflora when compared to samples collected from market. Out of these six fungal species *A. niger* was predominant (47.11 %) while, the least was *Penicillium* (0.44 %). Out of four districts surveyed the total incidence of mycoflora was high in

Mahabubnagar and low in Nizamabad. The fungal species *A. niger* was found to be associated with all the collected samples of four districts at maximum incidence when compared to other fungal species (Tables 1 and 2).

Seed mycoflora of farmer samples

Among the samples collected from farmers (Table 1), highest incidence was in Mahabubnagar (84.78 %) followed by Warangal (80.12 %), Karimnagar (72.23 %) and Nizamabad (56.68 %) district. Samples collected from Mahabubnagar district were high incidence of fungal species with *A. niger* (47.11 %) followed by *A. flavus* (34.44 %), *Alternaria* (0.89 %), *Penicillium* (0.89 %),

Fusarium (0.78 %) and *Macrophomina*. Samples collected from Warangal district were high incidence of fungal species with *A. niger* (43.55 %) followed by *A. flavus* (32.22 %), *Fusarium* (1.90 %), *Penicillium* (1.00 %), *Macrophomina* (0.78 %) and *Alternaria* (0.67 %). Samples collected from Karimnagar district were high incidence of fungal species with *A. niger* (43.11 %) followed by *A. flavus* (25.56 %), *Fusarium* (1.00 %), *Macrophomina* (1.00 %) *Alternaria* (0.89 %) and *Penicillium* (0.67 %). Samples collected from Nizamabad district were high incidence of fungal species with *A. niger* (39.11 %) followed by *A. flavus* (14.67 %), *Fusarium* (0.78 %), *Penicillium* (0.78 %), *Macrophomina* (0.67 %) and *Alternaria* (0.67 %) (Fig. 1).

Table.1 Extent of groundnut seed mycoflora in fresh harvest at farmers’ level collected from Karimnagar, Warangal, Nizamabad, Mahabubnagar districts of Telangana

DISTRICTS	<i>A. niger</i>	<i>A. flavus</i>	<i>Fusarium</i>	<i>Alternaria</i>	<i>Macrophomina</i>	<i>Penicillium</i>	TFC (%)
Karimnagar	43.11 (41.00)	25.56 (30.30)	1.00 (5.98)	0.89 (5.70)	1.00 (5.97)	0.67 (5.25)	72.23
Warangal	43.55 (41.20)	32.22 (34.54)	1.90 (7.61)	0.67 (5.26)	0.78 (5.45)	1.00 (5.97)	82.12
Nizamabad	39.11 (38.65)	14.67 (21.43)	0.78 (5.51)	0.67 (5.33)	0.67 (5.25)	0.78 (5.51)	56.68
Mahabubnagar	47.11 (43.46)	34.44 (35.85)	0.78 (5.51)	0.89 (5.62)	0.67 (5.97)	0.89 (5.70)	84.78
SE(m)±	2.11	1.69	1.08	0.91	1.05	0.96	
CD (0.05)	6.18	4.95	3.15	2.65	3.06	2.80	

Table.2 Seed mycoflora of groundnut market samples collected from Karimnagar, Warangal, Nizamabad, Mahabubnagar districts of Telangana

DISTRICTS	<i>A. niger</i>	<i>A. flavus</i>	<i>Fusarium</i>	<i>Alternaria</i>	<i>Macrophomina</i>	<i>Penicillium</i>	TFC (%)
Karimnagar	19.56 (26.22)	15.00 (22.75)	0.78 (5.53)	0.56 (5.06)	0.67 (5.25)	0.67 (5.26)	37.24
Warangal	21.89 (27.83)	19.22 (25.91)	0.78 (5.51)	0.78 (5.45)	1.00 (5.98)	1.33 (6.54)	45.00
Nizamabad	16.67 (23.99)	13.67 (21.61)	0.78 (5.45)	0.78 (5.51)	0.44 (5.25)	0.44 (4.79)	32.82
Mahabubnagar	25.00 (29.91)	26.22 (30.70)	1.44 (6.80)	0.78 (5.51)	0.67 (5.97)	0.67 (5.33)	54.78
SE(m)±	1.37	1.52	1.22	0.99	0.98	1.00	
CD (0.05)	4.00	4.45	3.58	2.89	2.73	2.93	

Fig.1 Seed mycoflora of groundnut farmer samples collected from Karimnagar, Warangal, Nizamabad, Mahabubnagar districts of Telangana

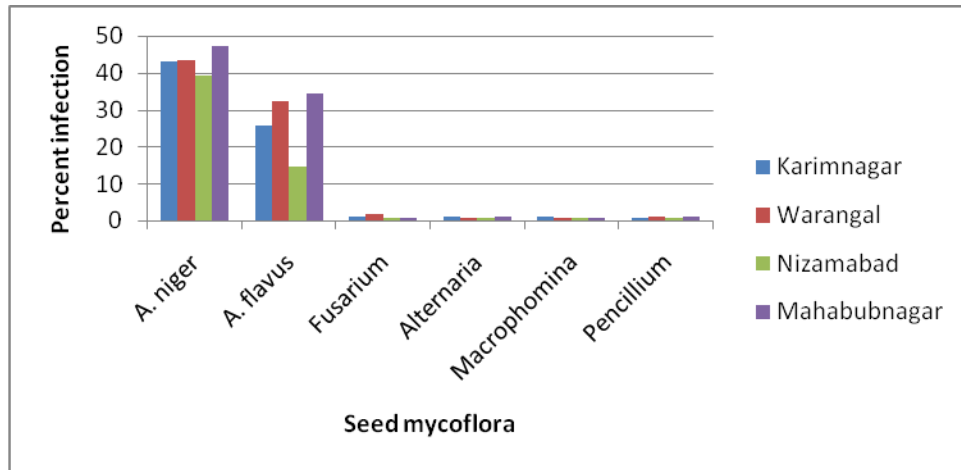
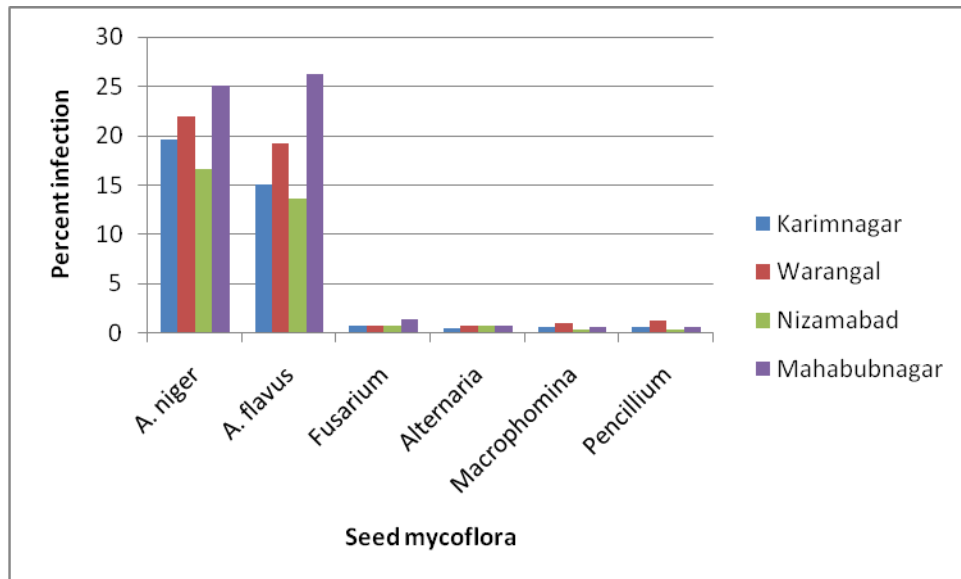


Fig.2 Seed mycoflora of groundnut market samples collected from Karimnagar, Warangal, Nizamabad, Mahabubnagar districts of Telangana



Seed mycoflora of market samples

Among samples collected from markets (Table 2) highest incidence was in Mahabubnagar (54.78 %) followed by Warangal (45.00 %), Karimnagar (37.24 %) and Nizamabad (32.82 %) district. Samples collected from Mahabubnagar district were high incidence of fungal species with *A. niger* (26.22 %) followed by *A. flavus* (25.00 %),

Fusarium (1.44 %), *Alternaria* (0.78 %), *Penicillium* (0.67 %), and *Macrophomina* (0.67 %). Samples collected from Warangal district were high incidence of fungal species with *A. niger* (21.89 %) followed by *A. flavus* (19.22 %), *Penicillium* (1.33 %), *Macrophomina* (1.00 %) *Fusarium* (0.78 %) and *Alternaria* (0.78 %). Samples collected from Karimnagar district were high incidence of fungal species with *A. niger* (19.56 %)

followed by *A. flavus* (15.00 %), *Fusarium* (0.78 %), *Macrophomina* (0.67 %), *Penicillium* (0.67 %) and *Alternaria* (0.56 %). Samples collected from Nizamabad district were high incidence of fungal species with *A. niger* (16.67 %) followed by *A. flavus* (13.67 %), *Fusarium* (0.78 %), *Alternaria* (0.78 %), *Penicillium* (0.44 %) and *Macrophomina* (0.44 %) (Fig. 2).

Risk and sensitive areas in Telangana

In Telangana, two surveyed areas were categorized under risk zone for kernel infection (%) by *A. flavus* and *A.niger*. Based on the mycoflora contamination in the pod samples, sampled in Mahabubnagar and Warangal districts were categorized as sensitive areas or risk zone. The other that fall in Nizamabad and Karimnagar districts were categorized as safe zone.

Our studies indicated that Telangana have significant levels of mycoflora contamination in kernels at farmers. Post-harvest contamination of groundnut pods during storage at farmers/markets in the present study is attributed to either the improper storage conditions or the carry over inoculums from field to farmers storage and ultimately to markets. Improper storage practices are the major factors during storage. Pod storage at high moisture levels increases post-harvest molding. Besides, storing the pods at optimum moisture levels, pod drying by farmers immediately after harvest also helps in reducing the infection during storage. Inverted windrowing is an ideal drying procedure of pods after harvest. It helps in proper drying of pods with adequate exposure to sunlight.

From among the *Aspergillus* species isolated in the current study, it is also agreement with Guchi *et al.*, (2014) *A. flavus* and *A. niger* were more prevalent species infecting

groundnut samples collected from farmers' fields than from farmers stores, market retailers and vendors in the five agro-ecologies of eastern Ethiopia. Present study also agreement with groundnut seeds collected from store house and oil mills of Warangal district were infested by different seed borne fungi varying in percentage different sample conditions and place of collection (Kalyani *et al.*, 2014). The finding of the present study is in agreement with the findings of Abdi and Alemayehu (2014) who reported that groundnut samples collected from farmers' fields and farmers store houses in Babile and Gursum districts had 80 and 70 % kernel contamination, respectively, by *Aspergillus* species. Present results showed that *A. flavus* and *A. niger* were the predominant fungi of groundnut which are similar to studies conducted by Mukherji *et al.*, (1992) and Naqui *et al.*, (2013). They also found that *A. flavus* and *A. niger* were the predominant storage fungi of groundnut seed and also compared with results found with Rasheed *et al.*, (2004). These results are also agreement with the *A. flavus* (72 %) gave highest percent incidence followed by *A. niger* (68 %) and *F. oxysporium* (55 %) (Rathod *et al.*, 2012).

In conclusion, the study has demonstrated a high prevalence of the fungi associated with groundnut kernels. *Aspergillus* sp. is an important fungi associated with groundnut seeds. However, their occurrence and level of infection is more or less similar among all the locations. The fungi *Aspergillus* sp. association with seed is a likely to be a threat in storage as it increases its infection under improper storage conditions. Hence, there is a need for reducing the mold growth and mycotoxin production by improving the storage conditions. There is also a need to increase public awareness among farmers in aspects of seed health because presence of so many pathogenic fungi could be an indicator

of poor soil health. Awareness in this direction can help the farmers to develop suitable management practices for improving the quality of harvest.

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