

Screening of *Aspergillus* And Aflatoxin From *Arachis hypogaea*

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Abstract: This is a preliminary investigation of the incidence and aflatoxins in *Arachis hypogaea*. The survey was based on groundnut samples, collected from local market. Most dominating fungi was found to be *A. flavus* followed by *Rhizopus* sp, *Mucor* sp. It was found that *A. flavus* produce aflatoxin G. From this study, it was clear that heavy fungal load in groundnut was present, however further research is needed in this field.

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important food and feed crop, which also serve as component of crop rotation in many countries (Pande et al., 2003; Upadhyaya et al., 2006). Groundnuts are also significant source of cash in developing countries that contribute significantly to food security and alleviate poverty (Smart et al., 1990). Developing countries account for 97% of the world's groundnut area and 94% of the total production (FAOSTAT, 2010). However, groundnut yield in this part of the world and particularly in Africa is lower than the world average due to prevailing abiotic, biotic and socio-economic factors (Pande et al., 2003; Upadhyaya et al., 2006; Caliskan et al., 2008). In warm climates grains are easily infected with toxigenic microorganisms like *Aspergillus* species. *Aspergillus* spp. are facultative parasites. They invade host plant tissues directly or attack tissues that have been predisposed by environmental stresses such as dry weather or damages caused by insects, nematodes, natural cracking, and harvest equipment (Pettit and Taber, 1968). They are distributed worldwide, mainly in countries with tropical climates that have extreme ranges of rainfall, temperature and humidity (Pettit and Taber, 1968). Many strains of this fungus are capable of producing aflatoxins that render the seed unacceptable due to high toxicity for human or animal consumption (Reddy et al., 1996). Aflatoxins are highly toxic metabolites associated with Reye's syndrome, Kwashiorkor and acute hepatitis (Wild and Hall, 1999; Wild and Turner, 2002). Mycotoxins, the secondary metabolites of toxigenic fungi, commonly occur in various agricultural products including food and feed stuffs and are a potential threat to the health of humans and animals. Because of the associated health risks of mycotoxin consumption, their presence in many foodstuffs has drawn the attention of scientists and

the general public in several parts of the world (Campbell and Stoloff, 1974). The objectives of this study were to: i) Isolation and identification of *Aspergillus* from *Arachis hypogaea*. ii) analysis of Aflatoxin produced by *Aspergillus*.

Materials and Methods

Collection of sample

Ground nuts were collected from local markets of selected locations and stored in sterile polythene bags.

Isolation and identification of Microorganism

Seeds were surface sterilized in 1% sodium hypochlorite solution for 10 minutes and then rinsed 5-6 times with sterile distilled water (SDW). The surface sterilized seeds were aseptically plated on 90 mm diameter petri plates containing modified Czapek Dox agar medium. Four seeds were placed on each petri plate. The plates were incubated at $28 \pm 1^\circ\text{C}$ for 7 days and then observed under a microscope for seed colonization by fungi. Based on cultural and morphological characteristics fungi were identified.

Extraction and Analysis of Aflatoxin and amino acid

Preparation of aflatoxin extract

CD broth was inoculated with spores from 8-day-old cultures of *A. flavus*. The spore concentration was adjusted to 10^5 spores/ml using a haemocytometer then flask was incubated at $28 \pm 1^\circ\text{C}$ on a mechanical shaker at 120 rpm for 20 days. The cultures were then filtered through Whatman's filter paper No. 1 and the culture filtrates were collected. To 20 ml of culture filtrate, 20 ml of chloroform was added and shaken for 5 minutes in a separating funnel. The chloroform layer was collected. Finally, the chloroform was evaporated to dryness on a rotary vacuum evaporator and the residue was dissolved in distilled water and used for TLC analysis.

Analysis of aflatoxin

Ten ml of sample was spotted on glass plates coated with activated silica gel G and the plates were developed with acetone: chloroform (12:88) and the plates were observed for fluorescence under UV light.

Analysis of amino acid

Sample was spotted on paper strip. Strip was kept in solvent system containing butanol:acetic acid : water(4:1:5). When solvent reached to maximum level on strip then it was removed from solvent. Strip was then air dried. Now paper strip was dipped in Ninhydrin solution then oven dried. Finally it was observed for spots developed on it.

Antibiotic sensitivity of *A. flavus*

Agar well-diffusion method was followed to determine the antifungal activity. Czapek dox Agar(CDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of fungi. Well (5mm diameter) was made in plate using sterile cork borer. Stock solution of antibiotic solution was prepared and 0.1 ml of antibiotic solution was transferred into the well. Plates were incubated at $28 \pm 1^\circ\text{C}$ for 6-8 days. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

Results

During this investigation following results were obtained :

Seed infection by fungi

Four different fungi were found to be associated with groundnut samples collected from local market. After staining it was cleared that three isolated fungi were of *A. flavus*, *Mucor* sp, *Rhizopus* sp and last one was unidentified. Most dominating fungi was *A. flavus* followed by *Rhizopus*, *Mucor*. Result has been shown in table 1.

Detection of Aflatoxin and amino acid

On spot was observed which exhibits bright green fluorescence when exposed to U.V. light this indicated the presence of Aflatoxin G in filtrate. It was found that *A. flavus* produced glycine, tyrosine, leucine, threonine and phenylalanine. Results has been tabulated in table 2, 3.

Antibiotic sensitivity

After measuring the diameter of zone it was found that *A. flavus* was sensitivity towards Fluconazole (6mm) and resistant against Tolnaftate. Results has been shown in table 4

Discussion

In the present investigation seeds were contaminated with fungi. Most prominent fungi was

found to be *A. flavus*. Pre-harvest aflatoxin contamination of groundnuts was reported to occur commonly in several parts of the world (Joffe, 1970). In a previous study, it was observed that average seed infection by *Aspergillus flavus* and *Fusarium* spp. was 11.9-18.3% and 5.6-12.8% in 1999, and 9.5-14.1% and 9.4-11.9% in 2000, respectively (Kishore et al., 2002). In present study it was found that *A. flavus* produce primary(glycine, tyrosine, leucine, threonine and phenylalanine) and secondary metabolites (Aflatoxin G) both. Similar results were found in previous study done by Bhat et al. (1996). They reported that among the samples collected from 11 states of India, 44.9% and 37.4% samples contain aflatoxins of over 5 mg/kg and 15 mg/kg, respectively, and among the samples collected from Andhra Pradesh, 15.2% were contaminated with aflatoxins. *A. flavus* produce aflatoxins during post-harvest stages, depending on the conditions of drying and storage. Improper drying of groundnuts allowing them to retain high moisture content and poor storage conditions favors post-harvest aflatoxin contamination.

Conclusion

Results of the current survey revealed heavy contamination of groundnuts by various fungi.

In combination with earlier report on aflatoxin contamination of groundnut, the current results should serve as a wakeup call to create awareness on toxicogenic fungi and associated mycotoxins. The Role of none chemical seeds treatments especially essential oils and that of biological control agents in reducing groundnut contamination should be studied to come up with a more effective and sustainable management strategy. In Previous study done by Trivedi and Singh (2014) reported that essential oil of *Cymbopogon citratus* inhibited the growth of *A. flavus*. Farmers' association and extension agents should also be encouraged in creating awareness about aflatoxins and management techniques.

Table 1. Enumeration of Fungi isolated from *Arachis hypogaea* seed

| S.NO. | Microorganisms | Cfu* (mean±SE) |
|-------|---------------------------|----------------|
| 1. | <i>Aspergillus flavus</i> | 10±0.8 |
| 2. | <i>Rhizopus</i> | 8±0.8 |
| 3. | <i>Mucor</i> | 7±0.3 |
| 4. | unidentified | 1±0.3 |

* data obtained is average of triplicates SE "Standard error"

Table 2. RF values of aflatoxin produced by *A. flavus*

| S.NO. | Sample | RF value | Florescence |
|-------|------------------|----------|-------------|
| 1. | Culture filtrate | 0.26 | Green |
| | | 0.11 | Green |

Table 3. RF values of amino acids produced by *A. flavus*

| S.NO. | Sample | RF value | Amino acid |
|-------|----------|----------|---------------|
| 1 | Sample A | 0.25 | Glycine |
| | | 0.45 | Tyrosine |
| | | 0.80 | Leucine |
| 2 | Sample B | 0.33 | Threonine |
| | | 0.69 | Phenylalanine |

Table 4. Antibiotic sensitivity of *A. flavus* towards various antibiotic

| S. NO. | Antibiotic | Concentration (mg/ml) | ZOI (mm) (mean±SE)* |
|--------|-------------|-----------------------|---------------------|
| 1. | Fluconazole | 0.015 | 6±0.5 |
| 2. | Tolnaftate | .005 | - |

* data obtained is average of triplicates
SE "Standard error"

References

- Bhat RV, Vasanthi S, Rao BS, Rao RN, Rao VS, Nagaraja KV, Rai RG, Prasad CA K, Vanchinathan S, Raja R, Subrata S, Mukherjee A, Ghosh PK, Tojeta GS, Saxena BN, Roy R, Saha S. Aflatoxin B1 contamination in groundnut samples collected from different geographical regions of India: a multicentre study. *Food Addit Contam* 1996; 13:325-331.
- Caliskan S, Arslan M, Arioglu H. Effects of sowing date and growth duration on growth and yield of groundnut in a Mediterranean-type environment in Turkey. *Field Cro. Res* 2008; 105:131-140.
- Campbell TC, Stoloff L. Implications of mycotoxins for human health. *J. Agric. Food Chem* 1974; 22:1006-1015.
- FAOSTAT (2010). Groundnut world production. <http://www.faostat.fao.org>.
- Joffe AZ. The presence of aflatoxin in kernels from five year groundnut crops and *Aspergillus* isolates from the kernels and soils. *Plant Soil* 1970; 33:91-96.
- Kishore GK, Pande S, Manjula K, Narayana J, Thomas D. Occurrence of Mycotoxins and Toxigenic Fungi in Groundnut (*Arachis hypogaea* L.) Seeds in Andhra Pradesh, India. *Plant Pathol* 2002; J. 18(4): 204-209.
- Pande N, Saxena J, Pandey H. Natural occurrence of mycotoxins in some cereals. *Mycoses* 2003; 33:126-128.
- Pettit RE, Taber RA. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. *Appl. Microbiol* 1968; 16:1230-1234.
- Reddy LJ, Nigam SN, Moss JP, Singh AK, Subrahmanyam P, McDonald D, Reddy AGS. Registration of ICGV86699 peanut germplasm line with multiple disease and insect resistance. *Crop Science* 1996; 36: 821.
- Smart MG, Shotwell OL, Caldwell RW. Pathogenesis in *Aspergillus* ear rot of maize: aflatoxin B1 levels in grains around wound inoculation sites. *Phytopathol* 1990; 80: 1283-1286.
- Trivedi B, Singh P. Antifungal activity of *Cymbopogon citratus* leaves extract against herbal drug contaminants. *WJPPS* 2014; 3(6): 768--775.
- Upadhyaya HD, Reddy LJ, Gowda CLL, Singh S. Identification of diverse groundnut germplasm: Sources of early maturity in a core collection. *Field Cro. Res* 2006; 97: 261-271.
- Wild CP, Hall AJ. Hepatitis B virus and liver cancer: Unanswered questions. *Can. Surv* 1999; 33: 35-54.
- Wild CP, Turner PC. The toxicology of aflatoxins as basis for public health decisions. *Mutagenesis* (2002); 17: 471-481.

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