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# TLC for the Detection of Aflatoxin in Groundnut (*Arachis hyposaea* L.) Kernels

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Cash crops such as groundnut, cashew, cotton, sesame, sugarcane, tobacco, turmeric and banana play vital role in the growth of economic and nutritional status in India. Of these, groundnuts (*Arachis hyposaea* L.) is the most important majer crops, which is energy rich and micro nutrient contents are relatively high compared with other corps. Out of total production, 12% is used as seeds, 8% for edible purpose, 70% extraction of oils and 10% for export and hence it is considered to be an economically important crop. This crop is found to be attacked by number of fungal diseases. Besides these fungal diseases groundnut is found to be affected by "Aflatoxins". They are probably the most serious fungal disease of groundnut on a worldwide scale. Aflatoxins are a group of highly oxygenated heterocyclic compounds with closely related structure of $B_1$, $B_2$, $G_1$ and $G_2$. These aflatoxins are closely related group of secondary fungal metabolites which have been epidemiologically implicated carcinogens in human being. The carcinogenic properties of aflatoxin have reinforced the concept that naturally occurring mycotoxins may be involved in the etiology of human cancer on a broad basis. Aflatoxin producing fungi are widely distributed in tropical and subtropical areas. Groundnut is highly susceptible to aflatoxin contamination. An attempt has been made to determine the possible variations in the amount of aflatoxin-B, present in the groundnuts grown in two different fields treated with chemical fertilizers and organic manure alone independently.

**Key Words: Thin layer chromatography, Aflatoxin, Groundnut.**

## INTRODUCTION

Four different varieties (VR-2, B-95, ICGV-86564, ICGV-90210) of groundnuts are grown in two different fields, *viz.*, chemical fertilizers and organic manure and the groundnuts kernels are collected field wise adopting standard sampling procedure. Thin layer chromatography (TLC) technique has been employed for the estimation of aflatoxin in groundnut kernels. According to TLC analysis, groundnut varieties grown in fields treated with organic manure are free from aflatoxin while groundnuts varieties from the fields treated with chemical

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fertilizers are found to contain a definite quantity of aflatoxin. *Results confirm* that except $B_1$ type aflatoxin, no other toxin is found in all the four groundnuts varieties treated with chemical fertilisers. The quantities of aflatoxin-B, are estimated and they found to vary from 0.015 to 0.030 ppm.

Aflatoxins are highly substituted coumarins containing a fused dihydrofurofuran moiety and are named because of their production by the fungus, *Aspergillus flavus* (*A. flavus* toxins). These substances are produced by the growth of *Aspergillus flavus* on food products when sufficient moisture is present. The term aflatoxin is used to a group of organic metabolites of certain strains of fungi belonging to the species *Aspergillus flavus*.[1] From the point of view of health and economics, it is imperative that only very low levels of aflatoxin are admitted. Of the different types of aflatoxins, aflatoxin-$B_1$ is one of the most potent hepatocarcinogens known to human, domestic animal, cells in culture, micro organisms and plants.[2]

Contamination of aflatoxin in groundnuts and groundnut products was first realised in the early 1960s and this lead to a renewed interest in research into mycotoxins. Groundnut is highly susceptible to aflatoxin contamination.[3] In India groundnut is consumed as raw, roasted, fried, salted, spiced in sweet toffee forms. Groundnut based items are popular and human exposure to aflatoxin occurs on consumption of these products.

In the recent past, a wide range of chemicals and bio-agents have been tested against pre- and post-harvest aflatoxin contamination of peanut kernels. Environmental conditions, especially water activity temperature and composition of intergranular air has been shown to influence succession of microorganisms on agricultural commodities. The environmental conditions to which these raw materials are exposed immediately after harvest in India are responsible for growth of *Aspergillus* spp. which has been reported in the raw materials like maize, groundnut cakes, oils and rice and also the presence of aflatoxin-$B_1$ is well recognised.

In order to protect the health and productivity of both man and animals aflatoxin in food and feeds has to be reduced to an acceptable level.

Prevention of aflatoxin formation has also been attempted by making use of plant extracts and fungus agents[4] but the process is expensive and tedious rendering it impractical. Hence approaches involving physical, chemical and biological methods have been made to detoxify aflatoxins in food and food stuff in the recent past. Several environmental factors influence the production of aflatoxin in the field and during storage when the conditions are favourable.

An attempt has been made in the present work is to detect and estimate some atoxigenic strains of *A. flavus* in groundnut kernels obtained from fields subjected to two different fertiliser treatments, namely chemical fertiliser and organic manure.

## EXPERIMENTAL

A wide variety of analytical methods have been developed for detecting the aflatoxins, using high performance liquid chromatography (HPLC), thin layer

chromatography (TLC), gas chromatography/mass spectrometry (GC/MS) and capillary electrophoresis (CE). In addition, a variety of immunochemical methods such as enzyme-linked immunosorbent assays (ELIASs) have been developed for the aflatoxins and their metabolites that allows for the rapid screening for this group of toxins in corn.

Many studies have been carried out on aflatoxin due to concern about their toxic effect on humans and animals especially their very strong mutagenicity and carciongenicity. The development of methods of analysis for aflatoxins was necessary for these studies and there is still a very strong demand for the development of new and improved techniques. Thin layer chromatography (TLC) has been used as the only practical method for the analysis of aflatoxin. The accurate measurement of aflatoxins of TLC plates by either visual or fluorodensitometric methods depends on adequate sampling of the agricultural product submitted for analysis. TLC method has been considered as semi quantitative, but the diffusion of sophisticated densitometer and sample application devices has significantly improved the accuracy of TLC analysis.

Early aflatoxin assay methods were based on the "dilution of extinction" technique. These methods involve visual comparison of intensity of fluorescence of sample to internal standard as spots on uniform absorbent layers on TLC plates.

**Sample Preparation**

In the present study, four varieties of groundnut kernels (VRI-2, B-95, ICGV-865664, ICGV-90210) obtained from Regional Research Station, Tamil Nadu Agricultural University, Virudhachalam, Tamil Nadu, India are taken and all the four varieties are grown in two different fields treated with chemical fertiliser.

$T_1$—*Treatment 1*: Gypsum 400 kg $ha^{-1}$ in two times, rhiylim 2 kg $ha^{-1}$, phosphobactor 2 kg $ha^{-1}$, micronutrient mixture 12.5 kg $ha^{-1}$, farmyard manure 12.5 tonnes $ha^{-1}$ and organic manure.

$T_2$—*Treatment 2*: Press mud 25 tonnes $ha^{-1}$, neem cake 250 kg $ha^{-1}$, rhizobium 250 kg $ha^{-1}$, phosphobactor 4 kg $ha^{-1}$.

Groundnut kernels comprising all the four varieties have been raised in these fields and are collected fieldwise by following standard procedure. The harvested groundnut kernels are dried at 60°C for 48 h and dried kernels are kept at room temperature in sealed plastic bags. Thin layer chromatographic (TLC) experiments are performed twice with treatment in each variety.

**Extraction and identification of aflatoxin $B_1$**
**[Derivative formation TLC plate, Ref. 5]**

50 g of the dried groundnut kernel (both chemical fertiliser and organic manure treated) samples are taken in a 500 mL glass stoppered Erlenmeyer flask containing 25 mL of $H_2O$, 25 g diatomaceous earth and 250 mL of $CHCl_3$. It is shaken well for about 30 min on wrist action shaker and then filtered through fluted paper.

The silica gel plate is divided into two equal vertical sections by scoring thick line on the plate. One section of the silica gel plate is covered with a clean glass

plate and the uncovered side is spotted two 1–10 μL aliquots of samples extract prepared for TLC containing 0.5–5 ng of standard aflatoxin $B_1$.

The extract plus standard and standard spots are added with 1 μL trifluoro acetic acid (TFA) and let it is allowed to react 5 min. with the use of blow warm air temperature 35–40°C for 10 min. The plate is developed in $CHCl_3$ with acetone (85 + 15) or saturated ethyl acetate with $H_2O$.

## RESULTS AND DISCUSSION

The TLC plates of the eight samples along with aflatoxin $B_1$ standard have been prepared, developed and observed under long wavelength ultraviolet light of 425 nm for the presence of fluorescence. Out of eight groundnut kernel sample extracts four ($T_1$-treatment) showed blue fluorescence along with $B_1$, standard. The aflatoxin $B_1$ content of the sample has been quantified.[6] The amount of aflatoxin $B_1$ has been tabulated in Table-1 which varied from 0.020 to 0.029 ppm. Among the different types of aflatoxins, aflatoxin $B_1$ is the only toxin invariably present in groundnut kernel samples collected from fields treated with chemical fertiliser alone.

TABLE-1
AMOUNT OF AFLATOXIN $B_1$ CONTENT IN GROUNDNUT KERNELS BY TLC METHOD

| Variety name | Fluorescence observed | | Type of aflatoxin identified | Amount of aflatoxin $B_1$ present (ppm/g) |
|---|---|---|---|---|
| | Standard aflatoxin $B_1$ | Sample extracts | | |
| $T_1$—Treatment VRI-2 | Blue | Blue | Aflatoxin $B_1$ | 0.029 |
| B-95 | Blue | Blue | Aflatoxin $B_1$ | 0.026 |
| ICG V-86564 | Blue | Blue | Aflatoxin $B_1$ | 0.020 |
| ICGV-90210 | Blue | Blue | Aflatoxin $B_1$ | 0.206 |
| TZ - Treatment VRI-2 | Blue | — | Absent | Negative for the presence of Aflatoxin $B_1$ |
| B-95 | Blue | — | Absent | — |
| IGGV-86564 | Blue | — | Absent | — |
| ICGV-90210 | Blue | | Absent | — |

The U.S. food and drug administration has currently established toxic levels (maximum) of aflatoxin at 20 ppb for human foods (except milk), 0.5 ppb for milk, 20 ppb for animal feeds[6]. As per regulations available from 53 countries, the aflatoxin limits vary from 0–50 ppb for all foods where as for India, it is up to 30 ppb.

Interestingly, the aflatoxin $B_1$ is totally absent in the groundnut kernel samples collected form fields treated with organic manure ($T_2$) alone. This total absence

of aflatoxin $B_1$, in all the four varieties point to the organic manure treatments which is only variant when compared with those varieties ($T_1$) which contain aflatoxin $B_1$. This shows that organic manure treatment ($T_2$) effectively contains the detoxic effect when compared with chemical fertiliser treatment ($T_1$). Similar result have been reported by earlier worker[7] about organic farming which is known to reduce toxic residues in food stuff and improves quality. Hence, TLC technique supports the possible presence of aflatoxin $B_1$ in $T_1$ groundnut kernels. The amount of aflatoxin $B_1$ estimated by this study is found to be below the Indian safe limit of 30 ppb.

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