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Aflatoxins Effect on Fungal Populations of Soil, Root and Leaf Surface of Peanut Plants in the Field with Special Reference to Aflatoxins Biodegradation

O.M.O. El-Maghraby, M.S. Youssef and Y.M. Ibrahim
Department of Botany, Faculty of Science, Sohag University, Egypt

Abstract: The research aimed to study the role of aflatoxins contaminated peanut seeds (4, 8, 16 and 32 μg g^{-1} seeds) on fungal populations of soil, rhizosphere, rhizoplane, phyllosphere and phylloplane of cultivated peanut plants for 15, 30, 60 and 120 days in the field, with special reference to aflatoxins biodegradation in soil. Mycological survey revealed that the total fungal count of soil (692.9 colonies mg^{-1} dry soil) was high compared with that of rhizosphere (602.9 colonies mg^{-1} fresh root system) and phyllosphere (103.12 colonies mg^{-1} fresh shoot system) based on dilution-plate method. As well as, phylloplane total fungal count (472 colonies/10 shoot segments) was rich than that of rhizoplane (288 colonies/10 root segments) based on plating-method. A total of 67 species in addition to two varieties belonging to 26 fungal genera were isolated and identified from soil (28 species of 12 genera), rhizosphere (43+1 variety of 16), phyllosphere (49+1 variety of 18), rhizoplane (15 of 10) and phylloplane (29 of 14) of peanut plants investigated (control and treated samples) on dextrose-Czapek's agar medium at 28±2°C. Treatment of peanut seeds with different doses of aflatoxin before planting resulted in a clear effect on total fungal count of both of rhizosphere and soil, while there was no clear effect on total fungal count of rhizoplane, phyllosphere and phylloplane after different cultivation periods. The rate of aflatoxin biodegradation was dose and time dependent, that after 3 days was 40, 70, 81.9 and 89.5% at (4, 8, 16 and 32 μg g^{-1} seeds), while after 7 days was 70, 81.3, 85.6 and 92.5%, respectively, whereas, after 15 days, no mycotoxin could be detected in the lowest dose (4 μg g^{-1}) and traces in the other remaining doses. After 30 days, completely disappearance of toxin was recorded at the different treatment doses.

Key words: Peanut seeds, aflatoxins, soil fungi, rhizosphere, rhizoplane, phyllosphere, phyloplane, aflatoxins biodegradation

INTRODUCTION

Peanuts (*Arachis hypogeae* L.) are unique among cultivated crops in that produce seed-bearing pods below the soil surface. Pods are in direct contact with soil fungal populations and the seeds are frequently invaded by soil fungi before harvesting. Infection of peanut by *Aspergillus* species, especially *A. flavus* group occurs under both pre-harvest and post-harvest conditions (Kumar *et al*., 2008).

Pre-harvest infection by *A. flavus* and *A. parasiticus* and consequent aflatoxin contamination is a major problem in the semi-arid tropic environment. These fungi are widespread in light sandy soils most suitable for peanut cultivation. Peanut pods when are in direct contact with spores of *A. flavus* in soil are frequently invaded before harvesting. The mode and extent of invasion by *A. flavus* depend on soil population density of *A. flavus*, soil moisture content and soil temperature during the pod development to maturity period (Craufurd *et al*., 2006).

Corresponding Author: M.S. Youssef, Department of Botany, Faculty of Science, Sohag University, Egypt

Post-harvest contamination may occur when stored products are not maintained at a safe moisture level. Also, these fungi can invade and produce toxins in peanut kernels before harvest, during drying and in storage. Owing to the toxicity and carcinogenicity of aflatoxins contaminated commodities destined for human or animal consumption pose a serious health hazard and are therefore, closely monitored and regulated. Apart from its effect on health, aflatoxin contamination also impacts the agricultural economy through the loss of produce and thus time and costs involved in monitoring and decontamination (Craufurd *et al*., 2006; Kumar *et al*., 2008).

Egypt exports shelled nuts at about 7 millions US$ and in shell nuts at about 4.4 millions US$, yearly. The major mycotoxins found in Egyptian peanuts were aflatoxins (El-Maghraby and El-Maraghy, 1988). Aflatoxin concentration is the most important quality problem in peanut worldwide with serious health implications for human as well as livestock (D'Mello, 2003). The climatic conditions as well as the food production chains are characteristic in most parts of Africa and the largest mycotoxin-poisoning epidemic in a decade was reported in Africa during the last 5 years (Lewis *et al.*, 2005; Wagacha and Muthomi, 2008).

The present investigation was designed to study the role of aflatoxins contaminated peanut seeds (4, 8, 16 and 32 $\mu g\ g^{-1}$ seeds) on fungal populations of soil, rhizosphere, rhizoplane, phyllosphere and phylloplane of the cultivated peanut plants for 15, 30, 60 and 120 days in the field, with special reference to the effect of aflatoxins on peanut seed germination, plant development and aflatoxins biodegradation in soil.

MATERIALS AND METHODS

Subculture of *Aspergillus flavus* and Toxins Preparation

Twenty-five grams of moistened (40% H_2O), autoclaved Egyptian rice in Erlenmeyer flask (250 mL) were inoculated with 5 mL spore suspension of *Aspergillus flavus* Link (IMI 102135), a highly aflatoxins B_1, B_2, G_1 and G_2 producer, purchased from International Mycological Institute (IMI, Kew, Surrey, England). A spore suspension (approximately, 7×10^7 conidia) was prepared on potato dextrose agar (PDA) to obtain a mass of conidia (7-10 days incubation) at 28°C. A spore suspension in sterile distilled water was used to inoculate the sterilized rice flasks and incubated at 28°C for 15 days.

At the end of incubation period the rice dried and weighed, then aflatoxins were extracted using 150 mL ethyl acetate (3 times). The ethyl acetate extracts were combined, dried over anhydrous sodium sulphate (5 g), concentrated in vacuum, transferred into brown glass vials and evaporated under stream of nitrogen. Then the crude extract of aflatoxins was cleaned up and purified in column of silica gel 60 F_{245} MERCK. On TLC, the fluorescent zones including standards were removed, dissolved in 10% methanol-chloroform and dried in air (El-Maghraby, 1989). The aflatoxins were weighed and kept in refrigerator for artificial peanut seed treatments.

Determination of Germinability of Seeds

Twenty-five seeds of each of peanut seed samples treated with different doses (4, 8, 16 and 32 $\mu g\ g^{-1}$ seeds) of aflatoxins, in addition to control samples were incubated at 25°C over a pad of moist sterile filter paper placed in sterile Petri dishes for 7 days. The seeds with healthy roots and plumules were counted and the counts were expressed as percentages of the numbers of tested seeds.

Determination of Mycoflora of Cultivated Peanut Plants

The aflatoxins used were obtained from *Aspergillus flavus* Link (IMI 102135). Five hundred grams of peanut healthy seeds were fumigated using formaldehyde (1 ml L^{-1} flask) for surface disinfection. The seeds (200 seeds) were soaked in aflatoxin solution at different concentrations (4, 8, 16 and 32 $\mu g\ g^{-1}$ seeds). The control sample (200 seeds) was soaked in ethanol-water only.

The soaked seeds were cultivated in the Farm of Botany Department, Faculty of Science, Sohag. Germination percentages for each group were calculated after 7 and 15 days. The plants with root systems and the soil surrounding the roots were transferred to the mycological laboratory into sterile polyethylene bags for immediately examinations. Fungi of each of soil, rhizosphere, rhizoplane, phyllosphere and phylloplane were determined after 15, 30, 60 and 120 days of cultivation.

Determination of Soil Fungi (Dilution-Plate Method)

Dilution plate method as employed by Youssef (2008) was used for isolation of soil fungi. Modified 1% dextrose-Czapek's agar medium (g L^{-1}; sodium nitrate 3.0, magnesium sulphate 0.5, potassium chloride 0.5, di-potassium hydrogen phosphate 1.0, iron sulphate 0.01, dextrose 10.0, agar agar 15.0- 20.0, pH 6.6±0.1) was used as cultivation and isolation medium. Choramphenicol (0.5 mg mL^{-1}) as bacteriostatic agent and rosebengal (30 ppm) to restrict for widespreading fungi because of stimulate slow growing fungi, were added to the medium.

Five plates were used for each sample tested. The plates were incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per g dry weight of each tested sample. The colonies of slow growing fungi as well as mycelial bits were transferred to slants with special media to ensure precise counting, then to plate for identification.

Taxonomic identification of fungi (based on purely morphologically macro- and microscopic characteristics) was carried out according to International Scientific Mycological References.

Determination of Rhizosphere Fungi

The roots were gently shaken to remove superfluous soil. One gram of the roots were placed into sterile flask (250 mL) containing 100 mL sterile distilled water and shaken well in a rotating motion for 10 min. Serial dilutions were carried out until obtain the desirable dilution. One milliliter of an appropriate dilution was transferred into sterile five Petri dishes and 1% dextrose-Czapek's agar medium (12-15 mL) just above the solidifying was poured. The dishes were rotated by hands to complete homogeneity, incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per gram fresh weight of roots of each tested sample.

Determination of Rhizoplane Fungi

The roots were cut into equal segments (~3 cm, each), subjected to a series of washing with sterile distilled water to remove any soil particles completely, then thoroughly dried between sterilized filer papers and ten segments of each sample were inserted aseptically onto the surface of agar medium (two segments in each Petri dish). The dishes were incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per 10 root segments.

Determination of Phyllosphere Fungi

Two grams of the shoot system were shaken by hand in sterile distilled water (100-200 mL) for 10 min. One milliliter of appropriate dilution was transferred into sterile five Petri dishes and 1% dextrose-Czapek's agar medium (12-15 mL) just above the solidifying was poured. The dishes were rotated by hands to complete homogeneity, incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per gram fresh weight of shoot system of each tested sample.

Determination of Phylloplane Fungi

The shoot segments (~3 cm, each), were serially washed in sterile distilled water. Thereafter, they were thoroughly dried between sterile filter papers and 10 equal segments were inserted onto the surface of agar medium (two segments in each Petri dish). The dishes were incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per 10 shoot segments.

Sample Preparation for Aflatoxins Analysis

Extraction Procedures

Twenty-five grams of each seed sample were defatted by extraction with cyclohexane (150 mL) for 10 h using Soxhlet type extractor. The defatted residue was extracted with ethyl acetate (three times, 50 mL/each). The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into a brown glass vial and evaporated under nitrogen stream.

Clean up of Crude Extracts

For cleaning up the crude extract (purified from interfering compounds); it was suspended in 1 mL chloroform and applied to 14×0.8 cm column containing 2.5 g kiesel gel 60, 70/230 silica gel (MERCK. Germany). The washing and eluting solvents (8 mL, each) for aflatoxins (n-hexane followed by ether and 3: 97 methanol-chloroform, respectively) were carried out according to Dorner ***et al.*** (1998).

Bioassay of Aflatoxins

Three bioassay tests for mycotoxins detection were used; brine shrimps (***Artemia salina*** L.) larvae, *Chlorella vulgaris* Bejerinck and ***Bacillus subtilis*** according to Korpinen (1974), Bean ***et al.*** (1992) and Földes ***et al.*** (2000), respectively.

Thin Layer Chromatography (TLC)

For qualitative detection of aflatoxins, thin layer chromatography technique was employed using precoated silica gel plates type 60 F_{254} TLC (E, MERCK, Germany). Aflatoxins B_1, B_2, G_1 and G_2, were used as standard references (Sigma). The plates were developed using methanol-chloroform (v/v, 3/97) for aflatoxins, the developed plates were viewed under short wave length UV (252 nm) light according to Dorner (1998).

For quantitative determination of aflatoxins, spectrophotometeric (Cecil, model 703) technique was used at molecular coefficient of 21800 at 260 and/or 366 nm UV light according to the method described by Bean ***et al.*** (1972).

Enzyme Linked Immuno-Sorbent Assay (ELISA)

For quantitative determination of aflatoxin B_1 (AFB_1), ELISA technique was employed according to Gathumbi ***et al.*** (2001) because World Health Organization has cited aflatoxins as the most potent naturally occurring carcinogens as well as International Agency for Research on Cancer (IARC) placed aflatoxin B_1 on the list of human carcinogens (Wu, 2004) and presence of aflatoxin B_1 kits.

Biodegradation of Aflatoxins in Soil

The peanut seeds (500 g) were fumigated using formaldehyde (4 ml L^{-1} flask) for surface disinfection and embryo death. The peanut (each, 100 seeds) were weighed and soaked in aflatoxins solution after dissolving (4, 8, 16 and 32 $\mu g\ g^{-1}$ seeds) in ethanol (0.5 mL) at different concentrations with completed the size to attain 40% MC with distilled H_2O. Also, 100 seeds were soaked in ethanol-water (25 mL) only and used as control.

The soaked seeds (each, 100 seeds) were immersed in 1500 gram soil and irritated by 500 mL water in the first day. After 3, 7, 15 and 30 days, 20 seeds of each concentration were removed including soil particles. After removing the seeds, the soil was irritated by water (100 mL). The seeds were carefully cleaned from soil particles, weighed and the aflatoxins were extracted, cleaned up and qualitative and quantitative determinated using TLC, UV spectrophotometric and ELISA analysis, respectively.

RESULTS AND DISCUSSION

Aflatoxins played an important role in peanut seed germination. The germination rate was good in control after 7 days of cultivation (73%) followed by relatively increased (75%) after 15 days. In treated seeds, the rate of germination was sharply retarded after 7 days (7, 5, 0 and 0%) at different doses (4, 8, 16 and 32 μg g^{-1} seeds) of aflatoxins, respectively. Whereas, after 15 days, the rate was sharply increased especially at 4 and 8 μg g^{-1} seeds (79%, each), decreased at 16 μg g^{-1} (71%) and at 32 μg g^{-1} (53%) as shown in Table 1.

As well as, after 120 days of cultivation, there was a correlation between aflatoxin doses and peanut growth, flowering and fruiting. The increase of aflatoxin doses led to decrease the development of the plants. A good growth with fruiting was recorded in control plants, followed by at 4 μg g^{-1}, the plants flowered and gave young pods. Whereas, at 8 μg g^{-1}, little leaves, little flowers with small pods were observed, while at other higher aflatoxin doses, dwarf in plants, some plants were wilted and died completely.

Mycological survey revealed that the total fungal count of soil (692.9 colonies mg^{-1} dry soil) was high compared with that of rhizosphere (602.9 colonies mg^{-1} fresh root system) and phyllosphere (103.12 colonies mg^{-1} fresh shoot system) based on dilution-plate method. As well as, phylloplane total fungal count (472 colonies/10 shoot segments) was rich than that of rhizoplane (288 colonies/10 root segments) based on plating-method on 1% dextrose-Czapek's agar medium. This finding in general accepted with the previous results obtained by Abdel-Hafez and El-Maghraby (1992).

Regarding the isolated fungal genera and species, a total of 67 species in addition to two varieties belonging to 26 genera were isolated and identified from soil (28 species of 12 genera), rhizosphere (43+1 variety of 16), phyllosphere (49+1 variety of 18), rhizoplane (15 of 10) and phylloplane (29 of 14) of peanut plants investigated (control and treated samples) on 1% dextrose-Czapek's agar medium at 28±2°C.

The most dominant fungal species in soil after peanut seed treatments with different doses (4, 8, 16 and 32 μg g^{-1} seeds) of aflatoxins B_1, B_2, G_1 and G_2 and cultivation for 15, 30, 60 and 120 days, were *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum* and *Emericella nidulans* with high frequency of occurrence (Table 2), while *A. niger*, *A. flavus*, *A. versicolor*. *E. nidulans* and *F. oxysporum* were the most prevalent species in both of rhizosphere (Table 3) and phyllosphere (Table 4). Whereas, *F. oxysporum* and *A. niger* were the most frequent species in rhizoplane (Table 5) and *A. niger*, *A. flavus*, *F. oxysporum* and *Myrothecium verrucaria* were the most common in phylloplane (Table 6). These results are in relatively agreement with those obtained by Abdel-Hafez and El-Maghraby (1992) that the most common species isolated from rhizosphere of *Zygophyllium coccineum* were *A. niger*, *A. fumigatus*, *A. terreus*, *P. citrinum* and *Rhizopus stolonifer*, while from phyllosphere were *Cladosporium herbarum*, *C. cladosporioides*, *A. fumigatus*, *A. niger*, *A. terreus* and *P. chrysogenum*, whereas from rhizoplane were *A. niger* and *A. fumigatus* and from phylloplane were *Alternaria alternata*, *A. fumigatus* and *A. niger*.

Table 1: Percentage of germination of peanut seeds treated with different doses of aflatoxins after two periods of cultivation

	Germination (%)	
Aflatoxins doses	After 7 days	After 15 days
Control	73	75
4 (μg g^{-1})	7	79
8 (μg g^{-1})	5	79
16 (μg g^{-1})	0	71
32 (μg g^{-1})	0	53

Table 2: Dominant fungal genera and species counts (colonies mg^{-1} dry soil) isolated from peanut soil on 1% dextrose-Czapek's agar at 28±2°C, after cultivation of aflatoxins (4, 8, 16 and 32 µg g^{-1} seeds) treated seeds for 15, 30, 60 and 120 days

	Cultivation period									
	15 days					30 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	71.9	59.4	53.7	47.1	27.6	55.0	53.8	31.6	28.6	16.6
Aspergillus (Micheli) Corda	62.1	50.2	42.1	40.5	17.0	19.0	23.6	8.4	11.6	5.2
A. niger van Tieghem	12.0	0.3	0.4	2.6	5.9	6.4	12.8	5.0	4.6	1.6
A. terreus Thom	-	-	-	0.8	0.7	3.2	0.4	1.2	1.0	-
A. versicolor (Vuill.) Tiraboschi	-	-	-	-	-	8.4	-	2.0	4.6	2.0
A. flavus Link	60.9	49.2	42.4	37.1	10.2	-	8.2	-	-	-
A. ustus (Bainier) Thom and Church	-	-	-	-	-	0.6	0.4	-	0.2	1.4
Fusarium Link	12.0	0.5	2.2	1.9	3.9	28.8	26.2	13.8	5.0	7.2
F. oxysporum Schlecht	12.0	0.5	2.2	1.9	3.9	28.6	26.2	13.8	5.0	7.2
Emericella nidulans (Eidam) Vuillemin	-	0.6	1.4	0.3	3.5	2.2	0.4	7.0	6.4	2.0
Mucor Micheli	-	0.2	0.8	12	12.0	0.6	3.0	1.2	3.0	0.8
M. racemosus Fresenius	-	0.2	0.4	12	12.0	0.6	3.0	1.2	3.0	0.8
Myrothecium Tode	-	-	-	-	-	4.4	0.4	0.4	1.4	0.8
M. verrucaria (Albertini and Schweinitz) Ditmar	-	-	-	-	-	-	0.4	0.4	1.4	0.8
Penicillium Link	8.6	7.6	6.6	25	1.0	-	-	0.6	0.4	-
P. citrinum Thom	8.6	7.6	4.1	25	0.9	-	-	0.6	0.4	-
Cladosporium cladosporioides (Fresen.) de Vries	-	-	-	-	-	-	-	-	-	-

	Cultivation period									
	60 days					120 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	26.2	25.2	17.6	16.6	12.6	11.0	25.2	24.2	44.0	45.6
Aspergillus (Micheli) Corda	20.2	14.0	14.6	11.4	10.8	3.2	3.2	9.6	34.6	25.0
A. niger van Tieghem	5.0	10.8	8.2	7.6	5.2	1.6	2.8	5.0	13.2	16.0
A. terreus Thom	0.2	-	-	0.4	0.2	-	0.2	0.2	-	3.0
A. versicolor (Vuill.) Tiraboschi	9.2	1.6	-	0.2	-	0.4	0.2	1.6	-	-
A. flavus Link	3.0	1.0	3.0	-	-	-	-	-	-	-
A. ustus (Bainier) Thom and Church	-	-	1.8	1.6	4.6	0.2	-	0.2	-	-
Fusarium Link	3.0	2.2	2.0	3.0	0.6	3.0	3.4	2.8	4.4	6.4
F. oxysporum Schlecht	3.0	2.2	2.0	2.0	0.6	3.0	3.4	2.8	4.4	6.4
Emericella nidulans (Eidam) Vuillemin	1.2	4.0	-	0.4	0.2	4.6	15.6	8.0	1.4	3.2
Mucor Micheli	1.0	2.4	-	0.8	-	-	-	-	-	-
M. racemosus Fresenius	1.0	0.4	-	0.8	-	-	-	-	-	-
Myrothecium Tode	0.8	1.6	-	-	-	-	1.6	2.6	1.2	10.2
M. verrucaria (Albertini and Schweinitz) Ditmar	0.8	1.6	-	-	-	-	1.4	2.6	1.2	10.2
Penicillium Link	-	0.4	-	-	0.6	-	-	0.2	-	-
P. citrinum Thom	-	0.4	-	-	0.6	-	-	-	-	-
Cladosporium cladosporioides (Fresen.) de Vries	-	0.2	0.6	0.2	0.2	0.2	1.4	1.0	2.4	0.8

Fungi of high occurrence (more than 10 cases out of 20 tested) and moderate occurrence (5-10 cases) were enclosed in the table, while fungi of low occurrence (3-4 cases) and rare occurrence (less than 3 cases) were omitted from the table. -: No fungal species isolated, C: Control sample

Treatment of peanut seeds with aflatoxins B_1, B_2, G_1 and G_2 (4, 8, 16 and 32 µg g^{-1} seeds) before planting resulted in varying in total fungal count as well as fungal genera and species diversity, through plant development after 15, 30, 60 and 120 days. A clear effect of the toxin was recorded in both of rhizosphere and soil total fungal counts, where the fungal counts decreased with increase the toxin doses through first, second and third periods in soil (Table 2) and first and second periods in

Table 3: Dominant fungal genera and species counts (colonies mg^{-1} fresh roots) isolated from peanut rhizosphere on 1% dextrose-Czapek's agar at 28±2°C, after cultivation of aflatoxins (4, 8, 16 and 32 μg g^{-1} seeds) treated seeds for 15, 30, 60 and 120 days

	Cultivation period									
	15 days					30 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 μg	8 μg	16 μg	32 μg	C	4 μg	8 μg	16 μg	32 μg
Total count	26.7	24.3	24.0	20.5	17.0	64.1	51.6	27.0	21.3	18.0
Aspergillus (Micheli) Corda	7.2	11.3	10.2	8.1	11.4	61.1	31.2	16.5	11.1	8.4
A. niger van Tieghem	4.2	2.4	0.9	1.2	1.5	30.7	5.1	12.9	7.8	3.9
A. flavus Link	2.4	4.8	7.8	3.3	6.3	29.8	-	2.1	2.4	1.5
A. versicolor (Vuill.) Tiraboschi	0.3	0.6	0.6	0.8	1.2	0.6	-	0.3	-	-
A. terreus Thom	-	1.2	-	-	-	-	21.0	-	0.6	0.3
A. ustus (Bainier) Thom and Church	0.3	1.1	-	0.3	1.8	-	5.1	1.1	0.3	2.7
A. candidus Link	-	-	0.6	0.3	-	-	-	-	-	-
Emericella Berkeley and Broome	-	0.9	-	1.2	1.2	2.7	10.5	0.3	2.1	2.4
E. nidulans (Eidam) Vuillemin	-	0.9	-	1.2	0.9	2.7	9	2.7	1.8	2.4
E. quadrilineata (Thom and Raper) Benjamin	-	-	-	-	0.3	-	1.5	0.3	0.3	-
F. oxysporum Schlecht	10.2	8.1	1.2	0.9	0.6	2.7	1.5	0.9	0.3	1.8
Penicillium Link	5.9	4.5	4.4	1.8	1.2	-	0.3	6.0	5.4	4.0
P. citrinum Thom	-	0.3	4.2	-	-	-	-	-	-	1.5
P. chrysogenum Thom	5.4	0.9	-	-	-	-	1.8	5.4	3.9	-
P. puberulum Bainier	0.6	0.3	0.3	0.3	-	-	0.3	-	-	-
Myrothecium Tode	0.3	0.6	0.9	0.6	-	-	0.3	-	-	-
M. verrucaria (Albertini and Schweinitz) Ditmar	0.3	0.3	0.9	0.6	-	-	-	-	-	-
Botryotrichum Saccardo and Marchal	-	0.6	0.6	0.6	0.3	0.3	5.1	1.5	0.6	-
B. piluliferum Saccardo and Marchal	-	-	0.3	0.6	-	0.3	5.1	-	0.6	-
Mucor Micheli	-	-	0.3	0.3	-	-	-	-	-	-
M. racemosus Fresenius	-	-	-	-		-	-	-	-	-

	Cultivation period									
	60 days					120 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 μg	8 μg	16 μg	32 μg	C	4 μg	8 μg	16 μg	32 μg
Total count	26.4	25.2	41.4	19.8	21.6	26.1	40.8	43.5	25.0	39.6
Aspergillus (Micheli) Corda	19.8	6.0	34.8	4.1	12.6	14.4	24.9	8.7	4.8	27.9
A. niger van Tieghem	6.0	3.3	34.5	12.9	7.8	5.4	7.8	5.4	2.4	11.1
A. flavus Link	0.3	-	-	0.3	1.2	-	2.4	-	-	0.3
A. versicolor (Vuill.) Tiraboschi	0.9	0.9	-	-	-	1.8	1.2	2.7	-	-
A. terreus Thom	12.6	1.8	-	0.3	1.2	-	-	-	-	3.0
A. ustus (Bainier) Thom and Church	-	-	-	-	-	0.6	-	-	0.3	-
A. candidus Link	-	-	-	-	-	0.6	13.5	-	-	3.6
Emericella Berkeley and Broome	3	4.5	0.3	1.8	0.6	5.7	9	2.4	0.9	0.3
E. nidulans (Eidam) Vuillemin	1.8	4.5	0.3	1.8	0.6	5.7	9	2.4	0.9	0.3
E. quadrilineata (Thom and Raper) Benjamin	1.2	-	-	-	-	-	-	-	-	-
F. oxysporum Schlecht	2.1	0.3	-	-	0.6	3.6	4.2	5.1	4.2	9.0
Penicillium Link	0.6	0.9	6.6	0.9	2.7	-	-	-	0.6	-
P. citrinum Thom	0.6	-	6.6	0.3	2.1	-	-	-	-	-
P. chrysogenum Thom	-	-	-	0.6	-	-	-	-	-	-
P. puberulum Bainier	-	-	-	-	-	-	-	-	0.3	-
Myrothecium Tode	-	-	-	-	-	1.2	2.7	27.3	2.1	2.4
M. verrucaria (Albertini and Schweinitz) Ditmar	-	-	-	-	-	1.2	2.7	27.3	2.1	2.4
Botryotrichum Saccardo and Marchal	-	-	-	-	-	-	-	-	-	-
B. piluliferum Saccardo and Marchal	-	-	-	-	-	-	-	-	-	-
Mucor Micheli	0.6	13.2	-	3	5.7	0.9	-	-	-	-
M. racemosus Fresenius	0.6	9.9	-	3	5.7	0.9	-	-	-	-

Fungi of high occurrence (more than 10 cases out of 20 tested) and moderate occurrence (5-10 cases) were enclosed in the table, while fungi of low occurrence (3-4 cases) and rare occurrence (less than 3 cases) were omitted from the table. -: No fungal species isolated, C: Control sample

Table 4: Dominant fungal genera and species counts (colonies/10 root segments) isolated from peanut rhizoplane on 1% dextrose-Czapek's agar at 28±2°C, after cultivation of aflatoxins (4, 8, 16 and 32 µg g^{-1} seeds) treated seeds for 15, 30, 60 and 120 days

	Cultivation period									
	15 days					30 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	14	11	15	13	15	21	16	14	14	16
Fusarium Link	10	11	9	9	6	8	10	10	10	10
F. oxysporum Schlecht	9	10	9	9	6	8	10	10	10	10
Aspergillus (Micheli) Corda	1	-	5	2	6	10	4	2	3	6
A. niger van Tieghem	-	-	4	2	6	5	2	1	3	4
A. flavus Link	-	-	1	-	-	5	-	1	-	2
Myrothecium Tode	-	-	-	-	-	1	-	2	1	-

	Cultivation period									
	60 days					120 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	13	13	21	9	16	14	15	13	13	12
Fusarium Link	10	8	10	9	9	10	10	10	9	10
F. oxysporum Schlecht	10	8	10	9	9	10	10	10	9	10
Aspergillus (Micheli) Corda	3	5	11	-	7	4	5	-	-	-
A. niger van Tieghem	3	5	6	-	5	4	5	-	-	-
A. flavus Link	-	-	5	-	2	-	-	-	-	-
Myrothecium Tode	-	-	-	-	-	-	-	3	4	2

Fungi of high occurrence (more than 10 cases out of 20 tested) and moderate occurrence (5-10 cases) were enclosed in the table, while fungi of low occurrence (3-4 cases) and rare occurrence (less than 3 cases) were omitted from the table. - = No fungal species isolated, C: Control sample

rhizosphere (Table 3). In this respect, fungal growth and subsequent mycotoxin degradation can be enhanced by physical environments such as aeration, cooling, modified atmosphere and others. The leaching and adsorption potential of aflatoxins in soil was 80-92% of total applied aflatoxin, which was retained in the upper 2.5 cm of soil columns. All of the aflatoxin was within the upper 20 cm of all tested soil types as reported by Goldberg and Angle (1985). Recently, aflatoxin B_1 was adsorbed by clays from water and corn meal as reported by Jaynes *et al*. (2006).

Regarding the results obtained concerning fungal species, *A. flavus* counts increased with increasing the toxin dose in the first period of cultivation in rhizosphere and decreased in the soil, whereas irregularly trend in the remaining doses with elapse of cultivation periods. In this respect, Griffin (1994) reported that the growth of fungal species enhanced by its metabolites. On the other hand, application of non-aflatoxigenic strains of *A. parasiticus* to peanut (*Arachis hypogeae* L.) plots reduced aflatoxin contamination of peanuts in three successive years (Dorner *et al*., 2003). Also, different rates of combined inoculums of non-toxigenic strains of *A. flavus* and *A. parasiticus* applied to soil in two years, reduced aflatoxin contamination of peanut from 74.3- 99.9% (Dorner *et al*., 1998). A success reduction in aflatoxin contamination of maize (Dorner *et al*., 1999) by applying a non-toxigenic strains of *A. flavus* in soil around developing cotton and maize, respectively.

Although, *Emericella nidulans* disappeared completely in control samples after 15 days of cultivation in both rhizosphere and soil, it flourished in rhizosphere (all doses) and soil (at 8 and 16 µg g^{-1} seeds) after the same period. The fungus continuously appeared in variable counts at different doses with elapse the cultivation periods (except, sample of soil after 60 days at 8 µg g^{-1} seeds). Based on findings of Griffin (1994) and Calvo *et al*. (2002) that the growth of fungal species

Table 5: Dominant fungal genera and species counts (colonies/g leaves) isolated from peanut phyllosphere on 1% dextrose-Czapek's agar at 28±2°C, after cultivation of aflatoxins (4, 8, 16 and 32 μg g^{-1} seeds) treated seeds for 15, 30, 60 and 120 days

	Cultivation period									
	15 days					30 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 μg	8 μg	16 μg	32 μg	C	4 μg	8 μg	16 μg	32 μg
Total count	3900	2475	4575	2025	4350	1650	2175	6600	4500	5100
Aspergillus (Micheli) Corda	3225	900	750	1050	2550	1050	1350	1425	2100	975
A. niger van Tieghem	675	300	450	525	1725	450	675	1125	1275	450
A. flavus Link	2025	375	150	150	225	600	75	150	375	-
A. versicolor (Vuill.) Tiraboschi	225	-	-	75	150	-	300	-	150	225
A. ustus (Bainier) Thom and Church	-	75	-	150	300	-	150	150	225	300
A. terreus Thom	-	75	-	75	75	-	150	-	-	-
A. fumigatus Fresenius	-	-	-	-	-	-	-	-	75	-
A. candidus Link	150	-	-	-	-	-	-	-	-	-
Emericella Berkeley and Broome	-	525	150	150	375	-	75	4575	2250	3150
E. nidulans (Eidam) Vuillemin	-	525	150	75	75	-	75	4200	1425	1500
E. quadrilineata (Thom and Raper) Benjamin	-	-	-	75	300	-	-	375	825	1650
F. oxysporum Schlecht	-	525	375	150	225	225	300	75	-	450
Penicillium Link	673	-	3000	225	675	150	150	75	75	75
P. chrysogenum Thom	225	-	-	150	150	75	-	-	-	75
P. puberulum Bainier	75	-	-	75	75	75	150	-	-	-
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	-	150	-	-	-	-	75	150	75	75
Cladosporium Link	-	75	225	-	-	-	-	150	75	-
C. cladosporioides (Fresen.) de Vries	-	75	-	-	-	-	-	75	-	-
Humicola Corda	-	-	75	150	150	75	-	75	300	75
H. grisea Traaen	-	-	75	150	150	75	-	75	300	75
Mucor Micheli	-	-	-	-	-	-	-	-	-	225
Ulocladium Preuss	-	75	-	-	-	-	75	-	150	-

	Cultivation period									
	60 days					120 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 μg	8 μg	16 μg	32 μg	C	4 μg	8 μg	16 μg	32 μg
Total count	2400	2775	6525	3075	8850	16275	10725	10725	1125	2400
Aspergillus (Micheli) Corda	2025	750	4500	1875	6075	8475	1200	5775	675	1350
A. niger van Tieghem	225	300	1125	1500	1950	3225	900	2925	600	1200
A. flavus Link	1350	150	675	-	2550	-	-	-	75	150
A. versicolor (Vuill.) Tiraboschi	-	-	-	225	75	4875	225	2700	-	-
A. ustus (Bainier) Thom and Church	150	225	-	-	-	-	-	150	-	-
A. terreus Thom	-	-	150	-	-	375	-	-	-	-
A. fumigatus Fresenius	225	-	2100	150	-	-	75	-	-	-
A. candidus Link	75	75	450	-	450	-	-	-	-	-
Emericella Berkeley and Broome	375	1650	2025	825	2700	1050	675	975	-	75
E. nidulans (Eidam) Vuillemin	375	1650	2025	825	2700	1050	675	975	-	75
E. quadrilineata (Thom and Raper) Benjamin	-	-	-	-	-	-	-	-	-	-
F. oxysporum Schlecht	-	300	-	-	-	1575	975	1575	-	975
Penicillium Link	-	-	-	75	-	-	-	75	-	-
P. chrysogenum Thom	-	-	-	-	-	-	-	-	-	-
P. puberulum Bainier	-	-	-	-	-	-	-	-	-	-
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	-	-	-	-	-	3675	7725	1350	-	-
Cladosporium Link	-	-	-	-	75	-	75	-	375	-
C. cladosporioides (Fresen.) de Vries	-	-	-	-	75	-	75	-	375	-
Humicola Corda	-	-	-	-	-	-	-	-	-	-
H. grisea Traaen	-	-	-	-	-	-	-	-	-	-
Mucor Micheli	-	75	-	75	-	-	1500	-	975	75
Ulocladium Preuss	-	-	-	-	300	-		-	-	-

Fungi of high occurrence (more than 10 cases out of 20 tested) and moderate occurrence (5-10 cases) were enclosed in the table, while fungi of low occurrence (3-4 cases) and rare occurrence (less than 3 cases) were omitted from the table. -: No fungal species isolated, C: Control sample

Table 6: Dominant fungal genera and species counts (colonies/10 fresh shoot segments) isolated from peanut phylloplane on 1% dextrose-Czapek's agar at 28±2°C, after cultivation of aflatoxins (4, 8, 16 and 32 µg g^{-1} seeds) treated seeds for 15, 30, 60 and 120 days

	Cultivation period									
	15 days					30 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	24	23	25	23	20	23	25	18	13	13
Aspergillus (Micheli) Corda	10	10	11	12	16	15	18	10	5	5
A. niger van Tieghem	3	6	6	7	7	8	10	4	4	2
A. flavus Link	6	4	4	1	2	6	3	5	1	1
A. ustus (Bainier) Thom and Church	-	-	2	1	-	1	-	-	-	2
A. versicolor (Vuill.) Tiraboschi	-	-	-	-	1	-	1	-	-	-
F. oxysporum Schlecht	2	2	4	3	2	4	4	1	3	-
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	3	2	3	-	-	2	1	-	-	-
Emericella Berkeley and Broome	-	3	2	3	5	-	-	6	2	8
E. nidulans (Eidam) Vuillemin	-	3	1	3	5	-	-	6	1	3
Mucor Micheli	2	-	1	2	-	-	-	-	-	-
M. racemosus Fresenius	2	-	1	-	-	-	-	-	-	-

	Cultivation period									
	60 days					120 days				
	Treatment dose					Treatment dose				
Fungal genera and species	C	4 µg	8 µg	16 µg	32 µg	C	4 µg	8 µg	16 µg	32 µg
Total count	21	23	28	21	28	31	42	30	21	20
Aspergillus (Micheli) Corda	19	11	14	9	18	13	18	12	8	12
A. niger van Tieghem	6	4	8	6	9	5	10	6	7	9
A. flavus Link	10	3	6	3	7	2	2	-	1	3
A. ustus (Bainier) Thom and Church	1	1	-	-	-	-	-	-	-	-
A. versicolor (Vuill.) Tiraboschi	-	-	-	-	-	6	6	6	-	-
F. oxysporum Schlecht	2	5	10	10	10	8	9	8	9	8
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	-	-	2	2	-	7	8	9	3	-
Emericella Berkeley and Broome	-	5	2	-	-	-	-	1	-	-
E. nidulans (Eidam) Vuillemin	-	5	2	-	-	-	-	1	-	-
Mucor Micheli	-	2	-	-	-	3	7	-	1	-
M. racemosus Fresenius	-	-	-	-	-	3	7	-	1	-

Fungi of high occurrence (more than 10 cases out of 20 tested) and moderate occurrence (5-10 cases) were enclosed in the table, while fungi of low occurrence (3-4 cases) and rare occurrence (less than 3 cases) were omitted from the table. -: No fungal species isolated, , C: Control sample

enhanced by its metabolites and the similarity in chemical formula between aflatoxin B_1, G_1 and sterigmatocystin that Bhatnagar *et al*. (1987) identified O-methylsterigmatocystin as an aflatoxin B_1 and G_1 precursor in *Aspergillus parasiticus*. Benneth and Klich (2003) reported that sterigmatocystin, a related dihydrofuran toxin, is a late metabolite in the aflatoxin pathway and is also produced as a final biosynthetic product by a number of species such as *Aspergillus nidulans* (*Emericella nidulans*) and *A. versicolor*. Cognate genes for aflatoxin pathway enzymes from *A. flavus* and *A. parasiticus* shown high sequence similarity to the sterigmatocystin pathway genes (Yu *et al*., 2000). The organization of the genes in the *A. flavus*, *A. nidulans* and *A. parasiticus* sterigmatocystin/aflatoxin pathway has been compared by Cary *et al*. (2001). Also, at least two genes, *aflR* and *aflJ*, play a regulatory role in sterigmatocystin/aflatoxin biosynthesis as reported by Yu *et al*. (1996) and Payne and Brown (1998). Several studies of *aflR* have demonstrated it encodes a sterigmatocystin/aflatoxin pathway-specific transcription factor (Todd and Andrianopoulos, 1997; Calvo *et al*., 2002). Then, biotransformation of

aflatoxin to sterigmatocystin may be carried out, which stimulated the flourishing of *Emericella* counts (*E. nidulans* and *E. quarinelleata*). As well as, the same trend was recorded in *A. versicolor* counts in rhizosphere after 15 days of cultivation, that it was well known as sterigmatocystin producer.

Aflatoxins had toxic effect against *Fusarium* in rhizosphere after 15, 30 and 60 days and in soil after 30 and 60 days of cultivation. Also, the toxin had toxic effect against *Penicillium* in rhizosphere and soil especially after 15 days of cultivation (decrease in counts) and low counts were detected at the remaining cultivation periods at different doses. Similarly mycotoxins (aflatoxins) had great effects on growth of some microorganisms including bacteria, fungi and algae (Smith and Moss, 1985; Bullerman, 1986; Bean *et al*., 1992).

Biodegradation of Aflatoxins in Soil

Based on the results obtained in Table 7 , the biodegradation rate of aflatoxins was dose and time dependent. After 3 days, the biodegradation varied between 40- 89.5% (40, 70, 81.9 and 89.5% at 4, 8, 16 and 32 μg g^{-1} seeds, respectively). Also, the rate of biodegradation increased with elapse of time that after 7 days (70, 81.3, 85.6 and 92.5%, respectively). The toxin sharply depressed after 15 days, where no aflatoxins could be detected at 4 μg g^{-1} seeds and traces with the remaining doses. Finally, completely disappearance of aflatoxins was recorded after 30 days.

The strategy of decontamination of mycotoxins is used based on some basic criteria. The mycotoxin must be inactivated (destroyed) by transformation to non-toxic compounds. The physical properties of raw material should not change significantly (Park and Liang, 1993). Although, aflatoxin formation has a serious problem occurring mainly under inadequate storage conditions of cereal grains and oil seeds such as groundnuts. Physical, chemical and biological detoxification of aflatoxins have been reported by Smith and Harran (1993) and Magan and Aldred (2007). Biological methods of aflatoxins detoxification have been widely studied but as yet do not constitute an acceptable approach. Studies have clearly demonstrated that food-grade *Rhizopus* species are capable of metabolizing aflatoxins and reducing inherent toxicity and mutagenicity. *Rhizopus oryzae* is able to detoxicate up to 70% of aflatoxins in peanut meal using solid substrate technique (Smith and Harran, 1993).

It is well documented that most fungi capable of producing mycotoxins, can also remetabolize them under appropriate conditions. In this respect, Ling *et al.* (1998) studied detoxification of aflatoxin B_1 by enzymes isolated from *Armillariella tabescens*. Most studies implicated enzymic activity in the degradation of aflatoxin B_1, but the rate of degradation was extremely slow and the possible involvement of enzymic byproducts was implicated (Smith and Harran, 1993). In this respect, Alberts *et al*. (2006) studied the biodegradation of aflatoxin B_1 (AFB_1) by extracellular extracts from *Rhodococcus erythropolis* liquid cultures. Results indicated that the degradation is enzymatic and the enzymes responsible for the degradation of AFB_1 are extracellular and constitutively produced. Furthermore, the biodegradation of AFB_1 when treated with *R. erythropolis* extracellular fraction coincided with a loss of mutagenicity, as evaluated by the Ames test for mutagenicity.

Dual cultivation of *A. flavus* and other microorganisms promoted decrease in formation of aflatoxins, continued incubation of dual culture for 5 days at 40°C with several microorganisms such as *Rhizopus stolonifer*, *A. niger* and *Bacillus stearothermophilus*, resulted in greater reduction of

Table 7: Biodegradation of aflatoxins in soil for 30 days of incubation

Initial dose of aflatoxin (μg g^{-1})	Aflatoxin concentration (μg g^{-1})			
	3 days	7 days	15 days	30 days
4	2.4	1.2	-ve	-ve
8	2.4	1.5	Traces	-ve
16	2.9	2.3	Traces	-ve
32	3.4	2.4	Traces	-ve

aflatoxin levels compared with a single culture of *A. flavus* (Smith and Harran, 1993). Peroxidase and cytochromo P450 monooxygenases have been shown to possess aflatoxin degradative ability and the degradation of aflatoxin levels was parallel to increase peroxidase activity (Smith and Harran, 1993).

In conclusion, it is clear evident that cultivation of contaminated aflatoxin peanut seeds led to disturbance in soil and rhizosphere mycoflora and consequently, affected on root and shoot systems development and led to wilt of plants and highly economic loss due to low quality crop yield and unhealthy pods with seeds. So, seeds must be subjected to quality control and good testing protocol for molds and mycotoxins contamination to ensure free of toxic levels of mycotoxins. Also, genetic modification resistant cultivars to fungal invasion and mycotoxin production is effective means beside to, biological control and others in prevention or minimizing mycotoxin synthesis on agricultural commodities in field, post-harvest and in storage. Therefore cultivation of suitable and selective resistant cultivars seeds and grains in different climatic and environmental conditions must be applied all over the world for high quality crop yields and protection human and livestock health from mycotoxins risk.

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