

# Tolerance Levels of Peanut Varieties against *Aspergillus flavus* Infection

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## Abstract

Peanuts (*Arachis hypogaea* L.) are usually infected by *Aspergillus flavus* and *A. parasiticus* during pre and post-harvest periods subsequently resulting in aflatoxin contamination. Thirteen peanut varieties were evaluated for kernel and pod colonization and infection by *A. flavus* in this study. The pods and kernels were examined under a microscope for *A. flavus* infection levels. Differences in mean ratings of infected peanut kernels and pods were observed after 10 days of artificial inoculation and incubation. More differences were observed among the mean ratings of peanut kernels and pods with invisible mycelial surface coverage. However, these mean differences were not statistically significant  $P \geq 5$ . Peanut varieties with the biggest mean ratings of kernels and pods with invisible mycelia or no visible sign of infection and smallest mean ratings of infected pods and kernels could be considered tolerant to *A. flavus* colonization and infection in this study. Therefore, there is a need to promote the cultivation of these varieties by farmers as they have low levels of infection and subsequently low level of aflatoxin contamination. The peanut varieties with the lowest mean ratings of kernels and pods with invisible mycelia which are considered to have good attributes warrant improvement through selection and breeding. This is because most farmers in Uganda store their peanuts in pod form which offers some protection against infection. In addition, peanut varieties with biggest mean ratings of kernels with invisible mycelia need to be promoted among traders since they are considered to have lower levels of *A. flavus* infection.

**Keywords:** Peanut; *Aspergillus flavus*; Mycelia; Aflatoxin; Contamination

## Introduction

Peanuts also known as groundnuts (*Arachis hypogaea* L) are commonly infected by *A. flavus* and *A. parasiticus* during pre-harvest and post-harvest periods [1]. *A. flavus* and *A. parasiticus* fungi attack peanuts when drying and under storage conditions subsequently resulting in aflatoxin contamination [2]. Furthermore, improper harvesting and storage practices also increases the levels of aflatoxin in peanuts [3]. The *Aspergillus* infection of peanuts during pre-harvest period is attributed to peanut pods being in direct contact with soil fungal populations [4]. In addition, high temperature, high relative humidity and insect damage also contribute to the high levels of pre-harvest infection [5].

Aflatoxin contamination of agricultural commodities leads to losses of the production especially in terms of costs of regulatory programmes designed to reduce risks to human and livestock health [6]. Therefore, in order to reduce aflatoxin contamination in peanuts and peanut based products, numerous approaches have been adopted by various countries. These aflatoxin management strategies include; adoption of good agronomic practices, proper harvesting and storage practices, chemical control, and biological control [7,8].

Adopting resistant cultivars is considered the most effective and low-cost component of aflatoxin management programme [3]. Peanut varieties with differing concentrations of aflatoxin during and after infection by *A. flavus* have been reported [9-12]. Four resistance strategies to *A. flavus* and *A. parasiticus* have been extensively studied [13-16]. And these four resistance strategies are; seed colonization by *A. flavus* (SCAF), field resistance to seed colonization by *A. flavus* (FSCAF), pre-harvest resistance to *A. flavus* contamination (PAC) and resistance to aflatoxin production. The need for empirical evaluation of the different peanut varieties to *A. flavus* infection tolerance levels and lack of known varieties resistant to aflatoxin contamination, justified this study. Therefore, recommended agronomical practices, post-harvest storage strategies and good processing practices need to be

promoted among farmers, traders, processors and other stakeholders in order to reduce the level of aflatoxin contamination.

The objective of this study was to evaluate the peanut kernels and pod shell against *A. flavus* infection. The pod-shell and kernel resistance to *A. flavus* infection is related to the combination of physical and chemical characteristics of the seed testa [3], while the resistance to pod shell penetration is thought to be related to the pod shell structure (reticulation), thickness and hardness [13]. Other research findings by Kushalappa et al. [17] show that pod resistance to *A. flavus* invasion was associated with undamaged peanut shells in addition to the presence of antagonistic fungal and bacterial microflora.

## Material and Methods

The experiment materials comprising of 13 peanut varieties as presented in Table 1.

## Experimental design

The experimental design was a Completely Randomized Design (CRD) with 3 replicates and each replicate comprised 10 health plump kernels and 10 unshelled peanut pods. The evaluation of the levels of *A. flavus* colonization of peanuts was rated as percentage mycelial growth surface coverage on peanut kernels and pods. The different levels of *A. flavus* tolerance against the different peanut varieties experiment was

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Varieties	Maturity (days)	Year of release	Other remarks
Red beauty	90–100	1966	Multiline of Red Valencia
Igola	125–130	1995	Spanish, tan seeded
Serenut 1	100–110	1998	Virginia, Red Seeded
Serenut 2	100–110	1998	Virginia, Tan
Serenut 3	90–100	2002	Spanish, Red Seeded
Serenut 4	90–100	2002	Spanish, Tan
Erudurudu	100–110	Landrace	Valencia
Acholi white	80–90	1966	Valencia
Gweri nut	100–110	1969	Red Manyema, Venezuela
Entry 99566	100–110	2010	Spanish, tan seeded
Entry 99527	90–100	Advanced line	Spanish, tan seeded
Entry 99528	90–100	Advanced line	Spanish, red seeded
Entry 93535	90–100	2010	Spanish, red seeded

Table 1: Peanut varieties used in the experiment.

conducted at the Department of Food Science Microbiology laboratory, Makerere University.

### Preparation of the inoculums

Peanut kernels were sterilized using 10% sodium hypochlorite and assayed in triplicates on malt extract agar for 3 days according to Pitt and Hocking [18]. *A. flavus* was isolated and identified morphologically and cultured on *Aspergillus flavus/ parasiticus* (AFPA) selective nutrient media [18,19]. Pure *A. flavus* fungi were obtained by sub-culturing using the aid of a sterile swab which was streaked over the entire malt extract on the petridish and incubated at 25°C until substantial sporulation was observed.

The *A. flavus* spores were washed off the Petri -dishes, dissolved in 1 litre of sterile distilled water in an Erlenmeyer flask and 2 drops of tween were added to ensure a uniform distribution of the spores. The  $1 \times 10^6$  spores per litre were used as inocula and were estimated using a haemocytometer.

### Inoculation of Peanut varieties with *Aspergillus* spores

Intact pods were selected at random, shelled and 10 healthy kernels from each sample lot in triplicates were assayed by direct plating technique for internal fungal infection [18,20,21]. The kernels were surface sterilized for 1 minute with sodium hypochlorite (10% commercial bleach, Jik®, Rickitt Benckiser, East Africa Ltd). They were rinsed 3 times with sterile water and hydrate to 20% moisture content by soaking in sterile distilled water for 10 minutes.

The 10 kernels and pod shells were aseptically placed in sterile Petri dishes of 9 cm diameter in triplicates and 1 ml of *A. flavus* spores ( $1 \times 10^6$  spores/per litre) in distilled water added. In addition, pods and kernels were washed with sterile distilled water as a control since sodium hypochlorite could compromise the integrity of seed testa constituents which are soluble in alkaline solutions [22]. The kernels and pods were rolled gently in the Petri dishes using a sterile inoculation loop to spread the inoculum evenly over the surface of kernels and pods. The Petri dishes were arranged in the semi rigid plastic boxes (chamber) with fitting lids to ensure constant humidity. The lids were sealed with cello tape to prevent cross contamination with open-air microorganisms and placed in an incubator at 25°C in darkness for 10 days. After 10 days of incubation, the kernels were examined under a Nikon stereoscopic microscope for *A. flavus* infection.

### Data collection and analysis

The data was collected 10 days after artificial inoculation and incubation. A 1 to 5 score scale according to Strange [23] with some modification was used to score *Aspergillus flavus* infection on different peanut, where; 1=invisible mycelial growth, 2=1 to 20% mycelial growth surface coverage on the kernel, 3=21 to 50% mycelial growth surface coverage on the kernel, 4=51 to 70% mycelial growth surface coverage on the kernels, 5=71 to 100% mycelial growth surface coverage on the kernels and shells. The data was analysed using GenStat discovery edition 3 and the means were separated by Fisher's protected t-test [24].

### Results and Discussion

The results of pods and kernels revealed that none of the kernels and pods from all the 13 peanut varieties was immune to *A. flavus* infection. However, kernels and pods of the 13 peanut varieties exhibited differences in mycelial growth surface coverage under different treatments as presented in Table 2 and 3. The differences in mycelial growth surface coverage were probably attributed to differences in physical and chemical features of the seed-coat, pod-shell thickness and reticulation. LaPrade et al. [25] and Liang et al. [26] reported that peanut resistance to *A. flavus* and subsequent aflatoxin contamination could have been attributed to seed coat thickness, permeability and seed testa constituents.

Among the peanut varieties sterilized with 10% sodium hypochlorite, the following varieties had invisible mycelia and highest mean ratings of kernel; Acholi white with 1.00, entry 99527 with 0.76, Serenut 1 and Serenut 2 with 0.62 each as shown in Table 3. The higher mean ratings of kernels with invisible mycelia (Acholi white, entry 99527, Serenut 1 and Serenut 2) are probably attributed to differences in the physical barriers constituents such as wax and cutin in the peanut seed testa which were sparingly soluble in sodium hypochlorite. Wotton and Strange [27] and Liang et al. [28] reported that wax and cutin isolated from seed testa play an inhibitory role against *A. flavus* colonization and invasion of peanut kernels.

Varieties	Invisible Mycelia growth	1-20%	21-50%	51 -70%	71-100%
Red beauty	0.49	0.15	5.00	2.00	3.00
Igola	0.49	1.03	5.50	3.50	1.00
Serenut 1	0.62	1.00	4.50	3.50	1.33
Serenut 2	0.62	0.50	2.21	3.50	3.50
Serenut 3	0.01	0.78	3.40	4.00	2.50
Serenut 4	0.50	0.40	4.00	3.00	2.50
Erudurudu	0.12	0.65	4.50	2.00	3.50
Acholiwhite	1.00	1.50	1.50	2.50	3.50
Gwerinut	0.13	0.34	2.50	1.50	6.00
99566	0.38	0.34	1.69	1.50	7.00
99527	0.76	1.19	4.50	1.50	2.00
99528	0.12	0.10	2.50	4.00	3.50
93535	0.28	0.34	5.50	1.33	3.50
Total	5.52	8.32	47.30	33.83	42.83
LSD (0.05)	1.34	2.02	11.48	8.21	10.40
CV, (%)	17.80	26.83	50.83	36.35	46.04

Scored on 1-5 rating scale where < 1=Invisible mycelial growth; 2=1-20% mycelial growth coverage on the kernel surface; 3=21-50% mycelial growth coverage on the kernel surface; 4=51-70% mycelial growth coverage on the kernel surface; 5=71-100% mycelial growth coverage on kernel surface.

Table 2: Mean ratings of infected peanut kernels sterilized with 10% sodium hypochlorite.

Varieties	Invisible mycelia	1 -20%	21 – 50%	51 – 70%	71 –100%
Red beauty	2.00	1.50	1.00	4.50	1.00
Igola	1.50	3.00	2.50	2.50	0.51
Serenut 1	2.50	2.50	2.50	2.50	0.37
Serenut 2	0.50	1.50	2.50	3.00	2.50
Serenut 3	0.00	4.00	2.00	3.00	1.00
Serenut 4	1.00	1.20	3.50	1.50	3.50
Eruduru	0.50	1.45	0.97	6.00	2.50
Acholi white	3.00	2.00	2.00	3.00	1.62
Totals	11.00	17.15	16.97	26.00	13.00
LSD	0.09	0.14	0.14	0.21	0.11
CV, (%)	20.00	31.11	31.11	46.67	24.44

Scored on 1-5 rating scale where < 1= Invisible mycelial growth; 2=1-20% mycelial growth coverage on the pod shell surface; 3=21-50% mycelial growth coverage on the pod shell surface; 4=51-70% mycelial growth coverage on the pod shell surface; 5=71-100% mycelial growth coverage on pod shell surface.

**Table 3:** Mean ratings of infected pods sterilized with 10% sodium hypochlorite.

However, under 71–100% mycelial growth surface coverage on the kernels category, Igola with 1.00, Serenut 1 with 1.10, entry 99527 with 2.00, Serenut 3 and Serenut 4 with 2.50 had the smallest mean ratings of infected kernels when sterilized with 10% sodium hypochlorite as observed in Table 2. The smaller mean ratings of infected kernels could have been due to the compact arrangement of palisade-like layers of the seed testa. The compact palisade-like layers of seed testa were reported to have reduced *A. flavus* colonization, invasion and subsequent infection of peanut kernels [29,30].

In general, the total mean ratings of kernels with invisible mycelia washed with sterile distilled water was higher than the total mean ratings of kernels sterilized with 10% sodium hypochlorite. However, the total mean ratings of infected kernels under 71–100% category which were washed with sterile distilled water were smaller than the total mean of infected kernels. The differences in total mean ratings of kernels with invisible mycelia and infected kernels is probably due to the dissolving action of sodium hypochlorite on the seed coat constituents especially the soluble wax and cutin in the alkaline solution of sodium hypochlorite whereas water has a minimum effect as it is neutral.

Sodium hypochlorite has a bleaching and dissolving effect on the soluble wax and cutin constituents which are embedded in the seed coat matrices and which are deposited on the external surfaces of the seeds [31]. Therefore by using 10% sodium hypochlorite as a sterilization agent, the seed protective mechanism against pathogen is broken down, consequently resulting in higher infection levels.

Among the varieties sterilized with 10% sodium hypochlorite, the biggest mean ratings with invisible mycelia was in Acholi white with 3.00, Red beauty with 2.00 and Igola with 1.50. Whereas Serenut 1 with 0.37, Igola with 0.57, Serenut 3 and Red beauty with 1.00 each of infected pods had the smallest mean ratings under 71–100% category as is presented in Table 3 below. The higher mean ratings of pods with invisible mycelial and the smaller mean ratings of infected pods could have been attributed to breakdown of resistance of pods against *A. flavus* colonization. According to Kushalappa et al. [32] pre-inoculation sodium hypochlorite sterilization of different peanut genotypes reduced their resistance against *A. flavus* infection. The sterilization action of sodium hypochlorite was reported to eliminate the antagonistic microflora which is responsible for peanut varietal resistance [33,34].

## Conclusion

The results from this experiment show differences in the mean

ratings of infected peanut kernels and pods. Differences were also observed in the mean ratings of peanut kernels and pods with no visible infection or invisible mycelial growth, however, these differences were not statistically significant  $P \geq 5$ . Peanut varieties with the highest mean ratings of kernels and pods with invisible mycelia and those with the smallest mean ratings of kernels and pods infected with *A. flavus* could be considered tolerant to *A. flavus* colonization and infection in this study. Since farmers store peanuts in kernel and pod form, peanut varieties with the biggest mean ratings of kernels (Entry 99527, Serenut 1, Serenut 2, Red beauty, Acholi white and Igola) with invisible mycelia and the varieties with the smallest mean ratings of infected kernels (Igola, Serenut 1, Serenut 2 and entry 99527) should be promoted as an aflatoxin management strategy. Therefore there is a need to carry out molecular elucidation for the cause of differences in the levels of *A. flavus* infection of the peanut varieties which have the biggest mean ratings of kernels with invisible mycelia and those with the smallest mean ratings of infected kernels. In addition, desirable peanut varieties with smaller mean ratings of invisible mycelia and those with the biggest mean ratings of infection by *A. flavus* warrant improvement by research.

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