Seed-storage Mycoflora of Peanut Cultivars Grown in Nigerian Savanna

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Summary

Storage of peanut is increasingly becoming important both among growers and users of the crop in Nigerian savanna. The aim is to sell the produce and maximize benefits accruing from the crops during scarcity. Very often, these envisaged advantages fail due to unfavourable market forces, thus compelling them to sell at a loss or store them across seasons for periods ranging from one to two years. However, information on fungi associated with storage of such peanuts in Nigerian savanna and its attendant problems are yet to be investigated. Thus, the seed mycoflora and viability seven common peanut cultivars stored under conditions similar to traditional settings were investigated using different isolating techniques. The peanut cultivars were RMP 12, RMP 91, RRB, 48-115B, M554-76, 55-437 Ex-Dakar and a local cultivar. None of these cultivars possessed resistance to in vitro *colonization by fungi.* Aspergillus niger, Aspergillus flavus *and* Rhizopus stolonifer *were consistently isolated from all the cultivars from almost all isolating techniques. Other fungi were* Fusarium chlamydosporium, F. roseum, F. oxysporium, Penicillium *spp.*, Curvularia *spp.*, Botryodiplodia theobromae, Macrophomina phaseolina *and* Sclerotium rolfsii. *Relative percentages, however, varied with individual fungi and peanut cultivars. The test with seven different types of growth media gave the highest fungi recovery rate than the blotter paper technique. Seed viability was lower with peanut seeds stored for two years. Also, the relative percentage occurrence of individual fungi was significantly higher with seeds stored for two years. While we recommend the use of growth media for recovery and study of seed mycoflora, peanut seeds should not be stored for more than one year.*

Résumé

Etude de la mycoflore lors de l'entreposage des graines de variétés d'arachide dans les savanes du Nigeria

L'entreposage de l'arachide est confronté à diverses contraintes qui concernent les producteurs et les consommateurs dans la région des savanes du Nigeria. Bien que l'objectif principal du producteur soit de vendre un produit de qualité afin de maximiser son profit, surtout lors des périodes de soudure, la commercialisation n'est pas toujours évidente. Cela est dû aux fluctuations du marché qui obligent les producteurs à vendre à perte ou à stocker leur récolte plus longtemps, jusqu'à deux ans, en attendant les jours meilleurs.

Cette étude, réalisée in vitro*, montre la présence des mycètes lors de l'entreposage sur les sept variétés d'arachide, les améliorées (RMP 12, RMP 91, RRB, 48-115B, M554-76, 55-437 Ex-Dakar), ainsi que la variété locale. Ces résultats montrent que toutes les variétés étudiées ont été sensibles à la colonisation des champignons et que toutes les techniques d' isolation utilisées ont permis d'isoler des champignons* Aspergillus niger*,* Aspergillus flavus et Rhizopus stolonifer*. D'autres champignons (*Fusarium chlamydosporium*, F. roseum,* Fusarium oxysporium, Penicillium *spp.*, Curvularia *spp.*, Botryodiplodia theobromae, Macrophomina phaseolina *et* Sclerotium rolfsii) *ont pu être identifiés mais leur fréquence dépendait du couple champignon/variété. En comparant les milieux de culture, les sept milieux étudiés ont donné une bonne croissance des mycètes par rapport à la culture sur du papier filtre humecté sans milieu de culture. Il a été également constaté après deux ans de stockage que le taux de viabilité des graines était faible alors que le pourcentage des mycètes augmentait significativement. Pour la bonne croissance des champignons, il est recommandé d'utiliser des milieux de culture et la conservation des graines d'arachide ne devrait pas dépasser une année.*

Introduction

Cultivated peanut (*Arachis hypogaea* L.) also known as groundnut, an annual legume and native to South America, is primarily cultivated in areas of the world between 40 ^{0}N and 40 ^{0}S (40). Approximately 80% of the world's production comes from developing countries where yields are usually low, ranging 0.5–1.0 t/ha compared to 2.7 t/ha in USA. Africa, and important continent for peanut production, produces 20% of the total world crop (11, 31, 40).

Peanut is an important cash and food crop in many parts of the tropics (14). In Nigeria, peanut is one of the most important leguminous crops second only to soybeans (*Glycine max* L.) (18). Nigeria which once produced up to 1.2 million metric tonnes per year and

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which was once an exporter of peanut is now an importer of peanut and peanut products (2, 18, 23). One of the main reasons for this setback are diseases during storage (23, 26, 43). Consequently, farmers are shifting to production of other grain crops.

Prince (35), McDonald (24), and Porter *et al.*, (34) examined pods from a range of varieties collected from dried stacked materials in the field and seeds in storage. Fungi isolated from seeds in descending order of importance based on frequency of occurrence included *Fusarium spp., Alternaria spp.*, sterile fungi, *Trichoderma viridae* Rufai, *Macrophomina phaseolina* Tassi, *Sclerotium rolfsii* Sac., *Diplodia natalensis* Pole-Everon, *Diplodia theobromae* Pat., *Penicillium spp.*, *Curvularia spp.*, *Fusoriella spp.*, *Rhizopus spp.*, *Rhizoctonia solani* Kuhn and *Scleroclaeta spp*. In a similar work utilizing different storage conditions of moisture regimes, Dienar (15) and Dange and Patel (13) observed that the most prominent fungi were *Aspergillus flavus* Link ex Fries, *A. tamerii* Kita and *Penicillium citrinum* Thom. Others species of frequent occurrence were *Aspergillus candidus* Link extr., *Cladosporium spp.*, *Torula sacchari* Carda, *Penicillium fumiculosum* Biourge, *Trichothecium roseum* pers. Link extr., *Rhizopus stolonifer (Ehrenb ex Link) Lind*, *Muccor spp.*, *Paecilomyces varietii* Bain, *Fusarium spp.*, and *Diplodia spp.* The findings of these research workers (13, 15, 34, 35) indicate that fungi associated with stored peanut may not be the same for different places and conditions. Also, differences in cultivars could have effects on colonization of peanut pods and seeds by fungi (8). For effective yield, viable seeds are among the most important things in agronomic practices. Global losses in food production due to seed borne diseases are important negative factors in world agriculture; hence seeds should be examined on a regular basis. Seeds have long been appreciated as the most important biological input to sustainable agricultural production and food security. It has been proved seed transmission is responsible for the perpetuation of plant diseases leading to drastic yield reduction (5, 16, 22, 28).

Mycoflora of peanut seeds have been reported on seeds of varieties NRRL 299, NRRL 502, NRRL 3357, NRRL 3239 in USA (6); TMV-2, TMV-7, GG2 and other varieties in India (13, 27, 36); a number of cultivars in South Carolina (35); Niger (42); Ivory Coast (33); Georgia (21); Virginia (17) and Brazil (20). However, there is no information yet on seed mycoflora of peanut cultivars commonly grown in the Nigerian savanna, the major peanut growing region of Nigeria. The aim of this study, therefore, was to investigate the seed-mycoflora of seven peanut cultivars cultivated in Nigerian savanna and their effect on seed viability.

Material and methods

-Sources of peanut seeds

Seeds of six peanut cultivars commonly grown in Nigerian savanna were obtained from Ahmadu Bello University, Zaria, Nigeria, the centre of the National Coordination on Groundnut Improvement Programme in Nigeria. The six cultivars were RMP 12, RMP 91, RRB, 48-115B, M554-76, and 55-437 Ex-Dakar. In addition, a local cultivar was procured from markets in Makurdi, Nigeria, making a total of seven cultivars employed in this investigation. The seeds were obtained manually from the pods. The seeds (13% R.H.) were stored in locally woven sacks made of fibre. The sacks were divided into two lots of five bags. Five of the bags were for one year and the remaining five for two years all at room temperature (28 ± 2 ^{0}C). Samples of seeds required for study were randomly obtained at the end of each storage period, one and two year(s) respectively. The fungi were isolated using seven different media and a blotter paper technique.

- Effect of storage period, media and blotter paper technique on the isolation of fungi associated with stored peanut seeds

Blotter paper technique (BPT) of Ito *et al.*, (20) was employed in this investigation. Moistened and sterile 9.00 cm diameter Whatman filter papers were placed in sterile Petri plates for the study.

The seven different media mentioned below were used to investigate the fungi. Potato (*Solanum tuberosum* L.) and cassava (*Manihot utilissima* L.) extracts were prepared by boiling 200 g each of both freshly peeled and sliced potato and cassava in 250 ml distilled water for 30 min. Beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) extracts were obtained by boiling 200 g of both ground beans and corn in 250 ml of sterile distilled water for 30 min. Extracts of tomatoes (*Lycopersicon esculentum* L.) and red sweet pepper (*Capsicum annuum* L.) and fresh leaves of fluted pumpkin (*Telfaria occidentalis* L.) were prepared by boiling 200 g of sliced fruits of tomatoes, red sweet pepper and chopped fresh leaves of fluted pumpkins in 500 ml of sterile distilled water for 30 min. Each filtered extract was respectively made up to 1l on cooling and supplemented with 20 g each of plain agar and glucose to make potato-dextrose-agar (PDA), cassava-extract-dextrose-agar (CAEDA), beans-extract-dextrose-agar (BEDA), corn-meal-dextrose-agar (CMDA), tomato-extract-dextrose-agar (TEDA), pepper-extract-dextrose-agar (PEDA) and fluted pumpkin leaf-extract-dextrose-agar (FPLEDA). All these media were autoclaved at 121 ^{0}C for 15 min. The media were upon cooling poured into 9.00 cm diameter Petri plates.

Each medium and blotter paper was seeded with four seeds of each cultivar/plate. To increase the surface area of seeds for isolation of fungi, similar experiments were performed for different parts of seed, which included testas, cotyledons, and embryos. The seed parts were respectively obtained through manual separation. Thus, a total of 1,152 samples (made up of 4 seeds, 4 testas, 4 cotyledons, 4 embryos) x 3 replications x 3 repetitions x 8 isolating techniques (seven different media + blotter paper technique) per cultivar of each storage period were used for the study. The experiment, which was arranged in a completely randomized block design, was incubated at 28 ± 2 ^{0}C and monitored daily for emerging fungus for 14 days, during which any fungus inhabiting the seed/seed parts would have emerged. Fungi species were determined by comparison with already stock

cultures that have been confirmed by International Mycological Institute, Kew, Surrey, England and deposited in our laboratory as well as references to relevant literatures and bibliographies on fungi. Relative percentage occurrence was calculated for each emerging fungus, as given in equation X= Y/Z; where Z= any fungus, Y= frequency of isolation of fungus X, Z= total number of seeds/seed parts in an experiment. The mean treatment total of each trial was used for further statistical analysis.

-Viability tests

The effect of storage period on viability of peanut seeds was determined on blotter paper as described for seeds. A seed is considered viable when it can germinate. Seeds are classified as having germinated if the radicle and coleoptile have grown out of the epicarp; a change from seed to seedling. Seed viability was also tested in the field.

For blotter paper, samples of four seeds/cultivar were placed on sterile moistened Whatman filter papers in sterile Petri plates following the procedures of Ito *et al.*, (20). Each experiment, which comprised of 50 plates (200 seeds; 4/plate of 50/cultivar) were completely randomised on a laboratory bench at 28 ± 2 ^{0}C and observed daily for seed germination.

On the field, the usual agronomic practices were followed for the planting of the seeds in a randomised block design. The seeds were sown on rows of 50 m long and 60 cm apart. A spacing of 10 -15 cm between plants within a row was used during planting according to recommendations of Subrahmanyam *et al.*, (40). The design was 7 cultivars x 3 replications and repeated thrice. The experiment whose moisture requirement was through the natural rain fed water was monitored daily for seed germination. Viability was measured by the percentage of germinated seeds/cultivar for each storage period. Data were analysed by analysis of variance and treatment means separated by Duncan's New Range Multiple Range Test at 5% probability level.

Results

-Effect of blotter paper technique and seven different media on the isolation of fungi associated with seeds of seven peanuts cultivars stored for one and two years

For blotter paper technique, fungi isolated and analysed from seeds stored for one year were *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Fusarium chlamydosporium* and *Curvularia spp. A. niger* and *F. chlamydosporium* had the highest and lowest relative percentage occurrence of 27.8% and 4.4% respectively (Table 1).

The same fungi organisms were also observed with seeds stored for two years. *A. niger* was highest with 67.5% while *F. chlamydosporium* was lowest with 4.4% (Table 2).

The result showed that, though, *A. niger* had the highest relative percentage occurrence. The percentage value of 67.5 was higher for seeds stored for two years than 27.8% for one year. Thus, the longer the storage period, the more the seeds become infected.

Table 1
Effect of blotter paper technique and seven different media on the isolation of fungi associated with seeds and seed parts of seven peanut cultivars one year after storage

Fungi	Blotter paper technique and media								
	BPT*	PDA	CAEDA	BEDA	CMDA	TEDA	PEDA	FPLEDA	MEAN TOTAL
Aspergillus niger	27.8**	41.3	44.4	38.5	37.3	44.1	38.9	44.4	39.6 (.11)a
A. flavus	19.8	28.2	44.1	35.3	28.2	37.7	35.3	28.2	32.1 (.10)ab
Rhizopus stolonifer	26.6	38.1	38.5	00	34.5	38.9	4.8	38.1	27.4 (.09)b
Fusarium roseum	00	6.2	00	00	4.0	10.2	00	00	2.6 (.03)c
F. chlamydosporium	4.4	12.3	9.1	00	00	15.5	00	11.5	6.6 (.04)c
F. oxysporium	00	00	00	10.3	00	00	00	00	1.3 (.02)c
Curvularia spp.	6.0	00	00	7.9	00	16.3	25.3	00	6.9 (.05)c
Penicillium spp.	00	00	00	00	00	00	00	16.7	2.1 (.03)c
Botryodiplodia theobromae	00	12.3	00	00	13.9	00	00	00	3.3 (.03)c
Sclerotium rolfsii	00	00	00	15.9	18.3	00	00	9.1	4.4 (.04)c

Keys:
* BPT= Blotter paper technique, PDA= Potato-dextrose-agar, CAEDA= Cassava-extract-dextrose-agar, BEDA= Beans extract-dextrose-agar, CMDA= Corn-meal-extract-dextrose-agar, TEDA= Tomato-extract-dextrose-agar, PEDA= Pepper-extract-dextrose-agar and FPLEDA= Fluted-pumpkin -leaf extract dextrose-agar.
** Each figure represents a mean percentage occurrence of each fungus from seven cultivars of peanut/isolating medium or technique. Figures in parenthesis are arc sine transformed values, arc sine= $\sqrt{\chi}$.
Means followed by the same letter are not significantly different from each other according to Duncan's multiple New Range Test (P≤ 0.05).

Table 2
Effect of blotter paper technique and seven different media on the isolation of fungi associated with seeds of seven peanut cultivars two years after storage

Fungi	Blotter paper technique and media								MEAN TOTAL
	BPT*	PDA	CAEDA	BEDA	CMDA	TEDA	PEDA	FPLEDA	
Aspergillus niger	67.5**	37.7	61.1	33.7	38.7	49.2	44.8	45.2	47.2 (.12)a
A. flavus	43.5	28.6	49.2	34.9	42.2	41.3	30.5	52.8	40.4 (.11)ab
Rhizopus stolonifer	63.5	37.3	51.2	00	29.2	19.1	17.9	48.8	33.4 (.10)b
Fusarium roseum	2.0	14.2	6.0	00	8.3	6.8	00	00	4.7 (.04)c
F. chlamydosporium	4.4	18.3	00	00	9.5	00	00	17.5	6.2 (.04)c
F. oxysporium	00	00	16.3	24.2	00	28.4	00	00	8.6 (.05)c
Curvularia spp.	6.0	20.2	10.3	20.0	00	00	17.9	00	9.3 (.05)c
Penicillium spp.	00	12.7	00	00	00	30.5	00	17.1	7.5 (.05)c
Botryodiplodia theobromae	00	00	00	29.0	8.0	00	00	00	4.6 (.04)c
Sclerotium rolfsii	00	00	00	00	10.0	04	00	00	1.8 (.02)c
Macrophomina phaseolina	00	11.1	00	00	00	00	00	00	1.4 (.02)c
Trichoderma spp.	00	00	8.7	00	00	00	00	00	1.1 (.02)c

Keys: as in table 1.

Results of isolation of fungi associated with peanut seeds using media indicated that *A. niger* had the highest relative percentage occurrence from seeds of seven peanut cultivars stored for one and two year(s). However, the percentage values varied with media. Cassava-extract-dextrose-agar (CAEDA) and fluted pumpkin leaf-extract-dextrose-agar (FPLEDA) were most favourable for isolation of *A. niger* with relative percentage occurrence of 44.4% each (Table 1). After two years of storage of peanut cultivars, the medium with highest percentage record of *A. niger* was CAEDA (Table 2). Following *A. niger* in order of frequency of isolation and occurrence were *A. flavus* and *R. stolonifer*. These three fungi were consistently observed over all isolating media and the cultivars except *R. stolonifer* which was not isolated when bean-extract- dextrose-agar was used as an isolating medium (Tables 1 and 2).

Similarly, some fungi were not observed with some peanut cultivars. For instance, *Sclerotium rolfsii* was

Table 3
Relative percentage occurrence of fungi associated with seeds of seven peanut cultivars one and two years of storage

Cultivar	Storage period	Fungi											
		Aspergillus niger	*A. flavus*	*Rhizopus stolonifer*	*Fusarium roseum*	*F. chlamydosporium*	*F. oxysporium*	*Curvularia* spp.	*Penicillium* spp.	*Botryodiplodia theobromae*	*Sclerotium rolfsii*	*Macrophomina phaseolina*	*Trichoderma* spp.
RMP 12	1 year	13.1*	13.9	10.4	2.8	2.9	3.8	1.4	2.8	0.9	0.0	0.0	0.0
	2 years	15.6	16.9	11.8	3.8	3.4	2.1	4.6	2.8	0.0	0.0	0.6	0.9
RMP 91	1 year	12.7	13.0	11.2	1.6	1.7	1.6	1.8	0.0	1.3	0.7	0.0	0.0
	2 years	15.1	17.0	11.9	4.2	3.1	1.6	5.2	0.0	0.9	1.0	0.0	0.0
RRB	1 year	12.7	11.0	9.5	3.0	2.7	0.5	0.4	0.0	1.4	0.9	0.0	0.0
	2 years	16.3	12.9	14.2	4.6	1.9	1.1	4.6	1.4	1.4	0.4	1.5	2.1
48-115B	1 year	14.6	10.9	8.0	1.3	1.4	0.0	1.8	1.4	1.6	0.0	0.0	0.0
	2 years	16.1	14.4	7.7	1.0	0.9	4.6	1.2	1.4	2.0	0.0	0.0	0.0
M554-76	1 year	14.0	11.6	10.0	0.6	0.7	0.8	1.4	1.2	0.8	1.5	0.0	0.0
	2 years	17.1	13.6	12.3	6.8	4.4	1.3	3.5	2.4	1.3	1.5	0.5	0.0
55-437 Ex-Daka	1 year	13.0	10.5	7.9	1.0	1.1	2.6	0.3	2.5	1.4	1.4	0.0	0.0
	2 years	14.2	15.4	6.6	1.2	0.8	2.9	0.9	1.8	2.6	0.0	0.0	0.0
Local Cultivar	1 year	12.9	10.0	7.6	1.0	0.0	1.2	0.0	0.0	0.0	0.9	0.0	0.0
	2 years	13.5	14.4	13.6	0.8	0.7	1.2	3.4	3.1	1.9	1.5	0.0	0.0
Mean Total	1 year	13.3(.06)a	11.6(.06)a	9.2(.05)a	1.6(.02)b	1.5(.02)b	1.5(.02)b	1.0(.02)b	1.1(.02)b	1.1(.02)b	0.8(0.02)b	0.0(.00)b	0.0 (.00)b
	2 years	15.4(.07)a	14.9(.07)a	11.2(.06)a	3.2(.03)b	2.2(.03)b	2.1(.03)b	3.3(.03)b	1.8(.02)b	1.6(.02)b	0.6(.01)b	0.4(.01)b	0.4 (.01)b

* Each figure represents an average of 1,152 samples of peanut seeds and seed parts per cultivar. Figures in parenthesis are arc sine transformed values, arc sine $\sqrt{\chi}$.

Means followed by the same letter along the horizontal column are not significantly different according to Duncan's New Multiple Range Test ($p \leq 0.05$).

not isolated from RMP 12 and 48-115B (Table 3). In other cultivars, some fungi were isolated from seeds only after a certain storage period. *Macrophomina phaseolina* was only isolated from seeds stored for two years (Table 3). Percentage occurrences of *A. niger, A. flavus* and *R. stolonifer* were significantly higher than other fungi (Table 3).

Viability values showed that seeds stored for two years had significantly (P= 0.05) lower values both in the laboratory and in the field than those stored for one year. However, percentage viability was generally higher in the field than in the laboratory for each respective cultivar. In the laboratory, percentage viability ranged from 25.0 - 32.4% and 1.9 - 4.6% for seeds stored for one year and two year(s), respectively (Table 4), whereas, the percentage viability for the corresponding periods was between 90 and 97% for seeds stored for one year, and 8 - 15% for seeds stored for two years (Table 4).

These data showed that the longer the storage period, the higher the attack by colonizing and associated fungi, and the lower seed viability (Table 4). On cultivar RMP 12, percentage viability of seed stored for one year was 28.7% in the laboratory using blotter paper technique. For this corresponding period and the same cultivar, viability was 90.3% in the field (Table 4). After two years of storage, viability of RMP 12 fell to 2.7% and 10.1% in the laboratory and in the field, respectively (Table 4).

derma spp. and *Penicillium spp*. in small and subdominant group, in this investigation is in contrast with the previous reports of Garren (17) for Virginia and Jackson (21) in Georgia. In the findings of these authors, Garren (17) and Jackson (20), *Trichoderma spp*., *Penicillium* spp., and *Fusarium* spp. were predominant while *Aspergillus spp*. were subdominant. The contrasts are most likely due to varietal and environmental and less to qualitative differences in the seeds mycoflora. Bass (7) reported that the storage potential of grain is influenced by inherent as well as external factors, especially genetic differences between genera, species and cultivars. Some of these fungi isolated in this work have been reported as being associated with seeds in Nigeria and other parts of the world (9, 10, 28, 30). However, this is the first record of seed storage-mycoflora of seven peanut cultivars that are cultivated in agro-ecological zone of Nigerian savanna.

The inconsistent isolation and absence of other fungi and consistent presence of *Aspergillus* and *Rhizopus spp*. may be an indication of adaptation and colonizing efficiency. *Aspergillus* and *Rhizopus* spp. colonized and adapted easily on the seeds, thus depleting nutrients that would have been made available for other fungi. Basha and Pancholy (6) reported a decrease in oil, iodine value, soluble carbohydrates and protein contents in groundnut seed infested with *Aspergillus* spp. This may be responsible for the inconsistencies in the isolation of other fungi that could not be observed from some other cultivars. *Macrophomina phaseolina* was isolated only from seeds stored for two years. Thus, the absence of some fungi may not be due to resistance to *in vitro* colonization of genotypes by certain fungi to reach and colonize the particular cultivar. Sherf and Macnab (38) and Coelho and Dhingra (12) found that *Macrophomina* is a weak pathogen with poor saprophytic ability and infects

Table 4
Viability tests of seeds of seven peanut cultivars one and two year(s) of storage

Cultivars	Location of tests			
	Laboratory		Field	
	One year old seeds	Two years old seeds	One year old seed	Two years old seeds
RMP 12	28.7 (.09)a*	2.7 (.03)a	90.3 (.17)a	10.1 (.06)a
RMP 91	25.0 (.08)b	2.7 (.03)a	95.0 (.17)a	14.0 (.07)a
RRB	27.8 (.09)a	1.9 (.02)a	96.4 (.17)a	8.7 (.05)a
48 – 115B	32.4 (.10)a	1.9 (.02)a	95.0 (.17)a	15.0 (.07)a
M554 - 76	28.7 (.09)a	1.9 (.02)a	97.6 (.17)a	15.5 (.07)
55 - 437 Ex-Dakar	32.4 (.10)a	1.9 (.02)a	94.2 (.17)a	10.2 (.06)a
Local cultivar	31.0 (.10)a	4.6 (.04)a	94.0 (.17)a	10.0 (.06)a

* Figure represents average percentage viability of seeds of different seven peanut cultivars. Figures in parenthesis are arc sine transformed values, arc sine $\sqrt{\chi}$.
Means followed by the same letter in the vertical column are not significantly different according to Duncan's New Multiple Range Test ($p \leq 0.5$).

Discussion and conclusions

The consistent isolation and presence of *Aspergillus spp*. from all the cultivars confirmed the earlier reports of Dienar (15) and Dange and Patel (13) that *Aspergillus* spp. were the prominent fungi isolated from stored groundnut seeds. The occurrences of other fungi organisms like *Fusarium* spp., *Tricho-*

through wounds. Other agents or penetration could ascribe the appearance of *Macrophomina phaseolina* after two years of storage to the inability of the fungus to infect and establish in seeds not until damage by other fungi (30).

An interesting observation was the absence of *Rhizopus stolonifer* when bean-extract-agar medium was used. A further study is required on the behaviour of *Rhizopus* spp. and bean-extract-derived media.

Mycoflora of peanuts are known to produce a large number of metabolites including aflatoxin. Aflatoxin contamination of groundnut is of significance in relation to public health and future export trade. Aflatoxin contamination of groundnut is one of the most important constraints to production in many West African countries (26). The toxin also constitutes a potential and even presents environmental hazard to animal and man alike (4, 32, 41). Aflatoxin is potent hepatocarcinogenic secondary metabolites produced by *Aspergillus* spp. These fungi occur on a number of agricultural commodities including peanuts. The use of stored food containing more than 20 ppb (µg/kg) aflatoxin for human consumption is prohibited in Mexico and other countries including United States of America. Grains with more than 20 ppb aflatoxin cannot be sold through interstate commerce, and some countries will not buy grain with contamination greater than 10 ppb (29, 37). Other effects of mycotoxins on human and animal health reported in medical literature include ergotism, hepatitis, tetratogenic, tremorgenic, skin diseases, gastroentritis, hemorrhage and vomiting (39). In view of the statements by Alpert and Davidson (3) that mycotoxins are the important causes of primary liver cancer, mouldy foods and beverages should be considered dangerous and not to be consumed. In this investigation, where there is no genotype with *in vitro* resistant to colonization by fungi suggests that these cultivars are a potential danger to animal and man if infested seeds are consumed or colossal losses of serious economic magnitude could be incurred if stored for two years.

Reduced viability of seeds with increased storage period may be associated with *Aspergillus* and *Rhizopus* spp., which were consistently isolated from all seven cultivars in almost all media. Reduced rice germination in the laboratory has also been associated with *Aspergillus* spp. (19). Our findings here therefore, suggest that some of the fungi were probably extraneous or just natural contaminants of seed. Jackson (21) and Mehan *et al.*, (25) pointed out that some of the these fungi were probably only casually associated with groundnut but a large number of them have been consistently reported as members of shell (pod) and seed mycoflora of sound and diseased pods and may be said to pose an affinity for the groundnut pod. This is consistent with the conclusions of Agarwal and Sinclair (1) that most seed-borne pathogens do not kill the seed immediately.

The higher percentage viability values in the field are indications of a more conducive environment for testing seed germination than the blotter paper technique, which has limited conditions in the laboratory. Also, the highest rate of fungi recovery from growth media showed that media are better use for testing and study of seed mycoflora than the blotter paper technique.

Studies are in advanced progress at our laboratory on the effect of different storage techniques on mycflora of groundnut seed in Nigeria.

Recommendations

To be able to achieve a more reliable production in groundnut, a lot of work is required to screen more cultivars for genotypes with *in vitro* colonization by fungi. One of the possible means of reducing aflatoxin contamination of groundnut is the introduction and cultivation of resistant cultivars. Although resistant cultivars such as ICGV 87084, ICGV 87094 and ICGV 87110 have been reported (42); whether they will adapt to Nigerian savanna environment is another subject of investigation when they are introduced. Long storage period by producers and users of groundnut seeds should not be encouraged to avoid losses.

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