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FUNGI ISOLATED FROM GROUNDNUTS IN SOME LOCATIONS OF WEST JAVA

O.S. DHARMAPUTRA

SEAMEO BIOTROP, P.O. Box 116, Bogor, Indonesia; and Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia

I. RETNOWATI

SEAMEO BIOTROP, P.O. Box 116, Bogor, Indonesia

ABSTRACT

One hundred and ninety eight groundnut samples were collected from freshly harvested groundnuts (FHG), farmer storage systems (FSS), middlemen warehouses (MW), wholesalers (WS) and retailer sample (RS) during the dry and wet seasons from Cidolog, Cianjur, Sukabumi and Bogor, West Java, Indonesia, in 1990/1991.

The moisture content (m.c.), intactness of kernels, and the percentages of groundnut kernels infected by each species of fungi were analyzed.

In genera), the m.c. of the samples collected during the dry season was lower than of those collected during the wet season. Also, the m.c. of samples collected from FHG, FSS and MW was higher than of those collected from WS and RS.

The m.c. of samples collected from FHG was the highest (12.5-45.75%), but the percentages of damaged kernels were the lowest (2.5-13.8%), because the samples were shelled manually.

A total of 25 species of fungi were isolated from samples collected from the 4 localities. They were *Acremonium strictum, Aspergillus candidus, A. flavus, A. niger, A. ochraceus, A. tamarii, A. wentii, Botryodiplodia theobromae, Cladosporium cladosporioides, C. sphaerospermum, Eumtium chevalieri, E. repens, E. rubrum, Fusarium equiseti, F. longipes, F. oxysporum, F. semitectum, Mucor* sp., *Papulaspora* sp., *Pestalotia* sp., *Penicillium aethiopicum, P. citrinum, Rhizapus* sp., *R. stolonifer* and *Syncephalastrum* sp.

The predominant fungi in samples collected from Cidolog and Sukabumi during the dry season were *Aspergillus wentii,* while those collected from Cianjur and Bogor were *A. niger.*

The percentages of kernels infected by *A. wentii* in samples collected from Cidolog and Sukabumi were between 30-100% and 36-100%, respectively, while those of kernels infected by *A. nigerin* samples collected from Cianjur and Bogor were between 34-93% and 14-98%, respectively.

The predominant fungi in samples collected from each location during the wet season were *A. flavus.* The percentage of kernels infected by the fungus in samples collected from Bogor was the highest (83-100%).

Key words: Indonesia/West Java/Stored product pests/Groundnut/Fungi/*Acremonium strictum/Aspergillus* sp./*Botryodiplodia theobromae/Cladosporium* sp./*Eurotium sp./Fusarium* sp./*Mucor* sp./*Papulaspora* sp./*Pestalotia* sp./*SPenicillum sp/Rhizopus* sp./*Syncephalastrum* sp./Moisture content.

INTRODUCTION

Groundnut is a major food legume and an important secondary crop after maize and soybean in Indonesia. Since Indonesia has a humid tropical climate, this commodity could be easily infected by fungi during storage when freshly harvested or even before harvest.

There are various practices of post-harvest processing and storage that could affect the moisture content and the intactness of the kernels and thus their susceptibility to fungal infection.

Aspergillus and *Penicillium* are the two common genera of fungi found on stored products. They can cause weight loss, seed discolouration, heating and mustiness, and production of mycotoxins, specially aflatoxins. The latter are toxic metabolic substances produced by *A. Flavus* and *A. parasiticus* and are known to be carcinogenic agents (Butler 1974).

In Indonesia, very few studies have been conducted on storage fungi of groundnuts and their ability to produce aflatoxin. This study shows the presence of fungi infecting goundnuts. Information on the percentages of groundnut kernels infected by each species of fungi, moisture content and damaged kernels of freshly harvested, post-harvest, stored and marketed materials during dry and wet seasons are also reported.

MATERIALS AND METHODS

Sample collection

A total of 198 groundnut samples were collected in Cidolog, Cianjur, Sukabumi and Bogor, West Java, Indonesia, in 1990/1991. Of the total, 100 were collected during the dry season (August, September and October, 1990) from farmer storage systems (FSS), middlemen warehouses (MW), wholesalers (WS) and retailer samples (RS), while 98 samples were collected during the wet season (February and March 1991) from freshly harvested groundnuts (FHG), FSS, MW, WS and RS. The samples consisted of shelled and unshelled groundnuts with the latter shelled manually.

About 1 kg of each sample was then divided into 4 subsamples for (1) moisture content analysis, (2) damage kernels analysis, 3) fungal analysis and 4) reserve sample.

Moisture content analysis

Percent moisture content of kernels (on the basis of water loss) was determined by the oven method (ISO 1968). Three replicates were used for each sample. The kernels were ground and dried in the oven at 130 C for 2 hours.

The moisture content was determined using the formula:

$$\text{Moisture content} = (Mo - Ml) \times \frac{100}{Mo}$$

where : Mo is the initial mass, in gram, of the test portion
Ml is the mass, in gram, of the dry test portion

Damaged kernel analysis

The kernels were classified as either intact or damaged. Damaged kernels are those cracked, broken, porous, shrivelled or insect- damaged. Three replicates were used for each sample. The percentage of damaged kernels was determined using the formula:

$$\text{Damaged kernels} = \frac{\text{weight of damaged kernels}}{\text{weight of the kernels analyzed for intact and damaged kernels}} \times 100\ \%$$

Fungal analysis

Fungi were isolated using direct plating methods on Dichloran 18% Glycerol Agar (DG 18). According to Hocking and Pitt (1980), DG 18 was especially used for the isolation of xerophilic fungi.

Before plating, samples were individually disinfected using 1% sodium hypo-chlorite for 2 minutes. One hundred kernels were then plated (10 kernels/plate) on the medium and incubated at 25 C for 7 days.

The fungi were identified using the publications of Samson *et al.* (1984), and Pitt and Hocking (1985) as the main references.

The percentage of kernels infected by fungal species on each medium was determined.

RESULTS AND DISCUSSION

Moisture content

Moisture content (m.c.) is the most important factor determining the development of storage fungi in seeds (Christensen & Kaufmann 1975; Neergaard 1979). According to WHO (1979) the minimum m.c. of groundnuts for the development of *Aspergillus flavus* was 9.0 - 10.0%.

The m.c.'s of samples collected from different localities during the **dry** season are presented in Table 1. The m.c.'s of samples from Cidolog, Cianjur, Sukabumi and Bogor were between 9.9 - 14.1%, 7.9 - 10.5%, 7.5 - 11.2% and 6.1 - 10.7%, respectively.

It seems that the m.c.'s of samples collected from the same location were not affected by their source (FSS and MW from Cidolog, WS and RS from Cianjur, Sukabumi and Bogor).

The m.c.'s of samples collected from different localities during the wet season are presented in Table 2. The m.c.'s of the samples from Cidolog were between 12.5 -45.7% (FHG) and 10.8 - 18.1% (samples from FSS and MW), while those from Cianjur, Sukabumi and Bogor were between 8.7 - 13.9%, 7.5 - 10.3% and 7.7 - 11.1%, respectively.

Table 1. Moisture contents and percentages of damaged kernels of groundnuts collected during the dry season

Date and place of collection	Source of sample	Nature of sample	Sampling no.	Moisture content (%)	Condition of kernel (% damage)
8 August 1990, Cidolog	Farmer Storage System (FSS)	Unshelled	14	9.9 - 13.7	12.0 - 51.8
		Shelled	1	12.1	18
	Middlemen Warehouse (MW)	Shelled	5	10.5 - 14.1	25.8 - 46.0
28 August 1990, Cianjur	Wholesaler (WS)	Shelled	2	9.0 - 10.1	14.5 - 24.8
	Retailer Sample (RS)	Shelled	18	7.9 - 10.5	10.2 - 38.0
18 September 1990, Sukabumi	WS	Shelled	5	8.5 - 11.2	19.5 - 27.9
	RS	Shelled	15	7.5 - 11.0	8.7 - 27.9
8 October 1990, Bogor	WS	Shelled	2	7.9 - 10.1	6.7 - 16.8
	RS	Shelled	38	6.1 - 10.7	1.5 - 26.0

Table 2. Moisture content and percentages of damaged kernels of groundnuts collected during the **wet** season

Date and place of collection	Source of sample	Nature of sample	Sampling no.	Moisture content (%)	Condition of kernel (% damage)
15 February 1991, Cidolog	Farmer Storage System (FSS)	Unshelled	14	10.8 - 18.1	9.9 - 21.0
		Shelled	1	12.6	31.7
	Middlemen Warehouse (MW)	Shelled	5	14.6 - 15.1	17.0 - 25.0
4 March 1991, Cianjur	Wholesaler (WS)	Shelled	1	12.4	31.4
	Retailer Sample (RS)	Shelled	19	8.7 - 13.9	13.0 - 61.0
11 March 1991, Sukabumi	WS	Shelled	2	9.1 - 10.0	18.0 - 24.0
	RS	Shelled	18	7.5 - 10.3	19.9 - 55.0
13 March 1991, Bogor	WS	Shelled	5	7.9 - 9.8	6.5 - 27.4
	RS	Shelled	15	7.7 - 11.1	7.7 - 56.0
30 March 1991, Cidolog	Freshly Harvested Groundnut (FHG)	Unshelled	18	12.5 - 45.7	2.5 - 13.8

In general, the m.c.'s of the samples collected during the **dry** season were lower than of those of the **wet** season.

The moisture content of samples collected on 30 March 1991 was the highest (12.5 - 45.7%), because they were not yet dried.

According to BULOG (1981) the maximum moisture content of stored groundnuts should be 7%. In this study, the moisture contents of the samples collected from farmers storage systems and middlemen warehouses were higher than 7%.

Damaged kernels

Damaged kernels gave a chance for fungi to infect the seeds. According to Christensen (1980) physical damage of seeds is one of the factors that affects fungal infestation.

The percentages of damaged kernels of samples collected from different localities during the **dry** season are presented in Table 1. The percentages of damaged kernels from Cidolog, Cianjur, Sukabumi and Bogor were between 12.0 - 51.8%, 10.2 - 38.0%, 8.7 - 27,9%, and 1.5 - 26.0%, respectively.

The percentages of damaged kernels of samples collected from different localities during the **wet** season are presented in Table 2. The damaged kernels from Cidolog were between 2.5 - 13.8% (FHG) and 9.9 - 31.7% (samples from FSS and MW), while those from Cianjur, Sukabumi and Bogor were between 13.0 - 61.0%, 18.0 - 55.0%, and 6.5 -56.0%, respectively.

The percentage of damaged kernels of samples collected on 30 March 1991 was the lowest because they were shelled manually, while the other samples were shelled either manually or using wooden/zinc sheller. It was assumed that handshelling prevented the occurrence of damaged kernels, but this method is time consuming. Proper tools should be used for shelling groundnuts to minimize the occurrence of damaged kernels.

Fungal analysis

Twenty three species of fungi were isolated from samples collected from Cianjur, Sukabumi and Bogor during the **dry** and **wet** seasons. The isolated fungal species belonged to the field fungi (i.e. *Botryodiplodia theobromae, Cladosporium cladospo-rioides, C. sphaerospermum, Fusarium equiseti, F. longipes, F. oxysporum, F. semitectum* and *Papulaspora* sp.), the storage fungi (i.e. *Aspergillus Candidas, A. flavus, A. niger, A. ochraceus, A. tamarii, A. wentii, Eurotium chevalieri, E. repens, E. rubrum, Penicillium aethiopicum* and *P. citrinum),* and fungi belonging to the Mucorales (i.e. *Mucor* sp., *Rhizopus* sp., *R. stolonifer* and *Syncephalastrum* sp.).

Aspergillus candidus, A. flavus, A. niger, A. ochraceus, A. tamarii, A, wentii, Botryodiplodia theobromae, Eurotium chevalieri, E. repens, Papulaspora sp. and *Penicillium citrinum* were always isolated from samples collected in each location during the **dry** season, while those isolated from samples collected during the wet season were always *A. flavus, A. niger, A. tamarii, A. wentii, B. theobromae, E. chevalieri, Papulaspora* sp., *Penicillium citrium* and *R. stolonifer.*

According to Dharmaputra and Rahayu (1988) the predominant fungal species isolated from groundnut samples collected from a market in Bogor, West Java, Indonesia belonged to the genera *Aspergillus, Eurotium* and *Penicillium,* i.e. *A. candidus, A. flavus, A. niger, A. restrictus, Eurotium* spp. and *Penicillium* spp.

The fungal species and the percentage of kernels infected by each species of fungi from samples collected from the three localities are presented in Table 3.

The percentages of kernels infected by the field fungi i.e. *F. longipes* (collected from Cianjur during the **dry** season), *C. cladosporioides* and *F. semitectum* (collected from Bogor during the **dry** season), *F. equiseti* and *F. oxysporum* (collected from, Cianjur during the **wet** season) and *C. cladosporioides, C. sphaerospermum* and *F. semitectum* (collected from Sukabumi during the **wet** season) were very low. They were between 0 - 4%, 0 - 8%, 0 - 5%, 0 - 1%, 0 - 9%, 0 - 3%, 0 - 5% and 0 - 2%, respectively.

Table 3. The fungal species and range of percentage of infected kernels of shelled groundnut samples collected from different localities and different storage systems

Fungi	Range of percentage of infected kernels											
	Cianjur				Sukabumi				Bogor			
	D		W		D		W		D		W	
	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS
Aspergillus candidus	3-10	0-8	0	0	3-18	0-9	0	0	5-6	0-39	0	0
A. flavus	34-58	9-98	42	41-100	18-60	10-85	36-81	59-96	31-77	8- 95	83-100	83-100
A. niger	34-61	51-93	50	35-92	16-43	9-100	15-29	24-91	5-87	14- 98	35-75	40-98
A. ochraceus	0	0-4	2	0-9	0-8	0-61	0	0-2	0-1	0-3	0-25	0-1
A. tamarii	10-15	0-70	34	13-85	0-59	3-63	44-56	8-100	0-2	0-74	34- 88	8-85
A. wentii	53-95	7-100	53	0-55	69-100	36-98	56-84	30-95	11-54	4- 91	35-91	19-69
Botryodiplodia theobromae	0-32	0-25	18	0-266	0-16	0-29	15-17	0- 31	0-3	0-20	0	0-12
Cladosporium cladosporiodes	0	0	0	0	0	0	0	0	0-3	0-3	0-8	00
C. sphaerospermum	0	0	0	0	0	0	0	0-5	0	0	0	0
Eurotium chevalieri	4-9	3-74	0	0-44	10-59	2-73	0-1	0-65	20-22	2- 81	20-86	0-82
E. repens	0-2	0-34	0	0-2	5-30	0-71	0	0-4	10	0-76	3-24	0-32
E. rubrum	0	0	0	0	0	0	0	0-2	0	0	0-19	0-20
Fusarium equiseti	0	0	0	0-1	0	0	0	0	0	0	0	0
F. longipes	0	0-4	0	0	0	0	0	0	0	0	0	0
F. oxysporum	0	0	0	0-9	0	0	0	0	0	0	0	00
F. semitectum	0	0	0	0	0	0	0	0	0-2	0-1	0-5	00
Mucor sp.	0-4	0-10	0	0	1-4	0-18	0	0	1-6	0-32	0	0
Papulaspora sp.	0-24	0-27	8	0-21	00-32	0-24	0-6	0-11	5-6	0-13	0- 1	0-11
Penicillium aethiopicum	0	0	0	0	0	0	0	0-46	0	0	0	0
P. citrinum	11-50	0-18	49	22-87	1-10	2-29	23-66	0-76	3-36	0-47	1- 9	2-50
Rhizopus sp.	20-21	0-30	0	0	3-7	2-17	0	0	0-25	0-30	0	0
R. stolonifer	0	0	+	0-+	0	0	+	0-++0	0	0	0-++	
Syncephalastrum sp.	0	0	0	0	0	0	0	0-++	0	0	0	0

D = Dry Season
W = Wet Season
WS = Wholesaler
RS = Retailer Sample

Among the storage fungi, *A. niger* was the predominant species of fungi infecting kernels collected from Cianjur and Bogor during the dry season. The percentage of kernels infected by the fungus in samples collected from both localities was between 34- 93% and 14 - 98%, respectively.

The percentages of kernels infected by *A. flavus* in samples collected from both localities were between 9 - 98% and 8-95%, respectively.

It was assumed that the moisture contents of the samples collected from the two localities were still favourable for their growth. They were between 7.9 - 10.5% and 6.1- 10.7%, respectively.

The predominant species of fungi infecting kernels collected from Sukabumi during the dry season was *A. wentii.* The percentages of kernels infected by the fungus in samples collected from that location were between 36 - 100%, while those of *A. flavus* were between 10 - 85%. The m.c. of the samples was between 7.5 - 11.2%.

Aspergillus flavus was the predominant species of fungi infecting kernels collected from Cianjur, Sukabumi and Bogor during the wet season. The percentages of infected kernels collected from the three localities were between 41 - 100%, 36 - 96% and 83 -100%, respectively. Pitt *et al.* (1993) reported that the predominant fungus infecting groundnut collected from farmer storage, middlemen and retailer in Thailand was *A. flavus,* followed by *A. niger.*

The percentages of kernels infected by *R. stolonifer* and *Syncephalastrum* sp. could not be determined, because their growth was very vigorous.

Twenty species of fungi were isolated from samples collected from Cidolog during the dry and wet season. They were *Acremonium strictum, Aspergillus candidus, A. flavus, A. niger, A. ochraceus, A. tamarii, A. wentii, B. theobromae, C. cladospo-rioides, E. chevalieri, E. repens, F. equiseti, F. longipes, F. semitectum, Papulaspora* sp., *Penicillium aethiopicum, P. citrinum, Pestalotia* sp., *Rhizopus stolonifer* and *Syncephalastrum* sp. (Table 4).

The predominant species of fungi infecting kernels collected from Cidolog during the dry season was *A. wentii,* with an infected percentage bertween 30 - 100%, while the percentage of kernels infected by *A. flavus* was between 0 - 91%. The m.c. of the samples collected from that location was between 9.9 - 14.1%.

During the wet season, the predominant species of fungi was *A. flavus.* The percentages of kernels infected by the fungus in samples collected from FSS and MW were between 17 - 96%, while those in FHG were between 51 - 99%, and their m.c's were between 10.8 - 18.1% and 12.5 - 45.7%, respectively. According to Christensen and Kaufmann (1969) *A. flavus* can grow in seeds which have a moisture content of at least 18%. On the other hand Martin and Oilman (1976) reported that optimum moisture contents of natural substrate, which permitted the growth of *A. flavus,* were between 15 -25%.

Table 4. The fungal species and range of precentage of infected kernels of groundnut samples collected from Cidolog in different storage systems

Fungi	Range of percentage of infected kernels						
	D				W		
	US	-S			US	S	
	FSS	FSS	MW	FHG	FSS	FSS	MW
Acremonium strictum	0	0	0	0-1	0	0	0
Aspergillus candidus	0-13	0	0-2	0	0	0	0
A. flavus	0-65	21	0-91	51-99	30-92	17	67-96
A. niger	0-42	7	0-80	12-78	3-50	5	14-69
A. ochraceus	0-24	0	0-4	0	0-21	5	0-2
A. tamarii	0-77	4	0-71	19-79	16-72	51	62-88
A. wentii	30-100	65	55-100	0-41	12-92	93	51-69
Botryodiplodia theobromae	0-46	0	10-34	0-41	4-36	20	6-19
Cladosporium cladosporioides	0	0	0	0	0-10	0	0-2
Eurotium chevalieri	0-75	49	22-49	0-3	0-27	13	2-10
E. repens	0-30	57	1-12	0-2	0	0	0
Fusarium equiseti	0	0	0	0-1	0	0	0
F. longipes	0	0	0	0-2	0	0	0
F. semitectum	0-24	0	0	0-1	0-4	0	0-1
Papulaspora sp.	0-44	5	12-27	0-23	1-27	5	7-15
Penicillium aethiopicum	0	0	0	0	0-29	11	11-43
P. citrinum	2-14	12	0-11	24-75	0-46	0	1-64
Pestalotia sp.	0	0	0	0-2	0-3	0	0-1
Rhizopus stolonifer	0	0	0	0-++	0-++	+	0-++
Syncephalastrum sp.	0	0	0	0-+	0	0	0

D = Dry season; W = Wet season; US = Unshelled; S = Shelled; FSS = Farmer Storage Systems; MW = Middlemen Warehouses; FHG = Freshly Harvested Groundnut

The percentages of kernels infected by *A. flavus* in FHG were higher than in samples collected from FSS and MW, because it was assumed that *A. flavus* infected the pods of groundnut before harves. According to Kozakiewicz (1989) *A. flavus* is cosmopolitan in distribution. It is found soil, and decomposes vegetation in stored cereals and other seeds as well as variety of food products. Dickens (1977) and Pitt (1989) reported that the invasion of groundnut by *A. flavus* take place before groundnuts are harvested. Damage of peanut kernels caused by insects and other small animals forces soil into close contact with kernels, and so facilitates the invasion of the fungus.

CONCLUSION

Fungi isolated from groundnuts collected during the dry and wet season from the farmer storage system (FSS), middlemen warehouses (MW), wholesalers (WS), retailer samples (RS), and freshly harvested groundnut (FHG) in Cidolog, Cianjur, Sukabumi and Bogor showed the presence of a large number of species.

A total of 25 fungal species were isolated from the 4 locations. The total fungal species isolated from samples collected from Cianjur, Sukabumi and Bogor during the **dry** and **wet** season were 23, while those from Cidolog were 20.

There were variations in the percentage of kernels infected by each fungal species from different sources and localities. The predominant fungi in samples collected from Cidolog and Sukabumi during the **dry** season were *Aspergillus wentii,* while those collected from Cianjur and Bogor were *A. niger.* The predominant fungus in samples collected from the 4 locations during the **wet** season was *A. flavus.*

The moisture contents of groundnuts collected from FHG, FSS and MW were higher than of those collected from WS and RS.

There were also variations in the percentages of damaged kernels of samples collected from different sources and localities.

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