STUDIES ON THE POPULATION OF TOXIGENIC FUNGI IN MARKET FOODS AND FOODSTUFFS. I. MYCOFLORA CONTAMINATION

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Summary

The fungi of 121 market foods and foodstuffs in Bangkok collected during March-June, 1975 were examined. The fungi were found in 97 (80.2 %) out of 121 samples whereas 24 (19.8 %) did not yield any fungi. Of the 97 fungal positive samples, 58 (47.9 %) samples were contaminated in the range of 76–100 % of the total number of cultured samples in each plate. The total number of 435 fungi, which involve more than 10 genera, were isolated. The major genera were ***Aspergillus*** *(58.2 %),* ***Rhizopus*** *(13.3 %),* ***Penicillium*** *(13.0 %) and* ***Fusarium*** *(3.7 %). Other genera such as* ***Helminthosporium*** *(2.3 %),* ***Mucor*** *(1.8 %),* ***Cladosporium*** *(1.2 %),* ***Cephalosporium*** *(1.2 %),* ***Heterosporium*** *(0.5 %),* ***Alternaria*** *(0.5 %),* ***Curularia*** *(0. 2%) and unidentified strains 2.3 %) were frequently isolated. Most of samples were contaminated with* ***Aspergillus,*** *except rice and beans were also contaminated with* ***Penicillium*** *and* ***Fusarium*** *respectively.* ***Aspergillus flavus*** *(33.2 %),* ***Aspergillus niger*** *(24.1 %) and* ***Aspergillus ochraceous*** *(17.4 %) were prevalent among 253 isolates of* ***Aspergillus.*** ***Penicillium citrinum*** *(28.1 %) and* ***Penicillium notatum*** *(12.3 %) were the major isolates found among* ***Penicillium.*** *On the basis of these results, its high incidence of fungal contamination suggests that most of market foods and foodstuffs, especially peanuts and corn, had not been kept under suitable storage condition.*

Introduction

Since the discovery of mycotoxins which are carcinogenic hepatotoxins, the importance of the existence and occurrence of these mycotoxins as contaminants of human foods and foodstuffs has been increasing in the field of food hygiene. Mycotoxicoses have been well decumented in both animal and man[1-7]. The problem of mycotoxicoses has now a worldwide significance in terms of public health, agriculture and economic. Therefore, it would be the great value of the studies on the mycoflora infested on rice, grains, beans, legumes and corn which represent the major components of the diets and major crops in Thailand, in particular to mycotoxin production. It was reported that the viable fungi contaminating market foods and foodstuffs in South-east Asia was highly variable, being lowest (13 %) in dried fish and shrimp, and highest (81 %) in peanuts. Among these, *Aspergillus* were often the most predominant molds. The group of *Aspergillus flavus* was found

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with high frequency in all foods and foodstuffs. *Penicillium*, *Fusarium* and *Rhizopus* were seldom found and they were not classified to the varieties of the genus[8]. This report presents the results of a portion of a screening program in which molds were routinely isolated from a variety of animal and human foods and foodstuffs, identified to the varieties, and tested for the productive ability of mycotoxin and antibiotic.

Materials and Methods

Collection of samples

A total of 121 samples of major raw and processed foods commonly used were obtained from selected market in Bangkok. All of the samples were collected during March-June, 1975. For the examination of mycoflora from a total of 11 different kinds of these foods and foodstuffs, sampling from various markets in Bangkok was taken into consideration, but samples from the origins were not considered. Upon receipt, all samples were immediately subjected to the chemical assay for aflatoxins and ochratoxin A. These results are reported elsewhere. The second portion of each sample was used for mycological examination and the third portion was used for moisture content determination.

Procedure of mycological examination

The portions of each sample were plated following surface-disinfectant with a 1.6 % solution of sodium hypochlorite (30 % Chlorox, from the Chlorox Co., Oakland, Calif.) for 1 min and then washed three times with sterile distilled water each for 1 min. Except for the flours, they were cultured without surface-disinfectant. The samples were cultured on Czapek's solution agar, Sabourouad dextrose agar and potato dextrose agar containing 10 mg% of chloramphenicol. The samples were incubated at 25–30°C, and inspected daily for 7 days. If no mold grew within 7 days, the samples would not be considered contaminated. As individual colonies developed, each colony was transferred to fresh agar medium of Czapek's solution agar, Czapek's solution agar (20 % sucrose) (Difco Laboratories, Detroit, Mich.), M40Y and/or malt extract agar[9] for final identification. All isolates were maintained in sterile soil tubes under low temperature until determination of their toxigenicity and antibiotic-ity was accomplished. *Aspergillus* and *Penicillium* isolates were identified by consulting gross and microscopic morphology described by Raper and Fennell[9], and Raper and Thom[10] respectively.

Moisture content

The moisture content was determined by measuring the difference of sample weights before and after drying in the oven at 80°C until the constant weight was obtained.

Results and Discussion

Of the 121 samples, the total number of 435 fungi was isolated from 97 (80.2 %) samples whereas 24 (19.8 %) samples were not contaminated. Table I summarizes

the relation between the frequency of occurrence of fungi isolated from various kinds of market foods and foodstuffs and the moisture content. The occurrence of fungi was low in a small number of samples. It was noted that only three samples were contaminated in the range of 1–25% of grains from which various mycoflora was grown. Higher occurrence of fungi in the range of 76–100% was in 47.9% of total fungal positive samples. It was indicated that most of the samples examined had not been kept under suitable conditions after harvesting, handling and even during storage. As this result shows, the moisture content of rice (13–15%) and beans (9–16%) may play an important role in higher occurrence of fungi. In the previous study, the critical moisture content for the better growth rate of molds on rice was somewhere between 14–16%[11]. However, the moisture content for the growth of molds on peanuts was higher than 6%,[12] but in this study, the viable molds were grown on peanuts with the moisture content as low as 3%. It was also found that there was no significant difference between the occurrence of fungi and the sampling areas.

TABLE I: TOTAL NUMBER OF FUNGI ISOLATED FROM MARKET FOODS AND FOODSTUFFS

Sample	No. of samples (contaminated/ examined)	No. of samples contaminated with fungi at various frequencies					Moisture Content (%)
		0%	1–25%	26–50%	51–75%	76–100%	
Rice	11/17	6	1	3	3	4	13.0–14.9
Glutinous rice	7/16	9	–	1	2	4	12.7–14.3
Rice flour	6/7	1	–	–	–	6	8.7–13.6
Raw peanuts	12/12	–	–	1	3	8	6.1– 8.5
Roast peanuts	11/11	–	–	5	2	4	2.8– 4.9
Ground peanuts	14/14	–	–	1	1	12	3.1– 7.0
Soybeans	13/17	4	1	3	5	4	9.1–12.5
Mung beans	6/10	4	–	–	1	5	9.1–13.0
Corn	9/9	–	1	4	–	4	12.3–15.7
Sorghums	2/2	–	–	–	1	1	11.6–15.1
Prepared foods[a]	6/6	–	–	–	–	6	4.8– 6.4
Total	97/121	24	3	18	18	58	–

[a]Prepared foods contain rice, soybean flour and sugar as major ingredients.

Table II presents the genera and the frequency of occurrence of fungi isolated from market foods and foodstuffs. Storage fungi were represented by *Aspergillus* (58.2%), *Rhizopus* (13.3%) and *Penicillium* (13.0%) which were the most predominant genera. Generally, storage fungi were more prevalent on the large grains than the small grains, but there was no difference in this study. Field fungi were represented by plant pathogenic fungi, *Fusarium* (3.7%) and *Helminthosporium* (2.3%), and the saprophytic fungi were represented by *Cladosporium* (1.2%), *Cephalosporium* (1.2%) and *Alternaria* (0.5%). The low incidence of *Alternaria* is to be expected as a prolonged storage generally reduces the levels of contamination by this mold[13, 14]. The

pattern of distribution, with high incidence of *Aspergillus* and *Penicillium* isolates, agrees in general with the results of several other surveys[8, 12, 13].

The species, genera and distribution of the fungi isolated are listed in Table III. Most of market foods and foodstuffs were highly contaminated, being lowest (44 %) in glutinous rice and highest (100 %) in peanuts and corn (Figure 1). The genus

TABLE II: LIST OF THE GENERAL AND THE FREQUENCY OF OCCURRENCE OF FUNGI ISOLATED FROM MARKET FOODS AND FOODSTUFFS

Fungi isolated	No. of isolates	% of occurrence
Aspergillus	253	58.2
Rhizopus	58	13.3
Penicillium	57	13.0
Fusarium	16	3.7
Helminthosporium	10	2.3
Mucor	8	1.8
Monilia	8	1.8
Cladosporium	5	1.2
Cephalosporium	5	1.2
Heterosporium	2	0.5
Alternaria	2	0.5
Curvularia	1	0.2
Unidentified	10	2.3
Total	435	–

TABLE III: MAJOR SPECIES OF FUNGI ISOLATED FROM MARKET FOODS AND FOODSTUFFS

Sample	No. examined	% contaminated	Major species of fungi isolates[b]								Total
			Af	*An*	*Ao*	*Ag*	*Ac*	*R*	*P*	*F*	
Rice	17	65	8	8	4	3	3	3	17	2	48
Glutinous rice	16	44	4	7	2	6	3	5	16	–	43
Rice flour	7	86	3	2	–	–	–	6	2	–	13
Raw peanuts	12	100	11	7	9	–	–	9	1	–	37
Roast peanuts	11	100	12	6	3	2	–	8	1	–	32
Ground peanuts	14	100	13	4	12	–	–	13	–	–	42
Soybeans	17	77	8	7	4	2	–	5	6	6	38
Mung beans	10	60	8	9	6	–	–	5	6	5	39
Corn	9	100	10	8	3	–	–	3	6	3	33
Sorghums	2	100	1	1	–	–	–	1	–	–	3
Prepared foods[a]	6	100	6	2	1	–	–	–	2	–	11
Total	121	–	84	61	44	13	6	58	57	16	–

[a]Prepared foods contain rice, soybean flour and sugar as major ingredients.

[b]*Af* = *A. flavus*, *An* = *A. niger*, *Ao* = *A. ochraceous*, *Ag* = *A. glaucus*, *Ac* = *A. candidus*, *R* = *Rhizopus*, *P* = *Penicillium*, *F* = *Fusarium*.

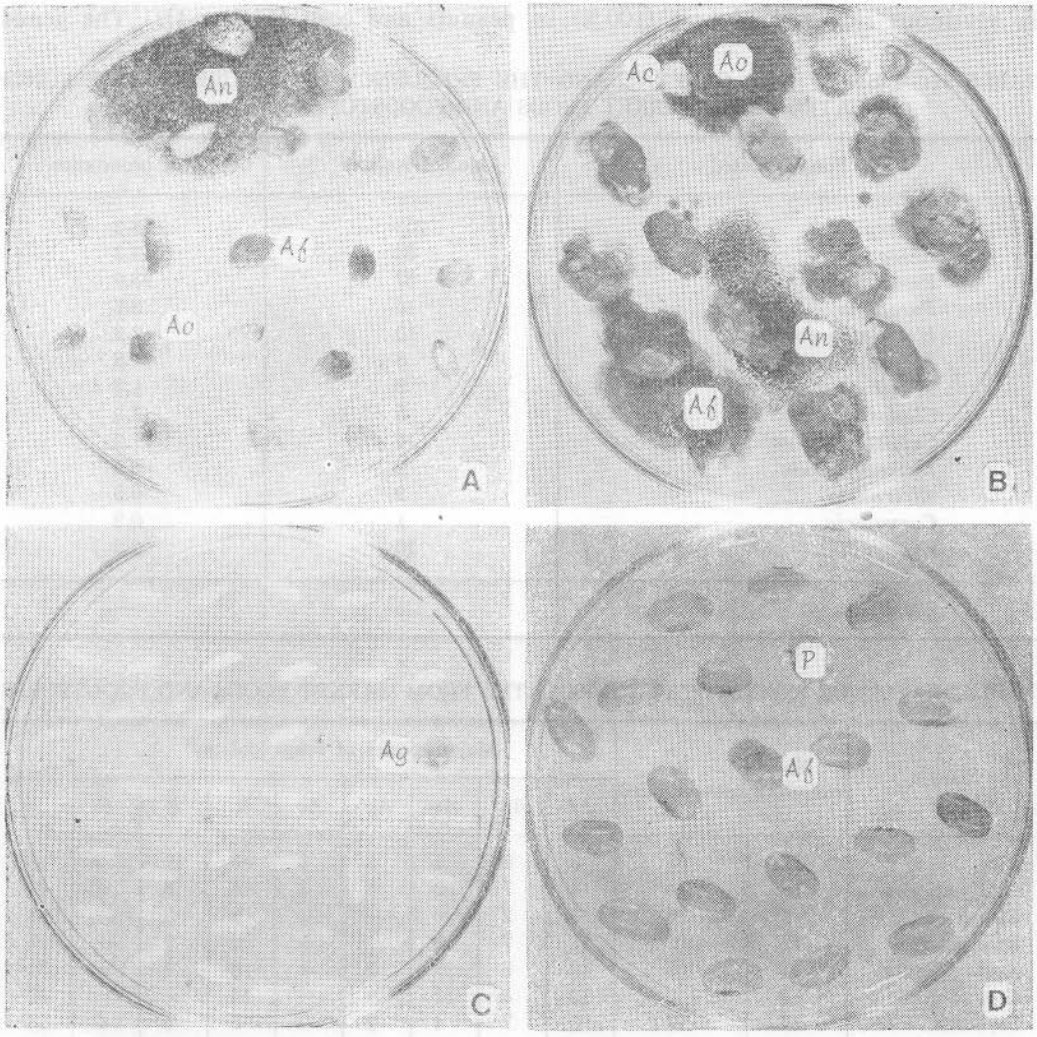

Fig. 1. Fungal contamination on various market foodstuffs cultured on Czapek's solution agar after 5 days' incubation at 25°C. A, corn. B, peanuts. C, rice. D, soybeans. *Af* = *A. flavus*, *An* = *A. niger*, *Ao* = *A. ochraceous*, *Ag* = *A. glaucus*, *Ac* = *A. candidus* and *P* = *Penicillium*.

Aspergillus (58.2 %) was most frequent fungi isolated from all samples examined. Among these, *Aspergillus flavus* (33.2 %), *Aspergillus niger* (24.1 %) and *Aspergillus ochraceous* (17.4 %) were found with high frequency in practically all foods and foodstuffs. *Aspergillus glaucus* (5.1 %) and *Aspergillus candidus* (2.4 %) were also found. *Penicillium* (13.0 %) species were less prevalent. However, rice and beans also showed high frequent contamination by *Penicillium* and *Fusarium* respectively. It is possible that high frequency of occurrence of numerous fungi on market foods and foodstuffs for human use may potentiate the public health problems. Furthermore, it may create the economic problem if the export rice, corn, beans and legumes are highly invaded and contaminated with fungi. As it was reported that Thai maize exported to Japan in 1966–1967 was highly contaminated with *Aspergillus* (92.4 %), especially *A. flavus*, *A. niger* and *A. ochraceous*, and *Penicillium* (72.6 %)[15].

Table IV presents a list of the species of *Aspergillus* found and indicates the frequency of occurrence on contaminated samples. A total of 253 isolates from 22 species of *Aspergillus* was obtained from the samples. *A. flavus* (57.7 %) was the most prevalent species. *A. ochraceous* (37.1 %), *A. tubingensis* (9.5 %), *A. awamori* (8.7 %),

TABLE IV: SPECIES OF *ASPERGILLUS* ISOLATED FROM MARKET FOODS AND FOODSTUFFS

Species of *Aspergillus*	No. of isolates	% of total no. of isolates	% of occurrence on contaminated samples
Aspergillus flavus Link	56	22.1	57.7
A. ochraceous Wilhelm	36	14.2	37.7
A. tubingensis Moss	24	9.5	24.7
A. awamori Nakazawa	22	8.7	22.7
A. parasiticus Speare	21	8.3	21.7
A. niger v. Tiegh	15	5.9	15.5
A. fumigatus Fresenius	8	3.2	8.2
A. nidulans Wint	8	3.2	8.2
A. terreus Thom	8	3.2	8.2
A. flavus var *columnaris* Link	7	2.8	7.2
A. candidus Link	6	2.4	6.2
A. chevalieri Thom & Church	6	2.4	6.2
A. alliaceous Thom & Church	4	1.6	4.1
A. clavatus Desm	4	1.6	4.1
A. japonicus Saito	4	1.6	4.1
A. vesicolor Tiraboschi	4	1.6	4.1
A. sulphureus Thom & Church	3	1.2	3.1
A. proliferans Smith	3	1.2	3.1
Other strains[a]	8	3.2	8.2
Unidentified	6	2.4	6.2
Total	253	–	–

[a]Other strains = *A. repens* de Bary (2), *A. resticus* Smith (2), *A. rnber* Thom & Church (2) and *A. tamarii* Kita (2).

A. parasiticus (8.3 %) and *A. niger* (5.9 %) were considered to be prevalent species. The high frequency of *A. flavus* and *A. parasiticus* contamination on peanuts (100.0 %), rice (50.0%), corn (100.0 %) and soybeans (47.1 %) may suggest the presence of aflatoxins in these foods and foodstuffs (see Table III). It was found to be the case for only peanuts which 45.0–50.0 % of all peanut samples contained detectable concentrations of aflatoxins. However, a small number of rice (2.0–3.3 %) and soybeans (0.5 %) were infrequently contaminated with only at low levels of aflatoxins eventhough the presence of viable *A. flavus* and *A. parasiticus* was often observed[8, 16]. Therefore, rice and soybeans seem to be seldom contaminated with aflatoxins. Ochratoxins, produced by more frequent species *A. ochraceous*, may present in a predictive quantity on these samples[17]. However, *A. clavatus* and *A. vesicolor*, cytochalasin E and citrinin, and sterigmatocystin producing fungi respectively, were less prevalent species.[18, 19]

TABLE V: SPECIES OF *PENICILLIUM* ISOLATED FROM MARKET FOODS AND FOODSTUFFS

Species of *Penicillium*	No. of isolates	% of total no. of isolates	% of occurrence on contaminated samples
Penicillium citrinum Thom	16	28.1	16.5
P. notatum Westling	7	12.3	7.2
P. islandicum Sopp	6	10.5	6.2
P. chrysogenum Thom	5	8.8	5.2
P. cyclopium Westling	4	7.0	4.1
P. funiculosum Thom	4	7.0	4.1
P. frequentans Westling	3	5.3	3.1
P. nigricans Thom	3	5.3	3.1
P. sclerotium v. Beyma	3	5.3	3.1
P. lanosum Westling	1	1.7	1.0
P. citre-viride Biourge	1	1.7	1.0
Unidentified	4	7.0	4.1
Total	57	–	–

The species and distribution of the genus *Penicillium* are listed in Table V. *Penicillium* species were less prevalent than the *Aspergillus* species. *Penicillium citrinum* represented 28.1 % of *Penicillium* isolated and its distribution on the contaminated samples was the highest (16.5 %). *P. notatum* (7.2 %), *P. islandicum* (6.2 %) and *P. chrysogenum* (5.2 %) were considered as common species. As it was mentioned above that aflatoxins were infrequently detected on rice, eventhough *A. flavus* species were frequently contaminated on these samples, but other known mycotoxins produced by *Penicillium* such as citrinin from *P. citrinum* and islanditoxin from *P. islandicum*, may be present on rice[20, 21].

The results in the present study reported a mycological survey of market foods and foodstuffs in Bangkok. It was found that most of the samples collected were invaded and contaminated with various species of fungi. Of high prevalence among

these fungi were mainly species of *Aspergillus* including *A. flavus*, *A. ochraceous* and *A. niger*. Moreover, some of the samples especially rice were also contaminated with species of *Penicillium* including suspect toxigenic fungi as *P. citrinum* and *P. islandicum*. The results of fungal counts showed that the average occurence of fungi was in the range of 76–100% isolates per total cultured grains and 47.9% of all samples were in this range. In this respect, market foods and foodstuffs were apparently in unfavorable hygienic condition. In the next phase of the study, precise determination on the importance of the toxigenicity of these fungi by bioassay will be subjected to further investigation.

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บทคัดย่อ

บทความนี้ได้บรรยายถึงการศึกษาของการกระจายและชนิดต่าง ๆ ของเชื้อราที่ขึ้นบนอาหารและอาหารสำเร็จรูปจำพวกเมล็ดพืชบางชนิดที่ซื้อมาจากท้องตลาดในกรุงเทพมหานคร ระหว่างเดือนมีนาคมถึงมิถุนายน พ.ศ. 2518 พบว่ามีเชื้อราขึ้นอยู่บนอาหาร 97 (80.2 %) ชนิดจากอาหารที่นำมาทดลองทั้งหมด 121 ชนิด ด้วยกัน อาหารจำนวน 58 (47.9 %) ชนิดมีเชื้อราขึ้นอยู่ระหว่าง 76–100 % ของตัวอย่างอาหารที่นำมาเลี้ยงบนวุ้น เชื้อราที่แยกได้ทั้งหมดมีจำนวนถึง 435 ชนิดซึ่งมาจากพวกใหญ่ ๆ มากกว่า 10 พวกด้วยกัน เชื้อราพวกนี้ประกอบไปด้วยแอสเปอร์จิลลัส (58.2 %) ไรโซปัส (13.3 %) เพนนิซิเลียม (13.0 %) และฟิวซาเรียม (3.7 %) เป็นส่วนใหญ่ นอกจากนี้ยังมีเชื้อราพวกเฮลมินโทสปอร์เรียม (2.3 %) มิวคอร์ (1.8 %) โมนิเลีย (1.8 %) แคลโดสปอร์เรียม (1.2 %) เซฟาโลสปอร์เรียม (1.2 %) เฮเทอร์โรสปอร์เรียม (0.5 %) แอลเทอร์นาเรีย (0.5 %) คิวรูลาเรีย (0.2 %) และเชื้อราที่กำลังจัดชนิดอยู่อีกด้วย อาหารเกือบทุกชนิดโดยเฉพาะอย่างยิ่งถั่วลิสงมีแอสปอร์จิลลัสขึ้นอยู่เป็นจำนวนมาก นอกจากนี้ข้าวและถั่วยังมีเชื้อราพวกเพนนิซิเลียมและฟิวซาเรียมขึ้นอยู่มากอีกด้วย เชื้อราในพวกแอสเปอร์จิลลัส ที่พบมากได้แก่แอสเปอร์จิลลัส แฟลวัส (33.2 %) แอสเปอร์จิลลัส ไนเจอร์ (24.1 %) และแอสเปอร์จิลลัส ออเครเซียส (17.4 %) สำหรับพวกเพนนิซิเลียมนั้นมีเพนนิซิเลียม ซิตรินัม (28.1 %) มากที่สุด จากผลการทดลองนี้จะสรุปได้ว่าอาหารและอาหารสำเร็จรูปจำพวกเมล็ดพืชบางชนิดมิได้เก็บให้อยู่ในสภาพที่ปลอดภัยจากการขึ้นของเชื้อรา