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**MYCOBIOTA ASSOCIATED WITH FOODSTUFFS  
COMMODITIES SPREAD IN JEDDAH  
(SAUDI ARABIA) WITH SPECIAL REFERENCE  
TO *ASPERGILLUS FLAVUS*  
(With 5 Tables and 5 Figures)**

By

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الاحياء الفطرية المصاحبة للمواد الغذائية المنتشرة بمدينة جدة  
بالمملكة العربية السعودية مع إشارة خاصة لفطره *اسبيرجيليس فلافس*

فردوس السيد معروف موسى بخارى

تم في هذا البحث اختبار ٥١ من المواد الغذائية المحلية المتداولة في مدينة جدة لمعرفة مدى تلوثها بالفلورا الفطرية. ووجد ان الفلورا الفطرية كانت من الفطريات التي تعطى دلالات على سوء عملية التخزين لهذه المواد الغذائية. وكانت اجناس الفطر الاكثر شيوعا هي الاسبرجيلس والفيوزاريوم والميوكر والبنسيليوم والريزوبس والتريكودرما. وكانت فطره اسبرجيلس فلافس هي الاكثر وفرة في انواع هذا الجنس حيث عزلت من جميع العينات التي تم فحصها.

**SUMMARY**

Fifty one samples of foodstuffs (seeds, pulses, spices, nuts, milk and dairy products) commonly used in Jeddah, Saudi Arabia were surveyed for fungal flora in order to detect the major foodstuffs contaminants. The mycoflora found consisted particularly of storage fungi which could indicate that poor storage facilities were used. The most dominant fungal genera were; *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma*. From *Aspergillus* species; *A. flavus* was the most abundant fungus which isolated from all the examined samples.

**Keyword:** Foodstuffs, Seeds, milk, *Aspergillus flavus*.

## INTRODUCTION

The growth of fungi on stored food and foodstuffs which have been implicated in human and animal illness could be a serious issue in Saudi Arabia, which imports most of its commodities and in which most of these food are stored for long periods before consumption. Jeddah region located in the western part of Saudi Arabia has a climate characterized by high relative humidity and high temperature very suitable for growth of the mould group *aspergilli* specially *Aspergillus flavus* species. In spite of the numerous researches studying the common fungi in soil, seeds and grains in Egypt and some Arab countries (Moustafa and Al-Musallam 1975, Abdel-Hafez *et al.*, 1977, Moubasher *et al.*, 1979, 1981, 1985, Abul-Nasr 1981, Mazen *et al.*, 1984 and El-Kady *et al.*, 1986) no attention has been given for similar studies in Saudi Arabia. Therefore, it was necessary to investigate samples of food materials such as seeds, pulses, spices, nuts, milk and dairy products purchased from Jeddah to investigate the spread of fungi in food commodities paying attention to *Aspergillus flavus*.

## MATERIALS and METHODS

### **1- Sampling, isolation and identification of isolated fungi:**

#### **i- Source of collected samples:**

All tested seeds, nut seeds, spices, milk and milk products were collected from different markets in Jeddah during 1997-1998. The collected samples were either immediately plated for fungal detection or stored at 3-5°C until mycofloral determination.

The tested seeds were Broad bean (*Vicia faba*), Ground nut (*Arachis hypogea*), Wheat (*Triticum vulgare*), Rice (*Oryza sativa*) and Sesame (*Sesame indicum*). Also, five nut seeds were tested; Almond (*Prunus dulcis*), Cashew (*Anacardium occidentale*), Hazelnut (*Corylus avellana*), Pine (*Picepa vulgaris*), Pistachio (*Pistacia vera*) and Walnut (*Juglans regia*).

The investigated spices samples were; Cloves (*Caryophyllus*), Thyme (*Thymus*), Cumin (*Cuminum*), Coriander seed (*Coriandrum sativum*), Oregano (*Origanum*), red and black Pepper seeds (*Capsicum*), Cardamon (*Elettaria cardamom*) and powdered dried Mint leaves (*Mentha viridis*).

Milk and milk products were also tested. Milk and yoghurt were obtained from different manufactories and five kinds of dried milk were used namely; Abu-walad, Carnation, Klim, Nedo and Regulaise. Processed and non-processed cheese were also tested for fungal contamination and they were Cottage cheese (local product), Saudi Fetta cheese, Parmesan cheese (French type) and yellow sliced Cheddar cheese.

**ii- Assay of plating medium:**

Potato dextrose agar (200 gm potato, 20 gm glucose and 20 gm agar per one liter of distilled water) was used for determining the total counts of developed fungi on the different examined samples; seeds, spices, milk and dairy products. Two hundreds of seeds were used in each particular treatment applied for assaying. The tested seeds which looked sound (visually healthy) were only used.

All tested samples (seeds, spices, milk and dairy products) were plated on PDA plates at 25-28°C for 6-8 days. The sterilized PDA agar medium was acidified with few drops of lactic acid to prevent bacterial contamination. Five or ten seeds were generally used for each plate according to the size of tested seeds. The developed fungi were almost usually inspected at 20-50x using a binocular microscope. Isolation experiments were usually duplicated.

**iii- Determination of moisture content of different samples:**

The tested seeds, powdered spices and milk samples were subjected to moisture content examination. The moisture content was determined by the air oven dry method specified by Association of official analytical chemists (1965). The samples were firstly weighed, oven dried at 70°C for 24 hours, then at 102°C to constant weighed. Moisture content of samples was then calculated on a wet weight basis.

**iv- Identification of the isolated fungi:**

The isolated fungi (borne-fungi) from each tested sample were verified by applying hyphal tip or single spore culture technique. Purified fungi were transferred onto slants of PDA medium at 25°C. Identification of fungi was based on comparison of growth colour, characteristics and colour of sporing structures with descriptions in Barnet (1972), Alexopoulos and Minis (1979), Webster (1980), Bokhari (1986) and in particular standard monographs by Raper and Fennel (1965), Malone and Muskett (1964), Cooney and Emerson (1964), Barron (1968), Ellis (1971) and Samson and van Reenen-Hoekstra (1988).

**2- Analysis of various samples for fungal detection:**

**i- Seeds mycobiota:**

The seeds were tested for fungal assay in two forms; either of disinfected surface or non-disinfected. Seeds disinfection was done by using sodium hypochlorite solution at 2% concentration for five minutes, then seeds were rinsed three times with sterile water then plated on PDA plates at 25°C ( $\pm$  2°C) for 6 to 8 days and were daily inspected for fungal appearance. Potato dextrose agar (plating medium) was supplemented with penicillin (20 units/ml medium) and streptomycin (0.05 mg/ml medium) for avoiding of bacterial growth on counts of developed seed-borne fungi especially *Aspergillus flavus*.

Two hundreds of seeds were used for each tested seed variety (15 seed varieties) in each particular treatment applied (either non-disinfected or disinfected seeds) on PDA medium. The seeds appeared sound have been randomly plated on medium plates. Five to ten seeds were used for each Petri-dish according to the size of tested seeds. Counts of developing fungal colonies were recorded after 6-8 days incubation period for each tested variety of seeds.

**ii- Mycological assay of spices:**

All fifteen different kinds of spices were ground to a powder before examination. Then, one gram from each sample was spread over three replicate PDA Petri-dishes with added antibiotics mentioned before. Plates were incubated at 25°C for 6-8 days, then enumeration of fungi appeared was counted.

**iii- Milk and milk products fungal assay:**

Twenty-one milk and milk products samples were used in this study. For milk and Labinah one ml was used from each sample where it is poured as 5 drops in each PDA plate used and four plates were used for each treatment. For dried milk or cheese one gram was used which was distributed in three PDA plates and five grams were used from each investigated sample. Plates were incubated at 25°C for 6-8 days and counted for fungal determination.

## **RESULTS and DISCUSSION**

**1- Fungi associated with seed and grain varieties:**

Non-disinfected and disinfected Broad bean, Barley, Yellow corn, American rice, Egyptian rice and Wheat seeds were used during this study. It was found (Table 1 and Figs. 1, 2) that, the total count of



fungi developed from non-disinfected seeds and grain varieties was higher than those in disinfected seeds as follow: 59 and 18 for Broad bean, 67 and 52 for Barley, 33 and 9 for Yellow corn, 32 and 12 for American rice, 37 and 13 for Egyptian rice and 52 and 41 for Wheat seeds. Also, the results presented reveal that *Alternaria sp.* were isolated only from disinfected seeds of Broad bean, Barley and Wheat and Wheat seeds amounted the highest percent of isolation. *Aspergillus flavus* was isolated in all seed varieties in both non-disinfected and disinfected ones. Its isolation was higher in non-disinfected samples except in American rice as the percentage of isolation in disinfected seeds was higher (25%) than in non-disinfected ones (6.3%). Other *Aspergilli* and *Penicilli* species and *Mucor* and *Rhizopus spp.* were frequently detected in all seed varieties in both treatments. *Trichoderma sp.* was only detected in disinfected Broad bean seeds.

It was found that the higher incidence of *Aspergillus flavus* was in Egyptian rice (84%) and the lowest was recorded in Broad bean (20%) in disinfected seeds whereas in non-disinfected ones Egyptian rice was the highest (54%) and American rice was the lowest (6.3%).

The results indicate that the incidence of fungi was different according to the kind of tested seed and this was obviously because of specificity of various fungi to invade seed of each crop and also because of the natural difference governing formation of seeds as reported by many other authors (Malone and Muskett 1964; Flannigan 1970; Miller and Trenholm 1997; Margw *et al.* 1988 and Lacey *et al.*, 1980). In addition, Bullerman (1979) and Smith and moss (1985) isolated the genera *Aspergillus*, *Penicillium* and *Fusarium* as a contaminants of foods and agricultural commodities.

#### **2- Fungi associated with oil and nut seeds:**

Nine oil and nut seeds which appeared normal in colour namely; Almond, Caju, Hazelnut, Pine, Pistachio (2 samples), Sesame (2 samples) and Walnut were used during this study.

An obvious variation in the incidence of the dominant fungi associated with the tested seeds as shown in Table (2) and Fig. (3). The total counts of isolated fungi ranged from 142 to 25 isolates and the Walnut seeds were the most contaminated samples and Sesame sample was the least. Seven genera and species, mostly common mold strong fungi, were isolated of which *Aspergillus spp.* was the most predominant genus and was isolated from all tested seed varieties. It comprised to 338 isolates, while *A. flavus* isolates were about 169. The most isolated fungi were; *Rhizopus* and *Penicillium*. In addition, Pistachio seeds contained

the highest percentage of *A. flavus* (78%) followed by Walnut seeds (56.9%). These findings are in accordance with the results found by Burdaspal *et al.*, 1990; Jimenez *et al.*, 1991; Abdel Gawad and Zohri 1993; Bokhari 1993 and Scholten and Spanier 1996.

### 3- Fungi associated with spices:

Fifteen spicy samples were tested and the results shown in (Table 3 and Fig. 3) indicate that the total viable fungal isolates ranged from 0 to 157 colonies/g dry powdered spices sample. It was found that Coriander, Cinnamon and Cardemom (sample 2) were the most contaminated sample whereas Mint, Oregano and Clove were the least as shown in Table (3). *Rhizopus sp.* was the most prevalent fungus (388 isolates) followed by *Aspergillus sp.* (299 isolates). *Aspergillus flavus* was highly isolated from black Pepper (sample 2) followed by Cardemom (sample 2), Cinnamon (sample 2) and Coriander (sample 1) amounted to 33.8, 33.3, 10.4 and 8.3%, respectively. While it was not detected from Cinnamon (sample 1), Clove, Coriander (sample 3), Mint, Oregano, Thyme and red Pepper. This findings may be attributed to the inhibitory effect of many spices on the growth of *Aspergillus flavus* which also affects the fungal toxicity as also proved by Postcr *et al.*, 1988; Olojede *et al.*, 1993; Bullerman *et al.*, 1977 and Doyle *et al.*, 1982.

### 4- Fungi associated with milk and dairy products:

Contamination by fungi with special reference to *Aspergillus flavus* was intensively studied in 21 samples of commercial milk and dairy products. Seven genera namely; *Alternaria sp.*, *Aspergillus spp.*, *Fusarium spp.*, *Mucor spp.*, *Penicillium spp.*, *Rhizopus spp.* and *Trichoderma spp.* were isolated from the tested milk and dairy products as indicated in (Table 4 and Figs. 4, 5 & 6). The highest frequency of isolated fungi in most samples was counted for both *Aspergillus flavus* and *Penicillium sp.* It was also observed that *A. flavus* was highly detected in the fresh Al-Saudi milk comparable to other isolated fungi (27 isolates out of 35 matching 77.7%). *Aspergillus flavus* was also detected in considerable percentage in old Almarai, Al-Safi fresh milk, Nedo dried milk, Labinah (Pinar), Opened sliced cheese and Fetta Saudi cheese and it was amounted to 21, 43, 12, 29.3, 63.8, 56.5 and 68%, respectively.

The isolated fungi were also previously isolated from different milk and milk products as reported by Nakae *et al.* (1976), Nelson (1981), Jarvis (1983), Aron and Eke (1987) and Lund *et al.* (1995). It was observed that *Fusarium* was not detected in all tested milk and milk

products while it was detected by Jordal *et al.* (1993) in Spanish raw and pasteurized milk with high frequency.

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Table 1. Fungi associated with direct plating method with non-disinfected seed on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds)

Isolated fungi	Seeds, Pulses					
	Barley	Broad bean	Yellow corn	American Rice	Egyptian Rice	Wheat
Alternaria	0	0	0	0	0	0
Aspergillus flavus	30	17	12	2	20	25
Aspergillus spp	14	23	8	1	1	11
Fusarium spp	0	0	0	3	3	0
Mucor spp	3	1	0	0	0	0
Penicillium	2	4	0	2	2	1
Rhizopus sp.	18	14	15	24	11	15
Trichoderma sp.	0	0	0	0	0	0

Table 2. Fungi associated with direct plating method with surface disinfected seed on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds)

Isolated fungi	Seeds, Pulses					
	Barley	Broad bean	Yellow corn	American Rice	Egyptian Rice	Wheat
Alternaria	2	1	0	0	0	11
Aspergillus flavus	16	7	2	3	11	2
Aspergillus spp	0	5	0	0	0	17
Fusarium spp	27	0	2	0	1	8
Mucor spp.	3	0	2	0	1	2
Penicillium	4	2	3	1	0	0
Rhizopus sp.	0	1	0	8	0	1
Trichoderma sp.	0	2	0	0	0	0

Table 3. Fungi associated with direct plating method for nine samples of oil seeds and nuts on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds):

Isolated fungi	Oil Seeds & nuts								
	Almond	Caju	Hazelnut	Pine	Pistachio (1)	Pistachio (2)	Sesame (1)	Sesame (2)	Walnut
Aspergillus flavus	6	5	27	8	78	8	1	1	33
Aspergillus spp	32	14	15	0	17	29	17	1	9
Fusarium spp	0	0	0	0	2	0	0	0	0
Mucor spp.	0	0	0	5	3	1	3	0	3
Penicillium spp.	3	5	0	0	0	27	0	2	1
Rhizopus sp.	9	23	100	100	0	0	4	0	12
Trichoderma sp.	1	0	0	0	0	0	0	0	0

Table 4. Fungi associated with direct plating method for 15 herbs and spices on PDA agar medium incubated at 28 C for 7 days

Isolated fungi	Herbs & Spices										
	Cardmon (1)	Cardmon (2)	Cinamon (1)	Cinamon (2)	Corander (1)	Corander (2)	Red Pepper	Black Pepper (1)	Black Pepper (2)	Dried Thyme	Wild Savager
Aspergillus flavus	3	35	0	16	3	0	0	0	22	0	2
Aspergillus spp	34	55	0	0	27	49	1	23	15	4	7
Mucor spp	0	6	0	50	2	3	0	0	12	0	0
Penicillium spp	20	1	0	0	0	0	2	0	1	0	0
Rhizopus sp.	7	8	4	87	4	105	92	1	15	0	50

Table 5. Fungi isolated from 18 samples of milk and dairy products on PDA agar medium incubated at 28 C for 7 days/one unit (ml or gm).

Milk and Dairy Products	Almarin children Milk		Al Sunat Milk	Al Saft Milk	Curmation dried Milk
	Isolated fungi				
	New	Old			
<i>Alternaria spp.</i>	0	0	0	0	1
<i>Aspergillus flavus</i>	1	21	27	23	0
<i>Aspergillus spp.</i>	2	3	2	6	0
<i>Fusarium spp.</i>	0	0	0	0	0
<i>Mucor spp.</i>	0	0	0	0	0
<i>Penicillium spp.</i>	1	75	4	37	0
<i>Rhizopus sp.</i>	0	0	2	0	0
<i>Trichoderma spp.</i>	0	0	0	0	0

Milk and Dairy Products	Nedo Dried Milk		Aken Waifal dried Milk	Khm. dried Milk	Regular dried Milk	Labanah Yogurt Pnr
	Isolated fungi					
	New	Old				
<i>Alternaria spp.</i>	0	1	0	0	0	0
<i>Aspergillus flavus</i>	2	12	0	1	1	23
<i>Aspergillus spp.</i>	1	2	1	1	0	0
<i>Fusarium spp.</i>	0	0	0	0	0	0
<i>Mucor spp.</i>	1	0	1	0	0	0
<i>Penicillium spp.</i>	0	1	28	5	2	13
<i>Rhizopus sp.</i>	0	17	0	0	0	0
<i>Trichoderma spp.</i>	0	8	0	0	0	0

Milk and Dairy Products	Sliced Cheese		Fatta Saudi Cheese		Cheddar Cheese	Cottage Cheese	Al Nadia Yogurt	Al Jomjoom Yogurt
	Isolated fungi		Old					
	New	Old						
<i>Alternaria spp.</i>	0	0	0	0	0	0	0	
<i>Aspergillus flavus</i>	13	17	6	0	2	4		
<i>Aspergillus spp.</i>	1	0	0	1	0	2		
<i>Fusarium spp.</i>	0	0	0	1	0	0		
<i>Mucor spp.</i>	0	0	0	0	0	0		
<i>Penicillium spp.</i>	9	8	5	0	1	0		
<i>Rhizopus sp.</i>	0	0	3	0	0	0		
<i>Trichoderma spp.</i>	0	0	0	80	0	0		

\* New = fresh just opened & tested.  
 \* Old = Opened & stored for 2-5 days & then tested.



Figure 1. Fungi associated with direct plating method with non-disinfected seeds on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds)

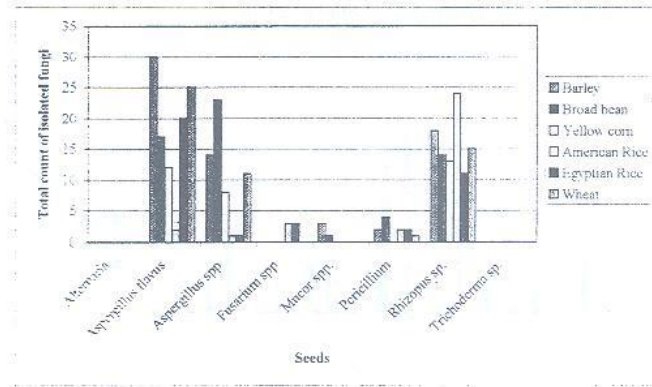


Figure 2. Fungi associated with direct plating method with surface disinfected seed on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds)

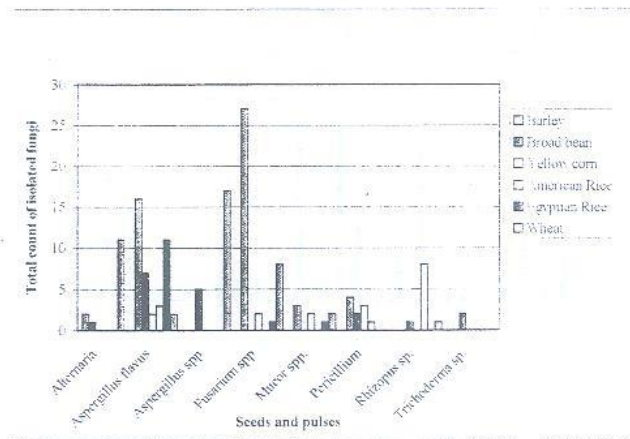


Figure 3 Fungi associated with direct plating method for nine samples of oil seeds and nuts on PDA agar medium incubated at 28 C for 7 days (counts for 100 seeds)

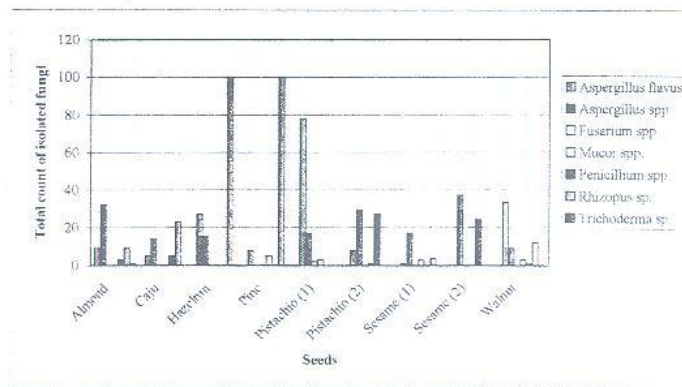


Figure 4. Fungi associated with direct plating method for 15 herbs and species on PDA agar medium incubated at 28 C for 7 days

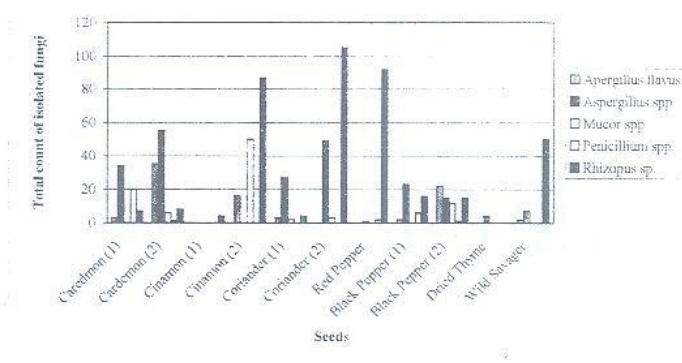


Figure 5. Fungi isolated from 18 samples of milk and dairy products on PDA agar medium incubated at 28 C for 7 days/one unit (ml or gm).

