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Isolation and Identification of Mycoflora Contaminated of Yemeni Coffee Beans

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ABSTRACT

This work was designed to study the mycoflora of stored Yemeni green and roasted coffee beans, in 50 samples (25 samples each) which were collected randomly from different markets in Sana'a city, Yemen during 2013 using direct plating technique. Our results showed that in the individual samples of green and roasted coffee beans, the counts of fungi were ranging from 110 to 236 CUF/ 100 beans and 8 to 249 CUF/100 beans, respectively. As well the broadest spectrum of fungal species were 55 species and 3 varieties belonging to 13 genera and 66 species and 3 varieties belonging to 23 genera in case of green and roasted coffee beans, respectively. *Aspergillus, Penicillium,* and *Rhizopus* followed by *Fennelliaand Fusarium* were the most common genera isolated from both the two types of coffee beans samples, whereas *Eurotium*was the most common genus only in roasted coffee bean samples, while it was absent in green coffee beans samples.

INTRODUCTION

The coffee tree or shrub belongs to the family Rubiaceae. Coffee beans are produced from the plant *Coffea* L. (Etienne, 2005; Belitz *et al*., 2009), of which comprises 103 species (Davis *et al.,* 2006). However, only two of these species are commercially explored worldwide: *Coffeaarabica* (Arabica), Considered as the noblest of all Coffee plants and providing 75% of world's production; and *Coffea canephora* (Robusta), considered to be more acid but more resistant to plagues, and provides 25% of world's production (Etienne, 2005; Belitz *et al*., 2009). Coffee can be cultivated only in subtropical, tropical, or equatorial climatic conditions . Arabica coffee is a highland species, adapted to high altitudes, where lower temperatures and reduced humidity prevail (Carvalho *et al.,* 1997).

Coffee ranks one of the world's most valuable and widely traded commodity crop and is an important export of a number of countries. Coffee represents an agricultural crop of significant economic importance to the coffee producing countries of the world. The global annual coffee production is estimated at 5.5 million tone of which 6.4% is produced in Ethiopia (Alemagehu *et al.,* 2007).

Yemen is one of the most historic coffee-producing nations, having launched the trade of what has become one of the world's most important agricultural commodities. Yemen mocha coffee is regarded as the most traditional coffee and still one of the world's greatest, uniquely delicious coffee.

It takes its name from the Yemen port city called Mocha (USAID, 2005). Coffee production in Yemen has been on a constant decline over the last decades (Nogaim *et al.*, 2013). Where According to the report of Food and Agriculture Organization (FAO) in 2011, surface cultivated with coffee in Yemen was about 34,686 hectares with yearly production of about 20,000 tones. It was generally acknowledged that more then 105,000 families of farmers are growing coffee in 15 governorates in Yemen.

As the case with other agricultural products, coffee beans are subject to various operations of contamination by micro-organisms during growth (while the beans are on trees), after harvesting (when the beans are de-hulled, washed and stored), processing, transport and storing. Microbial action detrimental to the quality and safety of the final product will depend on environmental conditions as well as crop and product management (Batista *et al.,* 2003).

Studies on the microbiology of coffee cherries and beans have shown that the main toxigenic fungal genera (*Aspergillus, Penicillium* and *Fusarium*) are natural coffee contaminants and are present form the field to the warehouse (Batista *et al.,* 2003). The presence of fungi in the coffee beans can cause loss of quality, producing foul odors and unpleasant flavor. They can sometimes produce toxic metabolites (mycotoxins), which can be harmful to consumers at certain concentrations (Chalfoun, 2010; Rezende *et al*., 2013).

Therefore, the present study aims to determine the mycoflora contamination of stored Yemeni green and roasted coffee beans (coffea Arabica), because of its importance and to ensure the safety of consumers who until now drinks coffee. There is also very little scientific research on this important economic crop.

MATERIALS AND METHODS

Collection of coffee samples

A total of 50 samples of stored Yemeni coffee beans including 25 green and 25 roasted (of the same type of the green ones) coffee bean samples were randomly collected from different markets and shops at Sana'a city, Republic of Yemen, during 2012. The weight of each sample was 250 g.

Each sample was put in a sterile polyethylene bag, sealed, put in another bag, and transferred to the laboratory, kept at 4^{o}C until mycological and mycotoxins analysis.

Isolation and identification of fungi

Isolation of fungi

Fungi were isolated using the direct plating technique as described by Pitt and Hoching (1997) and Samson *et al.* (2004). Samples were sub-sampled and then a total of 100 beans from each collected sample were planted directly (five beans per plate) onto Czapek dox agar medium (sucrose, 30g; $NaNO_3$,3g; KH_2PO_4,1g; $MgSO_4$. $7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4$, 0.01g; agar, 20g and 1000 ml of distilled water) plates, without surface disinfection, using five beans on each plate. The medium was supplement with chloromphenicol (500mgl^{-1}) as bacteriostatic agent. The plates were incubated at 28^{o}C for 7days.

The developing fungal colonies were observed, counted and calculated per 100 beans in every sample then isolated, purified and identified. Potato dextrose agar medium (potatoes, 200g; dextrose, 20g; agar, 15g and distilled water, 1000 ml) was used for purification of fungi. The purified fungi were transferred to slants of the same medium for the good sporulation, and kept in refrigerator at 4^{o}C (Bokhari, 2007; Alghalibi *et al.,* 2008).

Identification of fungi

Purified fungal isolate were identified morphologically (based on macroscopic and microscopic characteristics), following sub culturing on Czapek dox agar medium, Potato dextrose agar medium, Malt extractagar medium (malt extract, 30g; peptone, 5g; agar, 15g and distilled water, 1000 ml), and Oat meal agar(oat meal, 30g; agar, 30g and distilled water, 1000 ml) medium.

The identification of fungal genera and species was made with the help of the following references:

Booth (1971), for *Fusarium* species. Ellis (1971), for dematiaceous-hyphomycetes. Raper and Fennel (1977), for *Aspergillus* species. Pitt (1979), for the genus *Penicillium* and its teleomorphic states *Eupenicillium,* and *Talaromyces*. Moubasher (1993), for fungi in general. Samson *et al.* (1995), for fungi in general.

Some *Penicillium* isolates were identified at Mycological Center, Faculty of Science, Assiut University, Egypt.

Counting of identified fungi

Recovered fungal colonies were counted and expressed as colonies forming units per 100 beans(CFU per 100 beans). The isolation frequency of genera and species were calculated according to the method of Marasas *et al.* (1988) as follows:

$$\text{Frequency} = \frac{\text{No. of samples with occurrence of genus/specie} \times 100}{\text{Total No. of samples}}$$

RESULTS AND DISCUSSION

In this study, it was quite evident that all green and roasted coffee beans samples (100%) were invaded with different fungi. This result is in agreement with Leong *et al.* (2007) in Vietnam, who showed that, the majority of coffee beans in all samples were infected with one or more fungi.

Data in Table (1) indicate that it was possible to isolate 55 species and 3 varieties belonging to 13 genera and 66 species, one unidentified and 3 varieties belonging to 23 genera from green (G) and roasted (R) coffee beans samples respectively, on Czapek dox agar medium at 28^{o}C using direct plate method. The total counts of these fungi were widely varied from 110-236 colonies forming units per 100 beans (CFU/ 100 beans) and 8-249 CFU/ 100 beans for G and R coffee beans samples respectively, showing the highest count in sample No. 45 and 26 for G and R coffee beans samples, respectively. In this respect, in Egypt, Abdel-Hafez and El-Maghraby (1992) could isolate 26 fungal species belonging to 16 genera from coffee beans samples.

Also in a similar study, Nogaim*et al.* (2013) in India, who published that total fungi count in Yemeni green coffee ranged between 0 to 5.5 x10^2 CFU/g.

Also, during this study, the percentages of fungal isolation on Czaper dox agar medium at 28^{o}C were 65.57% and 34.43% found on green and roasted coffee beans, respectively (Fig.1).

Our result showed that, *Aspergillus, Penicillium,* and *Rhizopus* followed by *Fennellia*and *Fusarium*were the most common genera isolated from the two types of coffee beans samples. These results were similar to a great extent to results obtained by Abdel-Hafez (1984) who reported that coffee seeds were highly contaminated by the genus *Aspergillus*, followed by *Penicillium* and *Rhizopus*

Aspergillus occupied the first place occurring in 100% of the G and R coffee bean tested samples contributing 85.36% and 65% of total count of fungi, respectively. The genus fluctuated from 77-206 CFU/100 beans and 2-135 CFU/100 of the G and R coffee bean samples respectively, whereas the highest count recorded in G coffee bean samples No. 9 and 39 and in R coffee bean samples No.22 and 42. From this genus, 19 species, 3 varieties and 15 species, 3 varieties of the G and R coffee bean samples were identified of which *A. carbonarius, A. flavus* var. *flavus* and *A. ochraceus* the most prevalent species in both types from coffee beans. Whereas the rest species of *Aspergillus* were occurred in rare frequency of occurrence.

Table 1: Total count (TC), percentage of total count TC%, number of case of isolation (NCI), occurrence remarks (OR) of fungal genera and species recovered from roasted coffee beans samples.

Fungal genera and species	Green coffee beans					Roasted coffee beans				
	TC	TC%	NCI	NCI%	OR	TC	TC%	NCI	NCI%	OR
Alternaria	11	0.25	6	24%	L	3	0.13	3	12%	R
A. Alternata	6	0.14	3	12%	R	2	0.09	2	8%	R
A. chlamydospora	3	0.07	1	4%	R	1	0.04	1	4%	R
A. citri	2	0.05	2	8%	R					
Aspergillus	3738	85.36	25	100%	H	1510	65.68	25	100%	H
A. Aculeatus	28	0.64	10	40%	M	4	0.17	3	12%	R
A. aegyptiacus	1	0.02	1	4%	R					
A. awamori	17	0.39	6	24%	L	11	0.48	4	16%	L
A. candidus	1	0.02	1	4%	R	1	0.04	1	4%	R
A. carbonarius	2355	53.78	25	100%	H	858	37.32	23	92%	H
*A. flavus*var. *columnaris*	1	0.02	1	4%	R					
*A. flavus*var. *flavus*	389	8.88	24	96%	H	267	11.61	22	88%	H
A. fumigatus	4	0.09	3	12%	R	32	1.39	6	24%	L
A. glaucus	3	0.07	2	8%	R	4	0.17	3	12%	R
A. japonicus	34	0.78	10	40%	M	4	0.17	3	12%	R
A. malodoratus	2	0.05	1	4%	R					
A. niger	20	0.46	8	32%	M	46	2.00	5	20%	L
A. ochraceus	773	17.65	25	100%	H	140	6.09	14	56%	H
A. puniceus	34	0.78	10	40%	M	5	0.22	4	16%	L
A. speluneus	1	0.02	1	4%	R	1	0.04	1	4%	R
A. tamarii	69	1.58	19	76%	H	33	1.44	11	44%	M
*A. terreus*var. *aureus*	3	0.07	3	12%	R	10	0.44	6	24%	L
*A. terreus*var. *terreus*						1	0.04	1	4%	R
A. ustus	1	0.02	1	4%	R					
A. versicolor	1	0.02	1	4%	R	93	4.05	11	44%	M
A. wentii	1	0.02	1	4%	R					
Cephaliophoratropica						1	0.04	1	4%	R
Cheatomiumthermophilum						1	0.04	1	4%	R
Circinellamuscae						2	0.09	1	4%	R
Cladosporium	1	0.02	1	4%	R	11	0.48	7	28%	M
C. cladosporioides						1	0.04	1	4%	R
C. herbarum						7	0.30	4	16%	L
C. macrocarpum	1	0.02	1	4%	R	1	0.04	1	4%	R
C. tenuissimum						2	0.09	2	8%	R
Cochliobolus	4	0.09	3	12%	R	2	0.09	1	4%	R
C. australiensis	1	0.02	1	4%	R	2	0.09	1	28%	R
C. lunatus	3	0.07	2	8%	R					
Doratomycesstemonitis						1	0.04	1	4%	R
Emericella	1	0.02	1	4%	R	179	7.79	3	12%	R
E. nidulans	6	0.14	2	8%	R	177	7.70	2	8%	R
E. quadrilineata						2	0.09	2	8%	R
Eurotium						146	6.35	18	72%	H
E. amstelodami						126	5.48	17	68%	H
E. chevalieri						19	0.83	11	44%	M
E. rubrum	1	0.02	1	4%	R	1	0.04	1	4%	R
Fennellia	20	0.46	9	36%	M	17	0.74	4	16%	L
F. flavipes	20	0.46	9	36%	M	4	0.17	3	12%	R
F. nivea						13	0.57	2	8%	R
Fusarium	18	0.41	8	32%	M	7	0.31	5	20%	L
F. anthophilum	1	0.02	1	4%	R	1	0.04	1	4%	R
F. heterosporum	1	0.02	1	4%	R					
F. lateritum	1	0.02	1	4%	R					
F. nivale	1	0.02	1	4%	R	3	0.13	1	4%	R

Table 1: Continued.

F. oxysporium	9	0.21	5	20%	L	2	0.09	2	8%	R
F. sambuncinum	2	0.05	2	8%	R					
F. scirpi	1	0.02	1	4%	R					
F. solani	1	0.02	1	4%	R	1	0.04	1	4%	R
F. xylarioides	1	0.02	1	4%	R					
Humicola						5	0.22	3	12%	R
H. grisea						1	0.04	1	4%	R
H. hyalothermophila						3	0.13	1	4%	R
H. insolens						1	0.04	1	4%	R
Mucorracemosus						3	0.13	2	8%	R
Paecilomyceslilacinus	2	0.05	2	8%	R	1	0.04	1	4%	R
Penicillium	253	5.78	22	88%	H	294	12.79	24	96%	H
P. aurantiogriseum	7	0.16	3	12%	R	5	0.22	4	16%	L
P. brevicompactum	2	0.05	1	4%	R	2	0.09	2	8%	R
P. chrysogenum	19	0.43	8	32%	M	131	5.70	21	84%	H
P. citrenigrum	1	0.02	1	4%	R	2	0.09	1	4%	R
P. citrinum	27	0.62	11	44%	M	32	1.39	9	36%	M
P. crustosum	84	1.92	12	48%	M	50	2.18	13	52%	M
P. duclauxi						2	0.09	1	4%	R
P. expansum	1	0.02	1	4%	R					
P. funiclosum	29	0.66	8	32%	M	3	0.13	3	12%	R
P. glabrum	14	0.32	4	16%	L	23	1.00	9	36%	M
P. islandicum						1	0.04	1	4%	R
P. nigricans	1	0.02	1	4%	R	4	0.17	2	8%	R
P. pinophilum	1	0.02	1	4%	R					
P. purpurgenum	26	0.59	5	20%	L	16	0.70	5	20%	L
P. restrictum	41	0.94	9	36%	M	21	0.91	8	32%	M
P. variabile						2	0.09	1	4%	R
Phoma						6	0.26	4	16%	L
P. glomerata						1	0.04	1	4%	R
P. herbarum						4	0.17	2	8%	R
*Phoma*sp.						1	0.04	1	4%	R
Pithomyceatro-olivaceus						6	0.26	5	20%	L
Rhizomucorpusillus						2	0.09	1	4%	R
Rhizopus	313	7.15	24	96%	H	82	3.57	21	84%	H
R. oryzae	150	3.43	10	40%	M	68	2.96	15	60%	H
R. stolonifer	163	3.72	14	56%	H	14	0.61	8	32%	M
Scopulariopsisbrevicaulis						1	0.04	1	4%	R
Setosphaeriaholmii	2	0.05	1	4%	R					
Sterial mycelium	9	0.21	5	20%	L	16	0.70	6	24%	L
Thanaphoruscucumeris						2	0.09	1	4%	R
Trichodermaharzianum	1	0.02	1	4%	R					
Ulocladiumatrum						1	0.04	1	4%	R
Total count	4379	100	-	-	-	2299	100	-	-	-
Number of genera	13	-	-	-	-	23	-	-	-	-
Number of species	55	-	-	-	-	66	-	-	-	-

H = High occurrence (more than 14), **M** = Moderate occurrence (7-13); **L**= Low occurrence (4- 6).; **R** = Rare occurrence (less than 3).

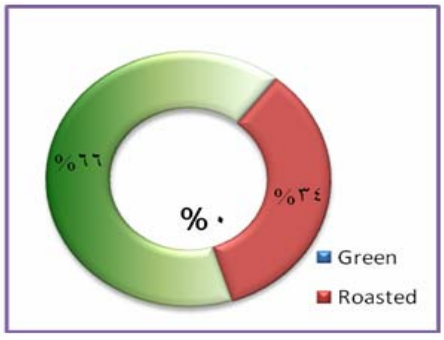

Fig. 1: Percentage of isolated fungal isolates from green and roasted coffee beans.

Penicillium was the predominant genus in coffee bean samples where it came second in R and third in G coffee bean samples (96% and 88% of the samples, respectively), contributing 5.78% and 12.79% of total fungi, and the counts of this genus fluctuated between 1-40 CFU/ 100 beans and 1-26CFU/ 100 beans of G and R coffee beans samples, respectively. The highest count was estimated in sample No. 13 and No. 18 of G and R coffee beans samples, respectively. Among the13 and 14 species of *Penicillium* isolated from G and R coffee beans respectively, *P. chrysogenum, P. citrinum, P. crustosum, P. funiclosum, P. glabrum*and *P. restrictum* occurred more frequently than others. our results in the field of predominance of *Aspergillus* and *Penicillium* in coffee beans agree with the result of Mislivec *et al*. (1983); Abdel-Hafez and El-Maghraby (1992); Nakajima *et al.* (1997); Silva *et al.* (2000); Panneerselvam *et al*. (2001); Noonim *et al.* (2008) and Bokhari and Aly (2009). In contrast to our results, Al-Kolaibe (2002) reported that *Penicillium* appeared in rare incidence on coffee fruits or beans.

Rhizopus (represented by 2 species)was also the most common genus in coffee beans samples where it came second in G and third in R coffee beans samples (96% and 84% of the samples, respectively), matching 7.15% and 3.57% of the total fungi and the highest count in this genus was 1-33 CFU /100 beans and existed in sample No. 45 and 15 CFU /100 beans and existed in sample No.2,6,8,10,12,14 and 28 for G and R coffee beans, respectively. *R. oryzae*and *R. stolonifer* appeared in high to moderate incidences in most of the samples. In contrast to our results, Al-Kohaibe (2002) who showed that *Rhizopus* was occurred in low to moderate frequency on coffee samples.

Fusarium respectively by 9 and 4 species isolated from G and R coffee beans samples, respectively. This genus was isolated in moderate and low occurrence emerged in 32% and 20% of the samples matching 0.415 and 0.31% of total fungal count from G and R coffee bean samples, respectively. This genus was counted 1-6 CFU/100 beans and 7 CFU/100 beans of G and R coffee beans samples, respectively. *F. oxysporium* was the most common species in G coffee beans, while the rest species of *Fusarium* were found in rare frequency in both types from coffee beans. This result is in agreement with Abdel-Kader and Hubaishi (1988) who showed that *F. oxysporium* was the most frequent species that was isolated from coffee fruits in Yemen. On the other hand, *Fennellia* represented by one and 2 species counting 20 CFU/100 beans and 17 CFU/100 beans,and existed in moderate and low occurrence (39% and 16% of the samples) contributing 0.46% and 0.74% of total count of fungi in G and R coffee bean samples, respectively. *F. flavipes* was isolated from both types of coffee beans, while *F. nivae*isolated only from R coffee beans.

Eurotium was isolated in high occurrence only from R coffee beans, whereas it was absent in green coffee beans samples. This genus was occurred in 72% of the samples constituting 6.35% of total fungal count. The counts of the genus varied from 1-27 CFU/100 beans giving maximum in sample No. 14. Three species were isolated from the genus of which *E. amstelodami* was recovered in high occurrence than *E. chevalieri* which recovered in moderate occurrence, while *E. rubrum* was found in rare frequency of occurrence.

Cladosporum was isolated in moderate frequency of occurrence from R roasted coffee beans, while *Alternaria, Phoma, Pithomyces*and Sterialmycelium were isolated in low frequency of occurrence. Finally, the rest of the genera were collected in rare frequency of occurrence.

Several reasons could be attributed for the biodiversity, variability and complexity of fungal load in coffee beans. According to a review by Silva *et al.* (2000) who showed that variation in climatic conditions, harvesting, processing method, and drying

could substantially affect degree of fungal infection in coffee beans. In addition, coffee seeds are liable to mould contamination especially, if not dried to safe moisture level or if rehydrated during any stage of drying, packing and transportation (Tsubouchi *et al.,* 1988). Beside that also this may be due to bad drying method used by farmers in Yemen as exposed of coffee for longer periods to the open air during harvesting, air drying on the surface of buildings as well as during storage in field so, coffee beans could be easily contaminated by fungal propagates dispersed in the air and air dust particles. Finally, may be due to the bad storage conditions in shops by sellers. Where, Lillehoj *et al.* (1980) and Hill and Lacey (1983) mentioned that storage conditions are one of the factors that determine the type and quantity of fungal contamination of fruits.

According to data obtained in this investigation, it was clear that there was an apparent difference in both fungal content and species in regard to green or roasted coffee beans. For example, the number of isolated fungi from green coffee beans was more than the number of those that isolated from roasted coffee beans (Fig. 1). On the other hand, the number of fungal genera and species that had been isolated from roasted coffee bean (66 species and 3 varieties belonging to 23 genera) was over than the number of those that had been isolated from green coffee beans (55 species and 3 varieties related to 13 genera) (Fig.2).

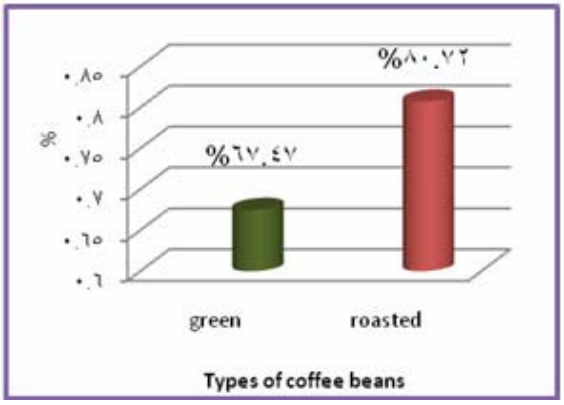

Fig. 2: Percentages of isolated fungal species from green and roasted coffee beans.

Increase the fungal content in green coffee beans compared to roasted coffee beans which were noticed in the present investigation could be explained according to the interpretation of (Trucksess *et al.,* 1999) who mentioned that fungi need a certain level of humidity within which they perform better and therefore decreasing humidity of coffee seeds by roasting could lead to changes in the range of latitudes at which certain fungi are able to complete.

Additionally, increase the number of fungal genera and species in roasted coffee beans compared to green coffee beans may be due to many reasons: (i) Loss of the silver skin of the coffee beans during roasting (Studer-Rohr *et al*., 1994); (ii) 30% reduction of caffeine content after the roasting step (Franca *et al*., 2005), because that caffeine was shown to inhibit the growth of the fungal (Buchanan *et al*., 1983); (iii) during roasting, some compounds such as carbohydrate, nitrogenous compounds, lipid, acids and esters are destroyed and other are formed (Farah,2012) ; (iv) over growth of A. niger on green coffee beans which led to obscure the growth of other fungi (Nehad *et al*., 2007), all of these reasons can help on invasion and the appearance of other fungal species on roasted coffee beans.

Finally, genera following: *A. citri; A. glaucus; A. aegyptiacus; A. awamori; A. puniceus; A. malodoratus; A. speluneus; C. tropica; D. stemonitis; E. rubrum; P.*

citreonigrum; P. restrictum; P. crustosum; F. anthophilum; F. heterosporum; F. lateritum; F. sambuncinum ; F. scirpi; F. xylarioides; H. insolens; P. atro-olivaceus; R. pusillus; R. oryzae; S. holmii T. cucumeris considered new record in yemen.

CONCLUSION AND RECOMMENDATION

All stored Yemeni green and roastedcoffee bean samples were highly contaminated by fungi. Also, the most common identified fungal genera in Yemeni green and roasted coffee beans were *Aspergillus, Penicillium* and *Rhizopus* followed by *Fennellia* and *Fusarium*. Special attention must be paid to the conditions under which these coffee beans are stored and also precautions must be adopted during storage to avoid their contamination by mycoflora.

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ARABIC SUMMARY

عزل وتعريف الفلورا الفطريه من حبوب البن اليمني

عبدا لرحمن عبدا لله حميد - سعيد منصر الغالبي- اشراق الخلقي
قسم علوم الحياة- كلية العلوم- جامعة صنعاء - اليمن

تم تصميم هذا العمل لدراسة الميكوفلورال ٥٠ عينة من حبوب البن الأخضر والمحمص اليمني المخزن (٢٥ عينة لكل نوع) التي تم جمعها عشوائيا من الأسواق المختلفة في مدينة صنعاء، اليمن خلال ٢٠١٣ باستخدام تقنية زراعة حبوب البن مباشرة على الأطباق المحتويه على الوسط الغذائي. وقد أظهرت نتائجنا أنه عدد الفطريات المعزول هفي عينات فردية من حبوب البن الأخضر والمحمص تتراوح من ١١٠-٢٣٦ 100/CUFحبة و٨-٢٤٩ 100/CUF حبة على التوالي. وكذلك اظهرت النتائج ان الاجناس الفطرية ساهم تبنطاق واسع من الأنواع الفطرية والتي كانت ٥٥ نوع و ٣ أصناف تنتمي إلى ١٣ جنسا و ٦٦ نوعا و ٣ أصناف تنتمي إلى ٢٣ جنسا في حالة من حبوب البن الأخضر والمحمص، على التوالي بالاضافه إلى ذلك ، كانت الاسبرجلس، البنسليوم، الريز وبستليهاالفينلاوالفيوزاريوم هيألاجناس الأكثر شيوعا المعزولة عن كلا النوعين من عينات حبوب البن ، في حين جنس اليوريتيوم كان جنس الأكثر شيوعا فقط في عينات حبوب البن لمحمص ، في حين أنه كان غائبا فيعينان حبوب البن الخضراء .