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ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH DIFFERENT SPECIES OF STORED GRAINES

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ABSTRACT

This study was conducted on different species of stored grains obtained from Duhok Province, Kurdistan Region, of Iraq to investigate the occurrence of seed borne fungi. Nine types of grains were collected and two methods were selected (agar plate method and blotter method) for fungal isolation. A total of 19 species assigned to 13 genera were identified. The high frequent genera were *Aspergillus* (4 species), *Penicillium, Alternaria* and *Fusarium* (2 species), while other genera include *Rhizopus, Cladosporium, Stemphylium, Ulocladium, Humicola, Bipolaris, Curvularia Phoma* and *Rhizoctonia* were represented only in a single species. The results showed a variation in the fungal species and contamination percentage according to the detection and incubation methods. The lowest occurrence percentage was detected in lentils seeds with only one genus represented by *Penicillium* spp., while the highest occurrence percentage was detected with Chickpea seeds represent by six genera. The most common fungal genera displayed by agar plate method in wheat and chickpea were *Rhizoctonia* sp. (34.1%) and *Penicillium* sp. (42.5%) respectively. The highest fungal detection by blotter method was recorded with barley seeds while the lowest was chickpea seeds. The most common fungal genera recorded by blotter method under room temperature after 7 days and 14 days was *Penicillium* spp, with a percent 100% from chickpea seeds followed by *Aspergillus* spp with percent of (66.7%, 47.6%) from barley and raisin seeds respectively. While the highest frequent fungus in seeds incubated at 25°C after 7 and 14 days was *Aspergillus parasiticus* (88.9%) from mash seeds and *Rhizopus sp.* (80.1%) from lima bean respectively. Blotter method considered an efficient and economically reliable method.

KEYWORDS: Stored grains, dried food, hosts, detection method, Iraq.

INTRODUCTION

Food products, especially grains are important nutrient origin worldwide and their chemical composition colonized by both fungal and bacterial (Samson et al., 2004; Pitt and Hocking, 2009). Seed born fungi represent as one of the restricted factors state the high-quality seed availability and escalation food crops production. They have the ability to affects seed quality criteria particularly seed health and may cut off the pant growth phases by make change in nutrients composition and seed biotechnology as well as become a source of inoculum in storage and field conditions (Mew and Gonzales, 2002, Ali, 2017). Some fungi isolated from stored grains are responsible on both pre-and post-emergence damping off of seeds and cause reduction in seed germination, they also cause myotoxic in life stock (Abdullah and Atroshi ,2014). The seeds should be harvested after maturity as soon as possible. Mechanical injury, during harvesting, threshing and cleaning will damage the seed coat. The storage fungi invade the damaged seeds easily and grow rapidly, during unsuitable seed storage conditions. Several types of fungi can remain variable causing deterioration or remain active to infect germinated seedlings. The genera of fungi mainly found in stored seeds are *Aspergillus, penicillium* and *Fusarium*. Many seed born fungi can produce mycotoxins in seeds that affect humans and animals. Fungi associated with grains can be pathogenic, weak parasite or saprophytic, therefore studying seed born fungi is very important to estimate the health of grains and to protect them from pathogenic fungi (Health grain crops association HGCA, 2012).

The aim of this study was to isolate and identify the occurrence of fungi with grains of different species from Duhok province using two different methods for detection.

MATERIALS AND METHODS

- Collection of post harvested stored grains and dried food

Nine samples of stored grains and dried food species as (Wheat, barley , corn, mash, lentils, chickpea, broad bean and lima bean) and dried food(raisin) were obtained from different seeds selling markets in Duhok Province, Iraqi Kurdistan Region. About 500 gm /sample of each species were obtained according of the international rules of seeds testing (ISTA 2009).

- Detection of seed born fungi

1- Agar Plate method

Fifty seeds of each grain species have been taken randomly from each working sample (except chickpea and broad bean which taken 25 seeds of each), and then were transferred aseptically in petri plate containing 20ml solidified sterilized potato dextrose Agar (PDA) medium in an autoclave at 1.2kg cm^{-2} pressure for 20 minutes. The tested seeds were placed at equal distance per plate by keep one seeds at the center and nine seeds at the periphery (chickpea and broad bean one in center and 4 at periphery). Then the plates were incubated under 12/12 hr. alternating light and darkened period at 25± 2°C for 7 days.

2- Blotter method

Seeds were washed and sterilized with a solution of sodium hypochlorite 1.5% for of 2-3min, and then washed with sterilized distilled water three times and dried by filter papers, after that transferred to misted blotter plate by sterile distilled water in five replication, 10 seeds in each (except chickpea and broad bean five seeds). Each blotter plate was incubated under alternating cycles of 12/12h light/dark at 25°C (and room temperature 18-22°C) for 7 and 14 days. Finally, each petri dishes and blotter plate were examined under light microscope for fungal identification . Percentages of occurrence were recorded after 7 and 14 days of incubation.

- Identification of isolated fungi.

After obtaining the pure culture of each fungus, identification was done according to keys and description provide by (Ellis (1971,1976): Klich (2002); Watanabe (2002) and Samson et al. (2000).

RESULTS AND DISCUSSION

A total of 19 species belong to 13 genera have been isolated from 9 samples (different grains and dried food species) using two different detection methods (Agar plate method and blotter method) and under room temperature and control treatments (25°C). The most common genera isolated were *Aspergillus* (4 species), *Penicillium, Alternaria* and *Fusarium* (2 species), while other genera include *Rhizopus* ,*Cladosporium* ,*Stemphylium, Ulocladium, Humicola* ,*Bipolaris, Curvularia* and *Phoma* were represented only in a single species (Table 1 and 3).

The lowest occurrence percentage was detected with lentils seeds (Table 1), with one genus represented by *Penicillium* spp. using agar plate method. The most common fungal genera recorded by agar plate method was *Aspergillus spp*, with percent of (88.2%,37.5%,33.9%,30%,25%,16.7%,4.9%) for raisin, broad bean, chickpea, corn, lima bean ,mash and wheat, respectively. Followed by *Penicillium* spp, with a percent of 100%,66.7%,42.5%,50%,12.5%,11.7% for lentils, mash, chickpea, lima bean, barley and raisin respectively (Table 2). The most common fungal genera displayed by agar plate method in wheat, chickpea, corn, lentils, mash, lima bean, raisin, barley and broad bean were *Rhizoctonia* sp (34.1%), *Penicillium* sp. (42.5%), *Aspergillus parasiticus* (40%), *Penicillium spp.* (100%,66.7%,50%), *Aspergillus niger* (88.2%), *Alternaria* sp. (62.5%) and *Aspergillus parasiticus* (37.5%) respectively (Table 2)

Table (1):-Detection of fungi from different grains species by using agar plate method

Fungi	**Wheat**	**Corn**	**Chickpea**	**Lentils**	**Mash**	**Lima bean**	**Raisin**	**Barley**	**broad bean**
Rhizoctonia solani	+		+						
Stemphylium sp	+								
Aspergillus parasiticus	+	+							+
Cladosporium sp	+								
Sterile mycelium	+		+					+	+
Curvularia sp			+						
Aspergillus niger		+	+			+	+		
Penicillium spp			+	+		+	+	+	+
Alternaria sp			+		+	+		+	
Aspergillus terreus		+			+				
Ulocladium sp						+		+	

Table (2):- Frequency of occurrence of fungi by using agar plate method

Species	**Isolated fungi**	**No.of colony**	**%frequency**
Wheat	*Rhizoctonia solani*	14	34.1
	Stemphylium sp	11	26.8
	Aspergillus parasiticus	2	4.9
	Cladosporium sp	2	4.9
	Sterial mycelium	12	29.3
Chickpea	*Rhizoctonia sp*	20	18.9
	Curvularia sp	1	0.9
	Aspergillus niger	36	33.9
	Penicillium spp	45	42.5
	Alternaria sp	2	1.9
	Sterial mycelium	2	1.9
Corn	*Aspergillus terreus*	6	30
	Aspergillus niger	6	30
	Aspergillus parasiticus	8	40
Lentil	*Penicillium spp*	4	100
Mash	*Penicillium spp*	4	66.7
	Aspergillus terreus	1	16.7
	Alternaria spp	1	16.7
Lima bean	*Aspergillus niger*	4	25
	Penicillium spp	8	50
	Ulocladium sp	2	12.5
	Alternaria sp	2	12.5
Raisin	*Asperegillus niger*	15	88.24
	Penicillium sp	2	11.76
Barley	*Penicillium sp*	1	12.5
	Alternaria sp	5	62.5
	Ulocladium sp	1	12.5
	Sterial mycelium	1	12.5
Broad bean	*Aspergillus parasiticus*	3	37.5
	Sterile mycelium	4	50
	Penicillium sp	1	12.5

The highest fungal detection by blotter method was recorded with barley seeds while the lowest was chickpea seeds (Table 3). The most common fungal genera recorded by blotter method under room temperature after 7 days was *Aspergillus spp*, with percent of(66.7,60,41.7,40,18.8,14.3) for barley, corn, raisin, wheat, lima bean and lentils respectively.(Table 4). Followed by *Penicillium* spp, with a percent (100, 62.5, 60, 10, 8) for chickpea, lima bean, wheat, corn and broad bean respectively.

The common fungal genera listed by blotter method incubated at 25°C after 7 days was *Aspergillus spp*, with 88.9% ,76.2%, in mash and lima bean respectively compared to 58.3% and 45.5% for mash and raisin respectively after 14 days (Table 4).

The common fungal genera listed by blotter method incubated at room temperature after 14 days was *Aspergillus* spp with a percent (50,47.6,38.4,36.4,33.3,15.8,4.8) for mash, raisin, barley, corn, wheat , lima bean and raisin respectively (Table 4). The most common fungal genera displayed by Blotter method at room temperature conditions after 7 days in wheat and Chickpea seeds were *Penicillium sp* (60% and 100%), in corn *Aspergillus niger* (60%), in lentils *Rhizopus stolonifer* (85.7%) , in mash *Penicillium sp., Aspergillus niger* (50%) ,in lima bean *Penicillium sp* (62.5%), in raisin *Rhizopus stolonifer* (58.3%), in barley *Aspergillus niger* (66.7%) and *Rhizopus* (92%) in broad bean, while the common fungal genera displayed by Blotter method incubated at 25°C in wheat was *Alternaria sp* (50%), in chickpea *Penicillium* sp (87%), in corn *Fusarium sp*(50%) , in mash *Aspergillus* (88.9%) , in lima bean *Rhizopus* (80.1%), in raisin *Rhizopus* (60.6%), in barley *Alternaria* (50%) and *Rhizopus* (70.5%)in broad bean (Table 4).

A total of 17 species belong to 11 genera were recorded using blotter method and 12 species were isolated by agar plate method. Species like *Aspergillus niger*, *A. flavus* , *A. parasiticus* , *Penicillium* spp, *Alternaria alternata*, *Cladosporium* sp. and *Rhizoctonia solani* were detected by both isolation methods. Species detected by Agar plate method were *Aspergillus terrus*, *Curvularia* sp and *Ulocladium* sp, while *Fusarium* sp, *Bipolaris sorokiniana*, *Humicola* sp, *Phoma* sp. and *Rhizopus* spp. were detected only by blotter method.

Table (3):- Detection of fungi from different grains species by using blotter method (two temperatures and duration).

Fungi	Wheat	Corn	chickpea	Lentils	Mash	Lima bean	Raisin	Barley	Broad bean
Penicillium spp.	+	+	+		+	+	+	+	+
Aspergillus niger	+	+		+		+	+	+	
Rhizopus stolonifer				+		+	+	+	+
Aspergillus parasiticus					+	+			
Aspergillus flavus		+					+		
Alternaria sp	+	+						+	
Cladosporium sp					+			+	
Humicola dimorphospora								+	
Fusarium oxysporum								+	
Bipolaris sorokiniana								+	
Rhizoctonia sp								+	
Stymphylium sp		+							
Fusarium sp		+			+				
Phoma like		+							
Fusarium solani			+						
Sterial mycelium					+				

Table (4):-Frequency of occurrence of fungi by using blotter method

Species	Isolated fungi	Room temperature		Incubator (25°C)	
		7days	14days	7days	14days
		Freq.%	Freq.%	Freq.%	Freq.%
Lima bean	*Penicillium sp.*	62.5	57.9	19	16.1
	Aspergillus niger	18.8	15.8	76.2	1.9
	Rhizopus stolonifer	18.8	26.3	4.8	80.1
	Aspergillus parasiticus				1.9
Raisin	*Penicillium sp*		4.8		2.3
	Aspergillus niger	41.7	47.6	36.4	45.5
	Rhizopus stolonifer	58.3	42.9	60.6	50
	Aspergillus flavus		4.8	3	2.3
Barley	*Alternaria alternata*	33.3	15.4	50	36.4
	Aspergillus niger	66.7	38.4	25	13.6
	Penicillium sp		7.7		6.8
	Rhizopus stolonifer		30.8		13.6
	Cladosporium sp		7.7		4.5
	Humicola dimorphospora				2.3
	Fusarium oxysporum				2.3
	Bipolaris sorokiniana				2.3
	Rhizoctonia solani			25	18.2
Broad bean	*Penicillium sp*	8	9.1	29.5	44.4
	Rhizopus stolonifer	92	90.9	70.5	55.6
Wheat	*Penicillium sp*	60	44.4	25	35.7
	Aspergillus niger	40	33.3	25	14.3
	Alternaria sp		22.2	50	50
Corn	*Stemphylium sp*			6.3	4.5
	Penicillium sp	10	9.1		
	Aspergillus niger	60	36.4	6.3	11.4
	Alternaria sp				2.3
	Fusarium sp	10	13.6	50	40.9
	Phoma sp.	20	31.8	31.3	34.1
	Aspergillus flavus		9.1	6.3	6.8
Chickpea	*Penicilliium sp*	100	100	87.5	80
	Fusarium solani			12.5	20
Lentils	*Aspergillus niger*	14.3	10		
	Rhizopus stolonifer	85.7	90		
Mash	*Penicillium sp*		50		8.3
	Aspergillus parasiticus		50	88.9	58.3
	Fusarium sp			11.1	25
	Cladosporium sp				4.2
	Sterile mycelium				4.2

Seed contamination occurs through small quantity of spore contamination when it going in to storage from harvesting in handlings and storage equipment or from spores which already present in storage structures (IRRI, 2006). The amount of inoculum can increase rapidly under high temperature and moisture. Seeds provide their mycobiota with microhabitats which are highly temporally inconstant. Consequently, it is credible to expect that fungal communities associated with seeds are also dynamic over time. The ways of temporal variation in fungal community composition in seeds reflect the inoculum that occurred at harvest as well as successive balancing among the population processes of emigration between internal and internal communities, and death for individual species; these processes are highly rely on the seeds physiological conditions, which are highly dynamic over time. In accordance with such expectations, we noticed sharp differences in

species between wheat and chickpea seeds comparing with other seeds.

Eleven fungal genera have been isolated from stored grains using blotter method, in other studies, same fungal genera were commonly isolated from contaminated stored grains (Logrieco and Visconti, 2004; Kocic-Tanackov and Dimic, 2013). The most frequent isolated fungal species in this study were *Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Aspergillus parasiticus* and *Penicillium* species. Further Prakash et al., (2015) have also reported the most fungi associated with stored food which include *Aspergillus* spp., *Penicillim* spp., *Fusarium* spp., *Alternaria* spp., *Curvularia* spp. and the member of Mucorales (Fakhrunnisa et al., 2006). They also produce mycotoxicosis in human and animals. The main genera of mycotoxigenic fungi are belong *Aspergillus, Penicillium,* and *Fusarium,* while *Trichoderma, Trichothecium* and *Alternaria* are also important as phytopathogenic or as food contaminants among others (Ashiq, 2015 and Richard, 2007). In nature, most fungi are toxigenic, and those that are not toxigenic may be during long storage, they impart a mouldy odor and taste. They are considered a significant factor in food spoilage, leading to substantial economic losses and a major public health risk worldwide through the processing of a wide range of mycotoxins (U.S. Dept. of Agriculture, 2003). Fungal mycotoxins contaminate foods and animal feeds and have significant agricultural, epidemiological, and economic impacts, representing a worldwide problem (Chulze, 2010).

This approach for isolation fungi let for more experimentation to answer various questions regarding the ecology, biology and evolution contributing to our understanding of the underlying mechanisms that determine the mycobiota composition and dynamics. The isolated fungi also perform valuable materials for pathological and plant-fungi interaction studies that will contribute to future disease management. Furthermore, the discovery of main mycotoxigenic fungal taxa as well as antagonistic species in this work indicated that the cultivation dependent approach remains an effective tool for studying the mycobiota associated with stored seeds because it provides in valuable insights that are impossible with cultivation-free approaches. Further studies should include the targeted tests of fungal species from large samples of seeds to recognize the relationship between seed physiological characteristics and fungal survival rates. The fungal communities of the same seed stocks for multiple time-intervals should be reisolated and recharacterized to reconstruct the mycobiota dynamics of seeds. Such studies may direct to novel strategies for prevention and management of disease and mycotoxins in stored seeds.

Governments of countries with high post-harvest losses should recognize the grains produced as a national asset and give significant resources to preserve them. Presently, in many nations, governments see grains as belong to farmers and therefore losing quality and quantity are farmers responsibility, but at the same time national governments import seeds to accomplish the demand of their citizens at a considerably higher price. If seeds were handled and secured as a national asset, demand for imports might be reduced and valuable foreign currencies could be saved. In near future. it is worthy that seeds production particularly wheat should be increased in order to cover the nutritional requirements of an increasing in human population this need certified and healthy seeds which are an important input for crop production, reduction of yield losses by seed born fungi is the major method to contribute to the food security in the world.

CONCLUSION

This study revealed that grain samples of the nine species as (Wheat, barley, raisin, corn, mash, lentils, chickpea, broad bean and lima bean) were contaminated with diversity of fungi Viz., *Penicillium sp, Aspergillus niger, A. parasiticus, A. flavus, Rhizopus stolonifer, Alternaria alternata, Cladosporium sp, Humicola dimorphospora, Fusarium oxysporum, Bipolaris sorokiniana, Rhizoctonia solani, Stymphylium, Phoma sp. , Fusarium solani, Sterialmycelial, Curvularia sp, Ulocladium* sp, which some of them caused plant disease in the field. Whereas the fungi such as *Aspergillus spp, Penicillium spp* and *Fusarium* spp producing mycotoxins. The results indicated that it could be vital to test the pathogenicity and toxigenic ability of some isolates. Our results indicated that using blotter method under control temperature conditions in alternating cycles of 12/12 h light/darkness at 25°C for mor than two weeks will encourage growing other types of

fungi such as *Humicola dimorphospora*, *Fusarium oxysporum*, *Bipolaris sorokiniana* and some *Aspergillus* species which found under not stable room temperature conditions (18 – 22C). Blotter method regarded as most efficient and economically reliable method. The traditional methods of seeds handlings during harvestings in the fields, drying process in relevant country and transferring it to other locations may lead to mechanical grains damages.

REFERENCES

Abdullah,S.K and Atroshi,H.I.M (2014). New records of fungi on wheat grains from Iraq.J.Univ.Zakho 2(A):256-265.

Ali, A.M, Activity of thuja (*Thuja orientalis*) alcoholic extract in inhibition *Aspergillus flavus* growth and detoxification of Aflatoxin B1 in contaminated corn seeds. Pak. J. Biotechnol. 14: 503-506 (2017)

Ashiq, S. (2015). Natural occurrence of mycotoxins in food and feed: Pakistan perspective. Comprehensive Reviews in Food Science and Food Safety, 14, 159–175.10.1111/crf3.2015.14.issue-2.

Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage: A review. Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure and Risk Assessment, 27, 651–657.

Ellis, M. B. (1976). More Dematiaceous Hyphon.rycetes,Comnonu'ealth Mycological Institute, Ke$,,England 507pp.

Ellis. M. B. (1971). Denatiaceous Hyphomycetes,Commonwealth Mycological Institute, Kew,England 608 pp..

Fakhrunnisa, M., Hashmi,H and ghaffar.A.(2006) Seed-borne mycoflora of wheat, sorghum and barley. Pak.J.Bot.38(10;185-192

HGCA (2012).Wheat disease n'ranagement guide.Agriculture and Horticulture Departrnent Board.HGCA publication, Warwickshire, U.K.

IRRI (2006). International Rice Research Institute: www.knowledgebank.irri.org/ppfm/storage/6. B.- fungi.htm

ISTA(2009).International Rules for Seed Testing.Annwx to chapter 7.seed health methods. International seed Testing association, Bassersdorf. Switzerland.

Klich, M.A. Identification of Common Aspergillus Species. Utrecht (NL): CBS (2002).

Kocić-Tanackov, D. S., Dimić, R. G. (2013). Fungi and mycotoxins - Food contaminants. Hem. Ind. DOI. 10.2298/HEMIND120927108K.

Logrieco, A., Visconti, A. (2004). An Overview on toxigenic fungi and mycotoxins in Europe. Dordrecht, Boston, London: Kluwer Academic Publishers.

Mew, T.W. and P. Gonzales, A Handbook of Rice Seedborne Fungi. Los Banos (PH): IRRI Pr (2002)

Pitt, J. and Hocking, A. (2009). Fungi and Food Spoilage. 3rd ed. New York: Springer.

Prakash, B.; Kedia, A.; Mishra, P.K.; Dubey, N.K. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities—Potentials and challenges. *Food Control* **2015**, *47*, 381–391.

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicosis-An overview. International Journal of Food Microbiology, 119, 3–10.

Samson, A.R., Hoekstra, S.E., Frisvad, C.J. (2004). Introduction to Food-and Airborne Fungi. 7th ed. Utrecht: Central bureau vor Schimmel cultures.

Samson,R.A.,Hoekstra,E.S.,Frisvad,J.C and Fitenberg,O (2000).Introduction to food and Air-borne Fungi. 6th ed. Baarn, The Netherlands.

U.S. Department of Agriculture (2003). Annual World Production Summary, Grains, http://www.usda.gov/ Agriculture crop production.

Watanabe, T. (2002). Pictorial Atlas of Soil and Seed Fungi. CRC Press, London,UK.486 p.

عزل وتشخيص الفطريات المصاحبة لانواع مختلفة من الحبوب المخزونة

الخلاصة

اجريت هذه الدراسة لمعرفة وتشخيص الفطريات المصاحبة لتسعة انواع مختلفة من الحبوب المخزونة في محافظة دهوك – اقليم كوردستان العراق باستخدام طريقتين للعزل (طريقة اطباق الاكار وطريقة الاوراق المبللة). تم خلال هذه الدراسة تسجيل 19 نوع تابع ل13 جنس واظهرت الدراسة اختلاف في انواع الفطريات المعزولة ونسبة التلوث اعتمادا على نوع الحبوب وطريقة العزل. تم تحديد إجمالي 19 نوعًا مخصصة لـ 13 جنسًا. كانت الأجناس المعزولة الأكثر شيوعًا هي *Aspergillus* (4 أنواع) ، *Penicillium*، *Alternaria* , *Fusarium* (نوعان) ، بينما تم تمثيل الأجناس الأخرى *Humicola* و *Bipolaris* و *Phoma Curvularia*, *Rhizopus*، *Cladosporium*، *Stemphylium* و *Ulocladium* فقط في نوع واحد. أظهرت النتائج تبايناً في الأنواع الفطرية ونسبة التلوث حسب طرق العزل التحضين. سجلت أقل نسبة تواجد في بذور العدس مع جنس واحد فقط يمثل Penicillium، بينما سجلت أعلى تلوث لبذور الحمص حيث تمثلت بستة أجناس. كانت الأجناس الفطرية الأكثر شيوعًا التي تم عزلها بطريقة الاكار في القمح والحمص هي .*Rhizoctonia* sp (34.1٪) و .*Penicillium* sp (42.5٪) على التوالي. سجلت أعلى نسبة تواجد فطرية باستخدام طريقة الاوراق المبللة لبذور الشعير بينما سجلت أقلها بذور الحمص. كانت الأجناس الفطرية الأكثر شيوعًا التي تم تسجيلها باستخدام طريقة الاوراق المبللة تحت درجة حرارة الغرفة بعد 7 أيام و 14 يومًا هي *Penicillium* spp بنسبة 100٪ من بذور الحمص تليها *Aspergillus* spp بنسبة (66.7٪ ، 47.6٪) من بذور الشعير والزبيب على التوالي. . بينما كان أعلى فطر متكرر في البذور المحتضنة عند 25 درجة مئوية بعد 7 و 14 يوم هو *Aspergillus parasiticus* (88.9٪) من بذور الهريس و *Rhizopus* sp (80.1٪) من الفاصوليا على التوالي. تعتبر طريقة الاوراق المبللة هي الاكثر فعالية والافضل اقتصاديا.