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RESEARCH ARTICLE

Investigation of mycotoxin Patulin in some types of dried fruits in Baghdad governorate

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Abstract

The results of mycotoxin Patulin (PAT) detection in 21 samples of dried fruits (grape and apricot) collected from different locations at Baghdad governarate and one sample from Karbala province revealed that all tested samples were contaminated with PAT ,samples K , D and C which collected from Karbala , Al-shoala and Palestine Street showed the highest contamination levels which recorded (30499.5, 2836.3 and 1795.3) µg/ml , while the samples I, S and G which collected from Al- Harthiya , Al- Sadr City and Al- Harthiya (2) showed the lowest contamination level which recorded (7.8 , 11.7 and 19.8) µg/ml. The results also showed that Aspergillus spp. recorded the high percentage of occurancy 100% in H sample and in samples (N , K , U , A , J) the percentage of occurancy is (94.12 , 94.44 , 95.45 , 95.56 , 96.55)% respectively. Also the percentage of Penicillium spp. occurancy rated from 0.0 to 46.15% , sample (H) recorded the lowest percentage of occurancy 0.0% while sample (M) recorded the highest percentage 46.15%. The results of toxigenic ability of 5 isolates include A.flavus and A.fumigatus and 3 isolates of P.expansum from three different samples include K , S and Z. Regarding A.flavus and A.fumigatus were not produced PAT, while P.expansum isolate from S and Z recorded the ability to produced PAT after 5 days , while P.expansum isolated from K produced PAT after 15 days. The highest ability to produce PAT recorded in S 16.5 µg/ml, and the lowest ability in K 13 µg/ml.

INTRODUCTION

Mycotoxins are natural secondary metabolites produce by microorganisms of kingdom fungi , commonly known as molds that are growing on agricultural commodities and have adverse effects on human , animals and crops , result in health and environmental threat beside economic losses (Richard , 2007) .More than 500 types of mycotoxins have been identified to present, which include the most commonly mycotoxins associated with food and feed that may be concern to consumer food safety: Aflatoxin , Ochratoxin A , Patulin and Trichothesences (Magan *et al*.,2004) .

Mycotoxigenic fungi may grow under certain limit climatic conditions to produce mycotoxins on any solid or liquid media support as soon as nutritional substances and moisture are present, hence the wide variety of contaminated foodstuff substrates are subjected to spoilage as a results of fungal growth. Natural occurrence of mycotoxins are found as natural contaminants in many feedstuffs including cereals, vegetables, fruits, oil seeds and foods consisting of or manufactured from, these products and intended for human or animal consumption (Abad *et al.* , 2002). Mycotoxins are highly toxic compounds of small molecular weight and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the food chain while keeping their toxic properties (Reddy *et al*., 2010).

Among the most important mycotoxins was (Patulin) PAT is a β unsaturated lactone and soluble in water and most polar organic solvents – soluble, produced by a number of fungal species belonging to the genera Penicillium ,

Aspergillus , Byssochlomys and Pacelomyces (Drusch *et al*., 2007). The main producer of PAT is the blue mold Penicillium expansum , which is considered as a wound pathogen (Lai *et al*.,2000). It is usually associated with fruits and vegetables especially in apple and apple products (Ritieni , 2003). PAT was first isolated as an antimicrobial active principle during 1940s from Penicillium griseofulvum , during the 1960s , PAT was reclassified as a mycotoxin which was toxic to both plants and animals (Andersen *et al*., 2004). The aim of the present study was to isolate and identify of PAT toxigenic fungi associated with dried fruits (grapes and apricots).

Materials and Methods

Isolation and Identification of Fungi :

Twenty grams of each sample was taken randomly cut in small pecies (0.5 cm) , surface sterilized with Sodium Hypochlorite solution for 2 min. and rinsed twice with sterilized distilled water. Samples were dried with sterile filter paper , cultured on Potato Dextrose Agar (PDA) plates supplemented with antibiotic (Chloramphenicol) in concentration 100 µg/ml. Three pieces in each plate , each replicate has 3 plates and each sample triplicated , incubated for 5 – 7 days at 25℃ . Growing of fungus colonies on dried fruits were sub-cultured by transferring a small mycelia plugs from the colony margins. Pure culture was obtained by sub-culturing many times then identified on the basis of their morphological characters by observing colony feature (colony and texture) and microscopically by staining with lactophenol cotton blue and observe under microscope for the conidia , conidiophores and arrangement of spores , the fungi were identified and classified depended on taxonomic keys (Raper and Fennell,1965; Simmons , 1967) , the percentage of occurancy and frequency of isolation to each isolated fungal genus and species were calculated according to the following formula:-

% occurrence of Genus = (colonies number of genus)/(total number of genera colonies)× 100

% occurrence of species = (colonies number of species)/ (total number of species colonies)× 100

% frequency of Genus =(Number of genus appearance in the sample)/ (total number of the genera appearance)×100

%frequency of species = (Number of species appearance in the sample)/ (total number of the species appearance)×100

Detection of Patulin in Dried apricot and grape Fruits

The method described by (MacDonald *et al*.,2000) with some modification in the detection of PAT in dried apricot and dried grape collected samples was carried out as following :-

1-Fifty grams of each sample of the dried fruit (apricot and dried grape) was weighted and soaked with 100 ml distilled water and left for 24 hours then blended by electric blender for 2 min.

2-The mixture was filtered using medical sterile gauze.

3-Ten ml of the filtrate was transferred to a separating funnel, 20 ml Chloroform was added then shacked for 10 min.

4-The top layer of chloroform was filtered through a bed of anhydrous Sodium Sulphate (Na2So4) and evaporated using rotary evaporator at 45℃ .

5-One ml of acetonitrile was added to extract then filtered by Millipore filters (0.45 µm) and collection in small tube specific to HPLC

6-0.2 microliter of filtrate was injected in HPLC device under U.V. Light at a wave length of (267 nm), with determination of Rotation time (RT) was 4 minutes .

7-The amount of PAT was estimated in comparison with standard PAT through the following formula (EEC, 1992)

PAT concentration = (Peak area of sample)/(Peak area of standard) ×Standard concentration.

Detection of Patulin Toxigenic Fungi

Glucose-Czapek`s apple medium (GCA) (Hasan, 2000) was used to stimulate PAT toxigenic fungi, conical flasks containing 50 ml of sterilized GCA inoculated with two discs (5 µm diameter) of 7 days old culture grown on PDA , with three replicates for each treatment , flasks were incubated at temperatures (15)℃ for three incubation periods (5 , 10 , 15) days in order to select the optimum temperature and incubation periods for production of PAT. Fungal biomass was separated by filtration through Whatmann No.1 filter paper , then the filtrate was refiltered through a Millipore filter (0.45 µm) to remove fungal spore , 10 ml of filter was washed with 20 ml of chloroform in separating funnel , shaken for ten min. the top layer of chloroform was filtrated then reduced at 45℃ then remained residue was dissolved in 1ml acetonitrile and kept in a deep freeze until used for PAT detection. PAT concentration was detected as mentioned in the same steps in (3-4-5).

Results and Discussion

Isolation and Identification of Fungi associated with Dried Apricot and Grape Fruits

The results of isolation and identification of fungi associated with 21 samples of dried apricot (12) and grape (9) , fruits showed that the number of fungal genera and species isolated, was varied in the percentage of occurancy and frequency of isolation according to sample kind and the local of collected samples (Table 1 and 2) , results of isolation and identification of fungi associated with 9 samples of dried grape fruit showed the isolation of *Aspergllus* spp. and *Pencillium* spp. with clear variation in the occurancy and frequency of isolation (Table. 6). The genus *Aspergillus* spp. was the highest occurancy fungus ranged from 64.71 % to 100 %. Samples H and J recorded the highest percentage of occurancy in 100 % and 96.55 % respectively. The lowest percentage of occurancy were 64.71 % and 69.23 % which recorded by Z and B respectively. The next genus was *Penicillium* spp. with occurancy ranged from 0.0 to 35.29 %. The highest percentage of occurancy recorded by sample Z 35.29 % , while the lowest percentage of occurancy 0.0% recorded by sample H .

Regarding fungal species *Aspergillus niger* recorded the highest percentage of occurancy 93.33 % in sample L , while the lowest percentage of occurancy recorded by sample Z 47.05 %. The next fungal species was *Aspergillus flavus* which recorded percentage of occurancy ranged from 2.22 to 20.45 % and the highest percentage 20.45 % recorded by sample H and the lowest percentage of occurancy recorded by sample A 2.22 %, followed by the species *Aspergillus fumigatus* which recorded a percentage of occurancy ranged from 8.33 to 17.65 % , the highest percentage 17.65 % recorded by sample Z , while the lowest percentage of occurancy recorded by sample K 8.33.%

Regarding *Penicillium* species , only *P.expansum* was recorded with the percentage of occurancy ranged from 0.0 to 35.29 % , the highest percentage 35.29 % recorded in sample Z and the lowest percentage of occurancy recorded by sample H 0.0.%

At the level of the fungal genus *Aspergillus* was the highest frequency isolated with percentage of 100% in the sample A,B,G,H,J,K,Land T , followed by the genus *Penicillium* which recorded the highest frequency in sample T 66.67 %.

Table 1. The percentage of fungi that associated with dried grape fruit.

Samples	Type of Fungi	Occurancy of Genus	Occurancy of Species	Frequency of Genus	Frequency of Species
	***Aspergillus* spp.**	**95.56**		**100**	
	A.niger		**77.78**		**44.45**
A	***A.flavus***		**2.22**		**11.11**
	A.fumigatus		**15.56**		**44.44**
	***Pencillium* spp.**	**4.44**		**22.22**	
	P.expansum		**4.44**		**22.22**
	***Aspergillus* spp.**	**69.23**		**100**	
B	***A.niger***		**61.54**		**77.78**
	A.flavus		**7.69**		**22.22**
	***Pencillium* spp.**	**30.77**		**11.11**	
	P.expansum		**30.77**		**11.11**
	***Aspergillus* spp.**	**86.36**		**100**	
G	***A.niger***		**86.36**		**100**
	***Pencillium* spp.**	**13.64**		**22.22**	
	p.expansum		**13.64**		**22.22**
	***Aspergillus* spp.**	**100**		**100**	
H	***A.niger***		**79.55**		**55.56**
	A.flavus		**20.45**		**44.44**
	***Aspergillus* spp.**	**96.55**		**100**	
J	***A.niger***		**86.21**		**77.78**
	A.flavus		**10.34**		**22.22**
	***Pencillium* spp.**	**3.45**		**11.11**	
	P.expansum		**3.45**		**11.11**
	***Aspergillus* spp.**	**94.44**		**100**	

	A.niger		**66.67**		**44.44**
	A.flavus		**19.44**		**33.33**
K	***A.fumigatus***		**8.33**		**22.23**
	***Pencillium* spp.**	**5.56**		**22.22**	
	P.expansum		**5.56**		**22.22**
	***Aspergillus* spp.**	**93.33**		**100**	
L	***A.niger***		**93.33**		**100**
	***Pencillium* spp.**	**6.67**		**22.22**	
	P.expansum		**6.67**		**22.22**
	***Aspergillus* spp.**	**77.14**		**100**	
T	***A.niger***		**77.14**		**100**
	***Pencillium* spp.**	**22.86**		**66.67**	
	P.expansum		**22.86**		**66.67**
	***Aspergillus* spp.**	**64.71**		**88.89**	
	A.niger		**47.05**		**66.67**
Z	***A.fumigatus***		**17.65**		**22.22**
	***Pencillium* spp.**	**35.29**		**33.33**	
	P.expansum		**35.29**		**33.33**

Regarding Isolation and Identification of fungi from dried apricot fruit (Table 2) , the results showed that the genus *Aspergillus* spp. was the highest occurancy fungus ranged from 53.85 to 95.45 %. Samples U and N recorded the highest percentage of occurancy in 95.45 % and 94.12 % respectively . The lowest percentage of occurancy were 53.85 % and 54.55 % which recorded by M and D respectively. The next genus was *Penicillium* spp. with occurancy ranged from 4.55 to 46.15 %. The highest percentage of occurancy recorded by sample M 46.15 % , while the lowest percentage of occurancy 4.55 % recorded by sample U.

Regarding fungal species *Aspergillus niger* recorded the highest percentage of occurancy 80% in sample S , while the lowest percentage of occurancy recorded by sample E 6.25 %. The next fungal species was *Aspergillus flavus* which recorded percentage of occurancy ranged from 5.56 to 30.77 % and the highest percentage 30.77 % recorded by sample P and the lowest percentage of occurancy recorded by sample C 5.56 %, followed by the species *Aspergillus fumigatus* which recorded a percentage of occurancy ranged from 5.56 to 62.5 % , the highest percentage 62.5 % recorded by sample E , while the lowest percentage of occurancy recorded by sample C 5.56.%

Regarding *Penicillium* species , only *P.expansum* was recorded with the percentage of occurancy ranged from 4.55 to 46.15 % , the highest percentage 46.15 % recorded in sample M and the lowest percentage of occurancy recorded by sample U 4.55 %. At the level of the fungal genus Aspergillus was the highest frequency isolated with percentage of 100% in the sample D and N , followed by the genus of the *Penicillium* which recorded the highest frequency in sample M and P 33.33.% .

Table 7.The percentage of fungi that associated with dried apricot fruit.

Samples	Type of Fungi	%Occurancy of Genus	%Occurancy of Species	%Frequency of Genus	%Frequency of Species
	***Aspergillus* spp.**	**61.11**		**77.81**	
C	***A.niger***		**50**		**55.59**
	A.flavus		**5.56**		**11.11**
	A.fumigatus		**5.56**		**11.11**
	***Pencillium* spp.**	**38.89**		**22.22**	
	P.expansum		**38.89**		**22.22**
	***Aspergillus* spp.**	**54.55**		**100**	
D	***A.niger***		**31.82**		**44.45**
	A.flavus		**9.09**		**22.22**
	A.fumigatus		**13.64**		**33.33**
	***Pencillium* spp.**	**45.45**		**11.11**	
	P.expansum		**45.45**		**11.11**
	***Aspergillus* spp.**	**68.75**		**77.78**	
E	***A.niger***		**6.25**		**11.11**
	A.fumigatus		**62.5**		**66.67**
	***Pencillium* spp.**	**31.25**		**22.22**	
	P.expansum		**31.25**		**22.22**
	***Aspergillus* spp.**	**81.81**		**88.89**	
F	***A.niger***		**36.36**		**33.33**
	A.fumigatus		**45.45**		**55.56**
	***Pencillium* spp.**	**18.19**		**22.22**	
	P.expansum		**18.19**		**22.22**
	***Aspergillus* spp.**	**53.85**		**88.89**	
	A.niger		**41.03**		**44.45**
M	***A.flavus***		**12.82**		**44.44**
	***Pencillium* spp.**	**46.15**		**33.33**	
	P.expansum		**46.15**		**33.33**
	***Aspergillus* spp.**	**94.12**		**100**	
	A.niger		**70.59**		**55.56**
N	***A.flavus***		**17.65**		**33.33**
	A.fumigatus		**5.88**		**11.11**
	***Pencillium* spp.**	**5.88**		**11.11**	
	P.expansum		**5.88**		**11.11**
	***Aspergillus* spp.**	**90**		**88.89**	
	A.niger		**80**		**77.78**
S	***A.flavus***		**10**		**11.11**
	***Pencillium* spp.**	**10**		**11.11**	
	P.expansum		**10**		**11.11**
	***Aspergillus* spp.**	**95.45**		**88.89**	
	A.niger		**68.18**		**44.44**
U	***A.fumigatus***		**9.09**		**22.22**
	A.flavus		**18.18**		**22.23**
	***Pencillium* spp.**	**4.55**		**11.11**	
	P.expansum		**4.55**		**11.11**
	***Aspergillus* spp.**	**72.97**		**88.89**	

	A.niger		**51.35**		**66.66**
R	***A.flavus***		**21.62**		**22.23**
	***Pencillium* spp.**	**27.03**		**22.22**	
	P.expansum		**27.03**		**22.22**
	***Aspergillus* spp.**	**80**		**88.89**	
Q	***A.niger***		**66.67**		**66.67**
	A.flavus		**13.33**		**22.22**
	***Pencillium* spp.**	**20**		**22.22**	
	P.expansum		**20**		**22.22**
	***Aspergillus* spp.**	**59.26**		**88.89**	
	A.niger		**59.26**		**88.89**
I	***Pencillium* spp.**	**40.74**		**22.22**	
	p.expansum		**40.74**		**22.22**
	***Aspergillus* spp.**	**69.23**		**77.78**	
	A.niger		**38.46**		**44.44**
P	***A.flavus***		**30.77**		**33.34**
	***Pencillium* spp.**	**30.77**		**33.33**	
	P.expansum		**30.77**		**33.33**

In dried grape sample H showed 100% occurancy for the fungus *Aspergillus* spp. and this might be return to saprophytic nature (live upon dead or decaying organic matter) of this genus beside it closely associated with agriculture and other human activities that make nutrients available to this highly competitive fungus . Also contamination reason may be due to bad storage conditions. It has been proven that high temperatures to more than 37℃ makes the genus of Aspergillus more predominate and this was confirmed by (Valero *et al*., 2005) . On the other hand numbers of *Aspergillus* spp. this genus possess the ability to grow will at a high osmotic concentrations (high sugar, salt, etc.) exists ,in addition to the colonies of genus *Aspergillus* were present on the berry skin from fruit setting and increase in amount from early variation to harvest, with a peak at ripening; however the incidence of colonized berries was highly related to climatic conditions during the ripening stage and to the geographical location(Visconti *et al*. 2008 ; Cozzi *et al.,* 2009).

The highly isolated frequency of *Aspergillus flavus* was due to the saprophytic nature of this fungus and its ability to utilize a wide range of nutrient sources. *A. flavus* has a capacity to produce a large array of enzymes to support biodegradation process of complex compounds. Indeed, when substrate utilization by *A. flavus* in compared to obligate pathogens, *A. flavus* was also found to have greater capacity for growth on both complex protein substrates (elastin and mucin) and complex carbohydrate substrates (Abdullah *et al.,* 2009).

The last recorded species belonged to the genus Aspergillus was *A. fumigatus* which it appeared in some samples, the reason of its present of *A.fumigatus* returned to its saprotrophic widespread in nature, it was typically found in soil and decaying organic compounds , such as compost heaps, where it played an essential role in carbon and nitrogen recycling and colonies of the fungus produce from conidiophores , although *A. fumigatus* occurs in areas with widely different climates and environments, the fungus was capable of growth at temperature 37℃ and can grow at temperatures up to 50℃ , with conidia surviving at temperature 70℃ conditions, it also regularly encounters in self-heating compost heaps. Its spores were found everywhere in the atmosphere (O'Gorman *et al*., 2008).

The second genus appeared in the tested samples was *Pencillium* spp. and only the species *P.expansum* was recorded, this due to the ability of this fungus to grow on grains and other stored foods rely on their propensity to grow in low humidity and colonize rapidly by aerial dispersion while the seeds were sufficiently moist (Pitt *et al.* 2000).

Some species of *Penicillium* affect the fruits and bulbs of plants, including P. expansum (Balgrie, 2003). *Penicillium* species were present in the air and dust of indoor environments, such as homes and public buildings (Larous *et al*., 2007).

*Penicillium expansu*m was a post harvest pathogen that affects number of different hosts, including some fruits such as apples, pears and cherries . The certain temperature to grow P.expansum essentially starts at optimum temperature range from 15-27 ℃ ,while some growth was still exhibited at temperatures lower and higher than this range but growth was much slower outside of this temperature range (Larous *et al.,* 2007).

Detection of PAT in Dried Grape and Apricot Fruits

The results of the detection of PAT in 21 samples of dried grape and apricot fruits , using HPLC , revealed the presence of PAT in all tested samples although the concentration was varied according to kind of sample and location of collection . (Table 3 and 4).

The results showed that all the samples of dried apricot fruit were PAT contaminated in range from (7.8 - 2836.3) µg/ml. Sample D collected from Al-shoala was showed the highest concentration level (2836.3) µg/ml followed by C collected from Palestine Street which recorded (1795.3)µg/ml . While sample I showed the lowest concentration level (7.8) µg/ml.

Table 3. The Quantitative estimation of PAT in dried apricot fruit sam ples collected from various local market in Baghdad and Karbala province

Samples	Location	Concentration of PAT. µg/ml
C	Palestine Street	1795.3
D	Al-shoala	2836.3
E	al-baladiat	187.7
F	Bab-almoazam	37.6
M	Karbala	47.1
N	Al- Utaifiyya	273.2
I	Al- Harthiya	7.8
Q	Al- Alawi	25.90
R	Al-shaab	388.9
U	Baghdadal jadeeda	299.3
P	Al- Kadhimiya	907.1
S	Al- Sadr City	11.7

The reason of low concentration of PAT in samples I and S might be due to good storage conditions and ventilation of dried apricots, and suitable temperatures and humidity, on the other hand the highest concentration of PAT was recorded in sample collected from Al-shoala 2836.3 µg/ml, might be due to the suitable storage conditions for fungus grow and production of PAT ,Trucksess and Scott , (2007) found that the fungal contamination of these products, was highly effected by sorting, storage, and processing.

Regarding dried grape fruits samples , the results showed that all tested samples were PAT contaminated in range from 19.8 to 30499.1 µg/ml, (Table.4) , the contamination was found to be dependent on the location of collection sample and fungal contamination. Sample K collected from Karbala was showed the highest concentration level (30499.1) µg/ml, followed by J which recorded (980.5) µg/ml. While sample G showed the lowest concentration level (19.8) µg/ml.

Table 4.The Quantitative estimation of PAT in dried grape fruit samples collected from various local market in Baghdad and one samples from Karbala province

Samples	Location	Concentration of PAT. µg/ml
A	Palestine Street	446.5
B	Baghdadal jadeeda	110.3
G	Al- Harthiya	19.8
H	Al-shaab	714
J	Al- Alawi	980.5
K	Karbala	30499.5
L	Al-shoala	130.3
T	Al- Kadhimiya	58.1
Z	Al- Utaifiyya	79

References

- **Abad, E.; Llerena, J.J.; Saulo, J.; Caixach, J. and Rivera, J. 2002**. Comprehensive study on dioxin contents in binder and anti-caking agent feed additives. Chemosphere, 46, 1417-1421.
- **Abdullah, S.K. and Al-Mousawi, K.A. 2009.** Incidence of *Aspergillus* in seeds of corn and sunflower cultivars grown in Iraq and aflatoxin-producing potential of *Aspergillus* section *Flavi*. Proc. 1st Scient. Conf. Biol. Sci. Mosul University, pp. 299-307.
- **Andersen, B., Smedsgaard, J. and Frisvad, J.C. 2004**. *Penicillium expansum*: consistent production of patulin, chaetoglobosins, and other secondary metabolites in culture and their natural occurrence in fruit products. J. Agricult. Food Chem., 52, 2421–2428.
- **Balgrie, B. 2003.** "Taints and Off-flavours in Food". CRC Press. p. 134.
- **Cozzi, G.; Haidukowski, M.; Perrone, G.; Visconti, A. and Logrieco, A. 2009.** Influence of *Lobesia botrana* field control on black aspergilli rot and ochratoxin A contamination in grapes. Journal of Food Protection 72 (4), 894–897.
- **Drusch, S.; Kopka , S. and Kaeding , J. 2007.** Stability of Patulin in a juice – like aqueous model system in the presence of ascorbic acid, food chemistry , 100 (1) : 192 – 197 .
- **EEC, .1992.**Official Journal of the European communies.L327/54 .13.11 .92.
- **Hasan, H. A. H. 2000**. Patulin and aflatoxin in brown rot lesion of apple fruits and their regulation. World Journal of Microbiology and Biotechnology, 16:607-612.
- **Lai, L.; Fuh , M. and Shih, C. 2000 .** Detection of mycotoxin Patulin in apple juice. J. Food and Drug Analysis, 8, P:85-96.
- **Larous, L.; Handel, N.; Abood, J.K. and Ghoul, M. 2007.** "The growth and production of Patulin mycotoxin by *Penicillium expansum* on apple fruits and its control by the use of propionic acid and sodium benzoate". Department of Biology, College of Science, University of Setiff. Setiff, Algeria.
- **MacDonald, S.; Long, M.; Gilbert, J.; and Felgueiras, I. 2000.** Liquid chromatographic method for determination of Patulin in clear and cloudy apple juices and apple puree: collaborative study. Journal of AOAC International, 83, P:1387-1394.
- **Magan , N. ; Aldred , D. and Sanchis , V. 2004.** The role of spoilage fungi in seed deterioration in: Arora , D.K. (eds.), Fungal Biotechnology in Agricultural , Food and Environmental Applications, P: 311-123.
- **O'Gorman, C.M. ; Hubert, T. F. and Paul, S. D. 2008.** "Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*". Nature , 457 (7228) , P: 471–4.
- **Pitt, J.I.; Basílico, J.C.; Abarca, M.L.; López, C.; Basílico; Abarca and López 2000.** "Mycotoxins and toxigenic fungi". Medical Mycology, 38 (vol. 1): P: 41–46.
- **Raper, K.B. and Fennell, D.I. 1965**. The genus *Aspergillus.* Williams and Wilkins Company.
- **Reddy, K.R.N.; Nurdijati, S.B. and Salleh, B. 2010 .** An overview of plant-derived products on control of mycotoxigenic fungi and mycotoxins. Asian Journal of Plant Science, Vol. 9, P: 126-133.
- **Richard, J.L. 2007.** "Some major mycotoxins and their mycotoxicoses—an overview". Int. J. Food Microbiol., 119 (1–2), P: 3–10.
- **Ritieni , A. 2003 .** Patulin in Italian commercial apple products. Journal of Agricultural and food chemistry , 51 (21), P:6086 – 6090 .
- **Simmons,E.G. 1967**.Typification of *Alternaria*,*Strephyllium* and *Ulocladium* . Mycological .59, P:67 -92.
- **Trucksess , M. W. and Scott , P. M. 2007 .** Mycotoxins in botanicals and dried fruits: A review. Food Additives and Contaminants, 25(2), P: 181–192.
- **Valero A. ; Marín, S. ; Ramos, A.J. and Sanchis, V. 2005.** Ochratoxin A-producing species in grapes and sun-dried grapes and their relation to ecophysiological factors. Letters in Applied Microbiology, 41, P:196–201.
- **Visconti A.; Perrone, G. ; Cozzi, G. and Solfrizzo, M.2008.** Managing ochratoxin A risk in the grape-wine food chain. Food Additives and Contaminants, Part A, 25 (2), P:193–202.