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COMPARISON BETWEEN THE MOST FREQUENT FUNGAL SPECIES COLONIZING GRAPEVINE BERRIES AT DIFFERENT MATURATION STAGES

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ABSTRACT

The study was undertaken to investigate the most frequent fungal species present on the surface of grape berries at different maturation at vineyards from Duhok governorate, Kurdistan region of Iraq. The associated fungi were detected both before and after surface disinfection on Dichloran Rose Bengal Chlormophenicol agar (DRBC) medium. The most abundant genera and species found on all the three stages of maturation by decreasing orders were *Aspergillus* (*A.niger, A.carbonarius, A.tubingensis*), *Cladosporium cladosporoides, Penicillium* (*P.brevicompactum,P.citrinum,P.glabrum*), *Alternaria alternata* and *Aureobasidium pullalans.* This result support the theory that risk of ochratoxin A contamination can be defined based on the mycobiota present on surface of healthy grape berries.

Keywords: *Grapes, Fungi, Iraq.*

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a crop of major economic importance used for the production of table and wine grapes, raisins, juices and vinegar. The grape cultivation area in Iraq is about 48 000 hectares with production of about 265 000 tons (FAO, 2003). Duhok province in Kurdistan Region of Iraq is a major area in Iraq for cultivation and production of grapevine.

Fungal conidia are usually present on berry skins from setting and increase in number from early version to harvest, with a peak at ripening (Serra *et al.*, 2006). Black aspergilli overwinter in soil and frequent soil cultivation can favor fungal infection in vineyard. The severity of *Aspergillus* rot is influenced by excessive irrigation prior to ripening, which causes berry splitting. Rain prior to ripening which causes berry splitting favoring *Aspergillus* infection. Berry damage caused by insects, birds or other fungal infection is the primary factor affecting disease development and ochratoxin A accumulation in berries (Cozzi *et al.*, 2006; Leong *et al.*, 2006).

Black aspergilli and other moulds are found on the surface of healthy grapes at all stages of maturation and are responsible for post-harvest decay of fresh fruits (Snowdon, 1990; Serra *et al.*, 2003, 2006). Black aspergilli are also the main source of Ochratoxin A contamination of grapes and grapevine products (Leong *et al.*, 2007).

The interior of grape berry is sterile, comprising fleshy mesocarp tissue (pulp) and seeds. The berry exocarp (skin) is the primary barrier to fungal infection, consisting of a waxy cuticle, and epidermal and sub-epidermal cells. The skin can be damaged by disease, pests and environmental conditions resulting in the splitting of the fruit (Amerine *et al.*, 1972). Grapes contain high levels of sugars and other nutrients, and although they possess ideal water activity for microbial growth, their acidic pH makes them particularly susceptible to fungal spoilage, because a large bacterial component is eliminated since most bacteria prefer near neutral pH.

Several factors influence fungal colonization of grapes. Climatic conditions were found to have a significant effect. OTA contamination of grapes and wines were found to vary from year to year even in the same vineyard (Rousseau, 2003).

The aim of the present study was to investigate the most frequent fungal species present on the surface of healthy berries at different maturation stages at vineyard from Duhok governorate.

MATERIALS AND METHODS

Grapes (Rashmew cultivar) were collected from June to September 2010 at three developmental stages of the berries. The maturation stages corresponding to pea berry (June/early July), early version (late July/

August) and ripe berry or harvest time (late August/ September).

At each sampling time in each vineyard, 20 bunches were collected by following the two diagonals (along two crossing diagonals transects). The samples were taken to the laboratory in closed paper bags, transported in cooled boxes and analyzed in the shortest time possible, usually within 24hours of collection.

Isolation from grape fruits samples:-

From each stage and from each sample 60 berries were randomly selected, and 30 of them were surface disinfected with sodium hypochlorite 2% for 1 min., then washed twice with sterile distilled water as described by Pitt and Hocking (1997), the other 30 berries were left without surface disinfection Ten berries from each of surface disinfected and non-disinfected lots were put per each plate containing Dichloran Rose Bengal Chloromphenicol Agar medium (DRBC) (Fluka-Germany). The plates were incubated at 25ºC for 7 days.

Identification of fungi:

Samples from soil, fresh berries and dried vine fruits were examined daily with the help of stereomicroscope for sporulating fungi. Pure colonies were established on appropriate media for identification. Majority of detected species were identified to species level based on morphological and cultural characteristics. Fungi other than the genera *Aspergillus* and *Penicillium* were identified according to the manuals of Domsch *et al.*, 1980 and Pitt and Hocking, 1997.

For identification of species in the genera *Aspergillus* and *Penicillium*, pure colonies were grown on four media according to Klich (2002) and Samson *et al.*, (2000). The media are as follows: Czapeck Yeast Extract Agar incubated for seven days at 25C˚ (CYA25), Czapeck Yeast Extract Agar incubated for seven days at 37C˚ (CYA37), Czapeck Yeast Extract Agar with 20% Sucrose incubated for seven days at 25C˚ (CY20S), Malt Extract Agar (MEA) incubated for seven days at 25C˚.

Ingredients and preparation of the above five media were mentioned in Klich (2002), Pitt and Hocking (1997). Each medium was supplemented with 50mg / ml chlorophenicol (SDI) to suppress bacterial growth. For each culture four plates were used, two of CYA and one each of CY20S, MEA. Each plate is inoculated at the center and incubated in the dark for seven days. One CYA is incubated at 37C˚. The rest are incubated at 25C˚.

All species identifications were according to the keys and descriptions provided by Klich (2002), Samson *et al.*, (2004) ; Frisvad and Samson; (2004) ; Abarca *et al.*, (2004) ; Samson *et al.*, (2007) .

Statistical analysis*:*

The data were expressed as mean ± standard error (mean ± S.E) and analyzed using an analysis of variance (ANOVA) followed by multiple mean comparisons. The data obtained from this study were converted to arcsine and analyzed using SAS program and means were compared using Duncan's multiple range test (SAS Institute Inc., Gary, NC, USA, 1999).

RESULTS

The most frequent genera detected from disinfected and non-disinfected berries at different maturation stages were *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium* and *Penicillium.*

Table (1) and Fig. (1) showed that there is no significance difference (P<0.05) in colonization of *Aureobasidium* at the pea berry stage compared to other stages.

In *Alternaria*, the colonization rate showed a *significance* difference (P<0.05) between the three maturation stages.

In *Cladosporium*, there is a significance differences between the three maturation stages at (P<0.05) in non-disinfected berries, whereas, in disinfected berries, colonization rate at harvest significantly different from the other two maturation stages.

Table 1. Comparison between genera isolated from grape at different maturation stages.

	Aureobasidium **disinfectant.**	***Aureobasidium*** **non disinfectant**	***Alternaria*** **disinfectant**	***Aternaria*** **non disinfectant**	***Cladosporium*** **disinfectant**	***Cladosporium*** **non disinfectant**
Pea berry	2.667±0.440[a]	4.000±0.342[a]	5.567±0.483[a]	6.267±0.744[a]	5.167±0.563[a]	6.367±0.816[a]
Early verasion	3.067±0.383[a]	5.500±0.569[b]	6.967±0.543[b]	8.000±0.681[b]	5.467±0.488[a]	7.667±0.675[b]
Harvesting	2.167±0.304[a]	4.333±0.251[b]	3.700±0.346[c]	4.000±0.437[c]	2.967±0.379[b]	3.533±0.439[c]

- Data presented as mean ± S.E.
- The same letters mean no statistical differences.
- The different letters mean statistical differences.

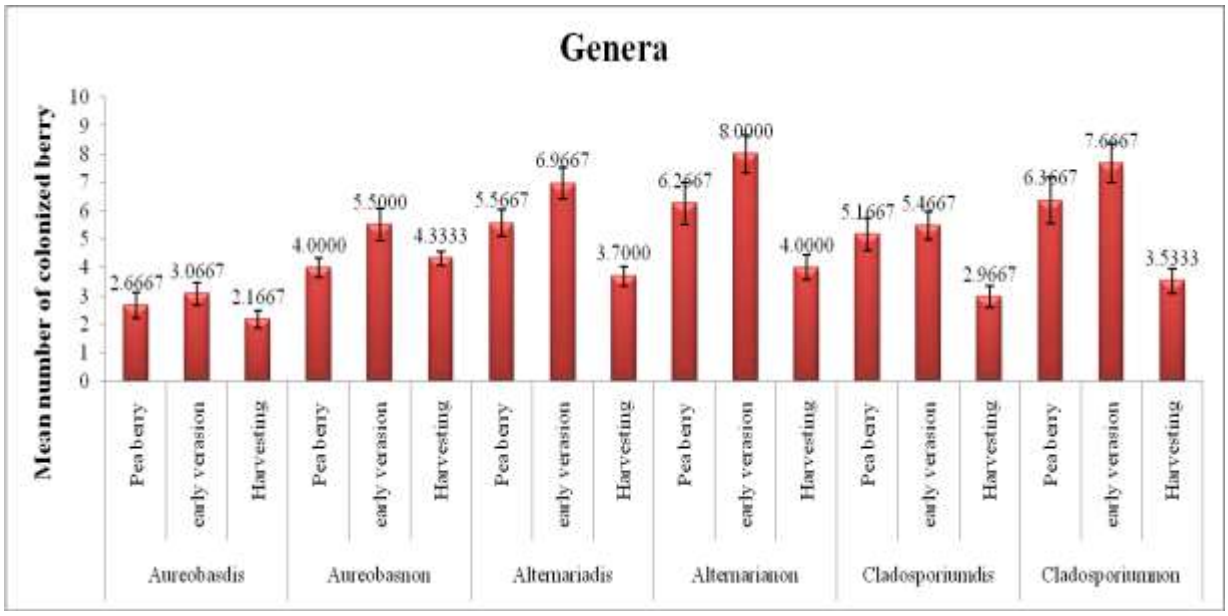

Figure1.Comparison between genera isolated from grape at different maturation stages

The most three abundant *Aspergillus* species, *A.carbonarius, A.niger* and *A.tubingensis* were detected from disinfected and non – disinfected grapevine berries at the three maturation stages(Table 2 and Fig. (2). Colonization rates of *A.carbonarius* in pea berry showed a significance differences at (P<0.05) from the other two stages of maturations in both disinfected at non-disinfected berries.

In *A.niger*, pea berry and early version stages showed a significant difference from harvest stage in disinfected berries, whereas, pea berry different significantly from early version and harvest stages in non- disinfected berries.

In *A.tubingensis*, there is a significant difference at (P<0.05) between pea berry and the two other maturation stages.

The most three dominant *Penicillium* species detected in both disinfected and non-disinfectant berries at three maturation stages were *P.brevicompactum*, *P. citrinum* and *P. glabrum* (Table 3and Figure 3).

There is no significant differences at (P<0.05) for colonization rate by *P.brevicompactum*, *P. citrinum* and *P. glabrum* on the disinfected berries between the three maturation stages ,whereas there is a significant differences between non-disinfectant berries colonized by *P. brevicompactum* and *P. citrinum* at the pea berry stage with both early version at harvesting .Moreover, there is no significant differences at (P<0.05) for colonization rate between non-disinfectant berries colonized by *P.glabrum* at the pea berry stage and early verasion, whereas both stages differ significantly from harvesting stages.

Table 2.Comparision between *Aspergillus* species during three maturation stages of grape.

	***A.niger* disinfictant**	***A.carbonarius* non disinfictant**	***A.carbonarius* disinfictant**	***A.niger* non disinfictant**	***A.tubingensis* disinfectant**	***A.tubingensis* non-disinfectant**
Pea berry	3.533±0.403[a]	6.333±0.728[a]	3.067±0.383[a]	7.000±0.650[a]	3.067±0.383[a]	4.000±0.342[a]
Early verasion	4.133±0.728[a]	7.667±0.448a[b]	3.667±0.353a[b]	8.067±0.383a[b]	3.233±0.364[b]	6.667±0.461[b]
Harvesting	6.333±0.342[b]	8.267±0.643[b]	4.500±0.302[b]	9.067±0.514[b]	2.967±0.370[b]	6.267±0.503[b]

- Data presented as mean ± S.E.
- The same letters mean no statistical differences.
- The different letters mean statistical differences

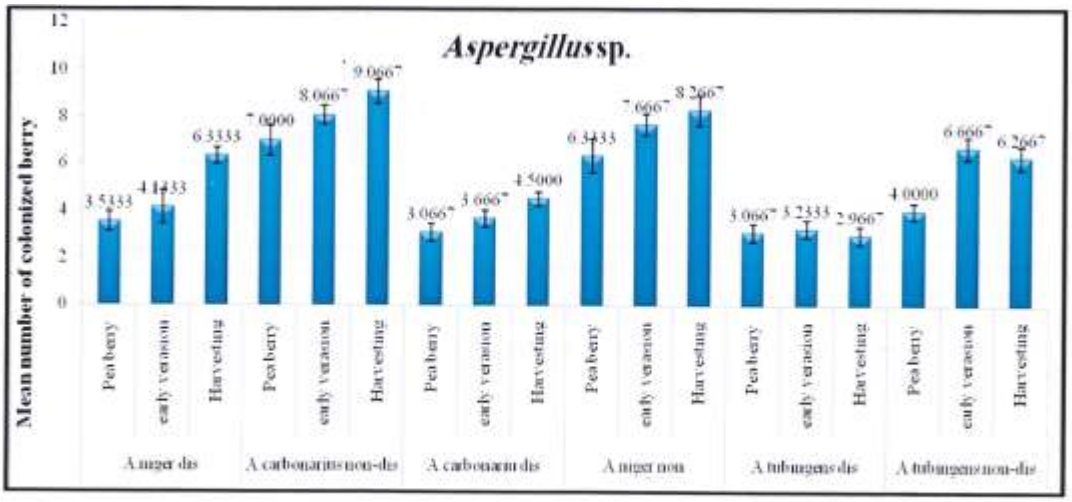

Figure 2 Comparisons between *Aspergillus* species during three maturation stages of grape.

Table 3. comparision between *Penicillium* species during three maturation stages of grape.

	***P.brevicompactum* disinfectant**	***P.brevicompactum* non-disinfectant**	***P.citrinum* disinfctant**	***P.citrinum* non disinfictant**	***P.glab*rum disinfectant**	***P.glabrum* non-disinfectant**
Pea berry	2.667±0.440[a]	3.067±0.383[a]	2.300±0.259[a]	2.667±0.347[a]	1.833±0.209[a]	2.767±0.164[a]
Early verasion	3.067±0.383[a]	4.400±0.306[b]	2.467±0.398[a]	3.833±0.339[b]	2.167±0.235[a]	3.300±0.199[a]
Harvesting	3.667±0.399[a]	4.833±0.365[b]	2.967±0.370[a]	4.333±0.305[b]	2.467±0.398[a]	4.033±0.344[b]

- Data presented as mean ± S.E.
- The same letters mean no statistical differences.
- The different letters mean statistical differences.

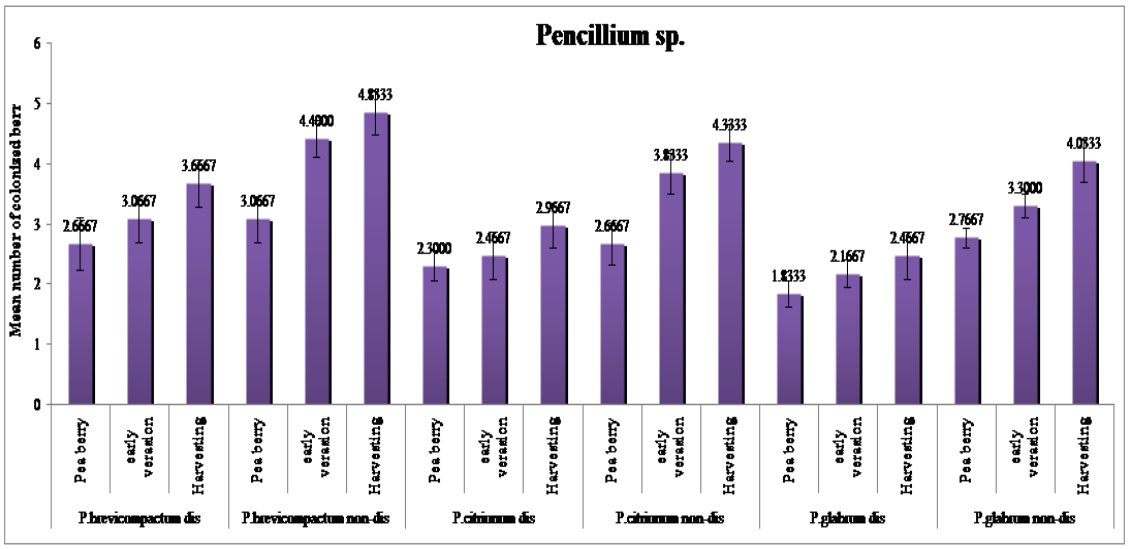

Figure 3.Comparisons between *Penicillium* species during three maturation stages of grape.

DISCUSSION

The most abundant genera found on all the three different stages of maturation by decreasing order were *Aspergillus, Cladosporium, Penicillium, Alternaria* and *Aureobasidium***.** These fungi are well known as field fungi which occur commonly in the air, plant surfaces, plant debris and soil.

Serra *et al.,* (2005) reported *Cladosporium* , *Alternaria* ,*Botrytis* , *Penicillium* and *Aspergillus* as the five most abundant genera found on grape berries in Portugal. They attributed the increase in colonization rates for the active spoilage agents such as *Aspergillus* and *Penicillium* , because conditions are more favorable for fungal invasion at harvest time ,when more damage to the berries is likely to occur. The majority of *Aspergillus* species belong to black aspergili (*Aspergillus* section *Nigri*). These include *A.carbonarius, A.niger* and A.*tubingensis.* Previous reports from different parts of the world, including Argentina and Brazil (De Rocha *et al.,* 2002; Magnoli *et al.,* 2003), and Europe (Serra *et al.,* 2005, 2006) supported our finding for predominance of black aspergilli on grape vine fruits.

Species of *Penicillium* showed high frequency of occurrence on different maturation stages were *P.brevicompactum, P.citrinum* and *P.glabrum.* The most frequent species of *Penicillium* reported on Portugies grapevine berries by Serra *et al.,* (2005) were *P.brevicompactum, P.thomi* and *P.glabrum*.

It is interesting to note that *Botrytis cinerea*, which is responsible for grey rot in grapes bunches, was not detected in our study .This is may be attributed to the dry and hot weather during grape vines berries maturation in our region.

Aureobasidium pullalans is anamorphic yeast – like fungus was also isolated from berries at different maturation stages with high colonization rate. The fungus is well known as a common inhabitant of the phylloplane of several plants .Recent studies indicated that some strains of *A.pullalans* used as biocontrol agents by preventing *A.carbonarius* infection to berries. Moreover, berries pretreated with the biocontrol agent and infected with OTA as compared to untreated infected control berries, the strains showed ability to degrade OTA to ochratoxin A in berries which is less toxic (De Felice *et al.,* 2008).

In conclusion,the presence of toxigenic fungi particularly black aspergilla on the skin of berries at different maturation stages will favor toxin production. The skin can be damaged by pests and environmental conditions resulting in the splitting of the fruit (Amerine *et al.*1972). Grapes contain high level of sugars and other nutrients; their acidic PH make them potentially susceptible for fungal spoilage and ochratoxin A contamination. Among black aspergilli , *A.carbonarius* is the most important as ochratoxin A producing isolates, followed by *A.niger* aggregate (Varga et al.2004).

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الخلاصة

اجريت هذه الدرسه لمعرفة اهم الانواع الفطرية المتواجدة علي سطح ثمار العنب لمراحل النضج المختلفة في محافظة دهوك اقليم كوردستان العراق عزلت الفطريات قبل و بعد تعقيم السطح التمار على وسط DRBC الاجناس والانواع الساعدة في جميع المراحل الثلاثة للنضج هي

Aspergillus (*A.niger, A.carbonarius, A.tubingensis*), *Cladosporium cladosporoides, Penicillium* (*P.brevicompactum , P.citrinum, P.glabrum*), *Alternaria alternata* and *Aureobasidium pullalans* دعمت هذة النتيجة النظرية القائلة يان التلوث بالسم الفطري ochratoxin A يحدد اعتمادا علي الانواع الفطرية الموجودة على سطح التمار السليمة.

پوختە

ئەڤ ڤەکولینە هاتنە کرن دا ڤەکولین لسەر هەبوونا کومێن کەرویالسەر بە رهەمێ تری د قوناغێن جورەجور دپێگەهشتێ دا ل پارێزگە ها دهوکێ، هەرێما کوردستانا عراقێ، ڤاڤارتنا کەروا هاتە کرن بەری و پشتی تاقیکرنا رەخورێت ئەوان ز پشتی هنگێ هاتەدانان زناڤ ئامانێن چاندنێ کو پێکهاتیە ژ DRBC **ئەو جورە گەزێن بە ربەڵاڤ د هەرسێ قوناغین پێگە هشتێدا ڤەدگرێن بو** *Aspergillus* (*A.niger, A.carbonarius, A.tubingensis*), *Cladosporium cladosporoides, Penicillium* (*P.brevicompactum ,P.citrinum,P.glabrum*), *Alternaria alternata* and *Aureobasidium pullalans* **ئەنجاما دیارکری ئەنجاما دیاری ئەو تیووریا دبێژیت پیسبوونا تری بژهرە کە روا** ochratoxin A **دهیتە دەست نیشان کرن ل جورێن کومەکە رویێن ل سە ر تریێ ساخلە م .**