

# BIODIVERSITY OF FILAMENTOUS AND YEAST FUNGI IN CITRUS AND GRAPE FRUITS AND JUICES IN ASSIUT AREA, EGYPT

Abdel-Aal Hassan Moubasher<sup>\*1,2</sup>, Mohamed Ahmed Abdel–Sater<sup>1,2</sup>, Zeinab. Soliman<sup>2</sup>

Address(es): Professor Abdel-Aal Hassan Moubasher,

1Assiut University, Faculty of Science, Department of Botany and Microbiology, P.O. Box 71516, Assiut, Egypt. 2Assiut University, Assiut University Mycological Centre, P.O. Box 71516, Assiut, Egypt.

\*Corresponding author: ahamaumc@yahoo.com

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ABSTRACT

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Mycobiota diversity associated with fruits and juices of citrus and grapevine plantations in Assuit Governorate, Egypt were evaluated during the period between April 2008 to February 2009. Identification of fungi was performed using the morphological and microscopical characteristics in addition to the biochemical in case of yeasts. In suspected isolates, molecular techniques were employed to confirm their identification. High counts of yeasts were recorded from the juice of both fruits (almost more than 95 % of total fungi), followed by citrus carposphere and carpoplane where they constituted about one-fifth to one-third of total fungi. High numbers of taxa were recorded from carposphere of both fruits than those recorded from carpoplanes or juices. The peak of total propagules of carposphere fungi was recorded in primordial fruit in citrus and in senescent fruit in grape, while the peaks of carpoplane fungi of both fruits and juices were recorded in mature fruits, while the troughs of all sources were regularly recorded in immature fruits. Aspergillus provided lower count in citrus than in grape carposhere and carpoplane while the reverse was recorded in juice. A. niger predominated in carposphere, carpoplane and juice of both plants, followed by A. aculeatus in all sources from grape and A. brasiliensis in citrus carposphere and carpoplane. Penicillium contributed small proportion of propagules in both plants. P. oxalicum was the most dominant species in all sources from grape but less common in citrus carposphere and carpoplane. P. digitatum and/or P. italicum were recorded in citrus only. Cladosporium contributed the highest counts (41.9 %-59.8 %) of all fungi in boh carpospheres, while contributing minor proportions in carpoplane and juice. It was recorded in high frequency in grape while less frequent in citrus for both carposphere and carpoplane but the reverse was recorded in juices. C. cladosporioides was the most dominant species in grape while C. sphaerospermum was the most dominant in citrus carposphere and carpoplane. The peak of yeast fungi was drawn in mature fruits of both citrus (December), grape carpospheres and carpoplanes; and juices of both fruits (Ocober). Of 22 yeast species recorded, only 2 were recovered from all sources of both plants (Hanseniaspora occidentalis and Issachenkia orientalis), 3 from carposphere, carpoplane and/or juice of citrus only (Candida catenulata, Geotrichum citri-aurantii and Kodemaea ohmeri) and 7 from grape only (Candida prunicola, Rhodosporidium paludigenum, R. diobvatum, Rhodotorula glutinus, Sporidiobolus pararoseus, S. ruinenniae and Sporobolomyces roseus). Ascomyceteous yeasts were dominant over basidiomyceteous ones in all subsrates. Since, mature fruits are succeptable to fungal attack, and almost all juice fungi, including yeasts and filamentous fungi, originated from fruit fungi, precautions during selecting fruits, transportation, handling and juice-making should be taken into accounts.

Keywords: mycobiota, juice, citrus, grape, fruits, biodiversity, rDNA sequencing

# INTRODUCTION

Grape berries are common niches for yeasts. Nevertheless, the yeast flora of grapes is surprisingly poorly documented (Loureiro and Malfeito-Ferreira, 2003; Ribereau-Gayon, 2005). The grape microflora may change in response to various factors such as: the climate, grape variety and geographical region (Sabate et al., 2002; Combina et al., 2005; Raspor et al., 2006). Botrytis infection resulted in a larger population and greater diversity of yeasts enriched with fermentative or spoilage species (Nisiotou and Nychas, 2007). Several studies of the occurrence of yeasts in grapes have already been published (Goto and Yokotsuka, 1977; Goto, 1980; Haridy, 1994). Sporobolomyces roseus, Cryptococcus albidus, Rhodotorula rubra and Candida were part of the natural microbiota of certain varieties of grapes in southern Spain (De la Torre et al., 1999). In Egypt, Haridy (1994) found that the most common spoilage yeast species of soft sound and unsound fruits (apple, grapes, dates, figs, strawberries, peach, apricot, plum, and guava) was Hanseniaspora valbyensis followed by H. vineae and Saccharomyces cereivisae. Metschnikowia pulcherrima, Torulaspora delbrueckii and Kluyveromyces marixianus were represented by considerable numbers of strains. Also, Hanseniaspora species wer reported as common yeast constituents on grapes (Phister et al., 2007), and on grapes and musts in Europe (Bioletti and Croiess, 1912).

The most frequent filamentous fungi found in grapes were species of Cladosporium, Penicillium, Botrytis, Alternaria and Aspergillus (Serra et al., 2005; Melki Ben Fredj et al., 2007). Several species of Aspergillus in section Nigri are common in vineyards and are often associated with bunch rots (Amerine et al., 1980). A. niger is reported to be the primary cause of Aspergillus rot in grapes before harvest (Nair, 1985; Snowdon, 1990), while A. aculeatus (Jarvis and Traquair, 1984) and A. carbonarius (Gupta, 1956) have also been reported. Melchers (1931) and Jones (1935) reported P. italicum and P. digitatum as causal agents of citrus-rot in Egypt, however Moubasher et al. (1971) and Elnaghy et al. (1973) reported that P. italicum was the sole incitant of Penicillum-rot in the Assuit area. Moubasher et al. (1971) found also that Cladosporium herbarum followed by A. niger and Alternaria species were the basic components on citrus fruits. In Washington, the most frequently encountered moulds from citrus fruit were Alternaria, Cladosporium, Penicillium and Fusarium, while Trichoderma, Geotrichum, Rhizopus and A. niger were isolated less often (Tournas and Katsoundas, 2005). Fungal spoilage of citrus fruit attributed to Alternaria, Alternaria citri, Fusarium, Penicillium digitatum, Penicillium italicum, Aspergillus, Geotrichum as well as to Botrytis was also reported (Splittstoesser, 1987; Ritenour et al., 2003). Studies have also been done on the processing of citrus fruits and juices from fruit concentrates (Parish

and Higgins, 1989, 1990; Deák and Beuchat, 1993). However, studies with yeasts in tropical environments have been rare.

Fruit juices are popular soft drinks with an important role in human nutrition. They are advertised as very healthy food supplements containing a variety of vitamins necessary for the good bodily function, and of the immune system in particular. Of freshly squeezed juices, citruses are the most popular (Arias et al., 2002). In general, the acidity (pH) of orange or grapefruit juices ranged between 3.5 and 3.9 and high sugar content (Bibek and Bhunia, 2004) creates favourable conditions for the growth of acidolactic bacteria, moulds, and yeasts. Sugar favours the development of a microbial biofilm. Before pasteurization, fruit juices contain a microbial load representative of the organisms normally found on fruits during harvest plus contaminants added post-harvest (during transport, storage and processing) that end up in the freshly squeezed juice offered in markets. Inadequate cleaning of fruit processors can pose a risk for consumers (Hatcher et al., 2001). Many reports have shown yeasts to be the predominant fungi involved in juice spoilage (Parish and Higgins, 1989; Hatcher et al., 2000). Yeast spoilage of fruit juice can result in formation of haze, production of CO<sub>2</sub> and off-odors, and changes in color (Grinbaum et al., 1994). Candida and Saccharomyces spp. have often been reported as spoilage-causing organisms in citrus juices (Hays, 1951; Grawmlich et al., 1986; Parish and Higgins, 1989; Teller and Parish, 1992). Many other yeast fungi such as Candida, Rhodotorula, Kluyveromyces, Pichia, Trichosporon, Kloeckera, Zygosaccharomyces have been also isolated from fruit juices, honey, milk and others (Cook, 1958; Jay, 1970; Ivo, 1982; Magalhães and Queiroz, 1991).

Arias et al., (2002) isolated 3 main species (Hansenula uvarum, followed by H. occidentalis and P. kluyveri) and 3 less common species (C. stellata, P. fermentans, and Saccharomycopsis crataegensis) from fresh-squeezed, unpasteurized orange juice. On the other hand, Cryptococus neoformans, Candida guilliermondii, C. famata, C. sphaerica, C. krusei, C. colliculosa, C. albicans, Kloeckera spp. and Trichosporon mucoides were the most common species identified in the orange juice from Zagreb, Croatia (Uhitil et al., 2009), however in the study of Hatcher et al. (2000) yeast species from in cirrus juices were Candida parapsilosis, C. stellata, Saccharomyces cerevisiae, Torulaspora delbrueckii, and Zygosaccharomyces rouxii, although species from the genera Rhodotorula, Pichia, Hanseniaspora and Metschnikowia were also common. Differently, yeasts commonly found in fruit juices in grapefruit juice in Washington were C. lambica, C. sake, Rhodotorula rubra, Geotrichum spp. and low numbers of Penicillium and Fusarium spp. (Tournas et al., 2006).

This study aimed at evaluating the yeast and filamentous fungi found in carposhere, carpoplane and fruit juices of both citrus and grapevine plants in Sahel Saleem city, Assiut Governorate. Identification of fungi was performed using the morphological and microscopical characteristics in addition to the biochemical in case of yeasts. In suspected isolates, molecular techniques were employed to confirm their identification.

# MATERIALS AND METHODS

#### Sampling location and collection of samples

This study was carried out in Sahel-Saleem city at approximately 25 km southeast of Assuit city. Sampling was conducted bimonthly over a twelve-month period from April 2008-February 2009. Three different plantations of citrus in the suburbs of Sahel-Saleem city and three of grapevine in El-Khawaled village (about 6 Km to the east border of the river Nile), in the northeast of Sahel-Saleem city were selected. A total of 31 fruit samples were collected from citrus (17) and grapevine trees (14) during the period from April 2008 to February 2009. Fruit samples were collected at random from different plants at each farm and put directly each into a clean plastic bag. Samples were brought into the laboratory and kept at 5°C till fungal analysis.

It should be mentioned that the dates of successive stages of development of fruit are as following: in citrus: primordial, in April; immature, in June and August; mature in October and December; senescent, in February (Figure 1), and in grape: primordial, in April; immature, in June and August; mature in October and; senescent, in December (Figure 2).

#### Isolation of carposphere fungi

In case of citrus, the fruits were peeled with a sterilized blade and a known weight of the peel was placed in 250 ml sterile Erlenmeyer flask containing 100 ml sterile distilled water. Flasks were shaken on an orbital shaker for 15 minutes. Ten ml aliquots of the suspension were transferred into sterile Erlenmeyer flasks containing each 90 ml sterile distilled water, then shaken for 5 minutes. In case of grapes a known weight of the fruits was mixed thoroughly as in the citrus fruits. The appropriate dilution which gave reasonable number of fungal colonies depends on the state of the fruits whether they were dusty or not was selected. One ml of the appropriate dilution was transferred into each sterile Petri-dish which was then poured with melted but cooled agar medium. Ten replicate plates were used for each sample (5 for each isolation medium type).



Figure 1 Developmental stages of citrus fruits.



Figure 2 Different developmental stages of grapevine fruits

### Isolation of carpoplane fungi

In case of citrus fruit, the peel after thorough washing with sterile distilled water and thorough drying was cut into small pieces of approximately 1 cm<sup>2</sup> and 4 pieces were thereafter placed on the surface of each agar plate.

In case of grapes, the whole fruits after thorough washing with sterile distilled water and drying were either inserted on the agar surface as a whole fruit when young or cut into two-halves when mature. Four parts were used in each of 5 replicate plates. Five replicate plates were used for each isolation medium type and for each plant type.

## Isolation of juice fungi

Fruits were surface washed by placing the whole fruits in a beaker containing sterilized water several times. The oranges were then sliced by sterilized cutter under sterile conditions and squeezed by hand into sterile universal tubes. In case of grapes, the berries after washing were squeezed by sterile lemon squeezer and the juice was collected into sterile universal tubes under aseptic conditions. One ml of the juice was transferred into each sterile Petri-dish which was then pour plated with melted but cooled agar medium. Ten replicate plates were used for each sample (5 for each medium type).

## Media used for isolation of fungi

a- Dichloran yeast extract malt extract agar (DYM) (**Moubasher** *et al.*, **2016**) Yeast extract malt extract agar (**Wickerham**, **1951**) was modified by **Moubasher** *et al.* (**2016**) by addition of 1 ml/l of 2 mg of dichloran dissolved in 10 ml ethanol which restricts mucoraceous growth without affecting the other species.

b- Dichloran rose bengal chloramphenicol agar, DRBC (King *et al.* 1979) to which rose bengal (25  $\mu$ g/ml) and chloramphenicol (100  $\mu$ g/ml) were used as bacteriostatic agents (**Smith and Dawson, 1944; Al-Doory, 1980**).

# Identification of filamentous fungi

The identification of fungal taxa based on macroscopic and microscopic features (Raper and Fennell, 1965; Rifai, 1969; Ellis, 1971, 1976; Pitt, 1979; Sivanesan, 1987; Moubasher, 1993; Gams and Bissett, 1998; Schroers, 2001; Zare and Gams, 2004; Leslie and Summerell, 2006; Crous *et al.* 2007; Domsch *et al.*, 2007; Samson and Varga, 2007; Seifert *et al.*, 2011).

Identification of fungal genera and species was performed using the morphological and microscopical characteristics in addition to the biochemical in case of yeasts. In suspected isolates, molecular techniques [Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using primers

ITS1, ITS4] were employed. Fungal diversity was observed in all the samples (Table 1).

#### Identification of yeasts

# Morphological characters

Formation of pseudomycelium and true mycelium (Wickerham. 1951) and the ability to form ascospores on three sporulation media (corn meal agar, potato glucose agar and yeast extract malt extract agar, YMA, at 25°C) (Barnett *et al.*, 2000) were carried out.

# Physiological characters

Fermentation of sugars and oxidative-utilization of carbon compounds were performed according to **Barnett** *et al.* (2000). Assimilation of nine nitrogen compounds was determined (Suh *et al.*, 2008). Test for hydrolysis of urea, growth at high osmotic pressure, growth at different temperatures, growth in the presence of cycloheximide, diazonium blue B (DBB) test and production of extracellular starch-like compounds were alo performed. Identification keys of **Barnett** *et al.* (2000) were followed to assign each isolate to its species level. Confirmations of these identifications were carried out using the molecular technique.

#### Molecular methods

The fungus was grown on CYA plates and incubated at  $25^{\circ}$  C for 7 days (for filamentous isolates) and on YMA plates and incubated at  $25^{\circ}$  C for 2 days (for yeast isolates). A small amount of fungal growth was scraped and suspended in 100 µl of distilled water and boiled at 100° C for 15 min and stored at -70° C. These preparations were sent to SolGent Company, Daejeon, South Korea, for PCR and rDNA sequencing.

Fungal DNA was extracted and isolated using SolGent purification beads in SolGent Company. Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using the universal primers ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then amplification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5  $\mu$ l, 10 mMdNTP (T) 0.5  $\mu$ l, primer (F-10p) 1.0  $\mu$ l, EF-Taq (2.5U) 0.25 $\mu$ l, template1.0  $\mu$ l, DW to 25  $\mu$ l. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at

50 °C for 40 s and extension at 72 °C for 1 min, with a final extension step of 72 °C for 5 min.

The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1% agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the forward and backward direction.

Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those retrieved from GenBank database were subjected to Clustal W analysis using MegAlign (DNAStar) software version 5.05 for the phylogenetic analysis. Sequence data were deposited in GenBank and accession numbers are given for them.

#### RESULTS AND DISCUSSION

Higher numbers of taxa were recorded from carposphere of both citrus and grape fruits (54 genera, 120 species + 2 varieties) than those recorded from carpoplanes (48genera, 99 species + 1 variety) and juices of both plants (27 genera, 54 species).

The peak of total propagules of carposphere fungi was recorded in April (primordial fruit) in citrus and in December (senescent fruit) in grape, while their trough was recorded in August (immature fruit) in citrus and in June (immature fruit) in grape, while the peak of total fungi was recorded in December (mature fruit) in citrus carpoplane and in October (mature fruit) in grape carpoplane and in both citrus and grape juices, while their trough was regularly recorded in June (immature fruit) in both carpoplanes. Total counts of all fungi were higher in grape carpoplane and juices than their respectives in citrus, while the reverse was true with the carposphere.

The genus Aspergillus (25 species + 1 variety) was the most common fungus. Its peak was recorded in December on DYM and in December and February on DRBC in citrus carposphere and carpoplane and in October or August on both media in grape carposphere, carpoplane and juices of both plants. Its propagules were fewer in citrus than in grape carposphere. The highest percentage of Aspergillus propagules was recorded from grape carpoplane (42.31% and 40.11% of total fungi on DYM and DRBC respectively). Leachates exuding outside the fruit surface could be favourable for carposphere and carpoplane fungi in grape than their respectives in citrus fruits. In fresh citrus juice, Aspergillus propagules exceeded those in fresh grape juice, contributing 1.24 % - 4.54 % in citrus juice and 0.25 % - 0.82 % in grape juice of total fungi respectively and this is a contrary to that observed in the carposphere and carpoplane. Most common species isolated were related to section Nigri (A. niger followed by A. aculeatus from carposphere and carpoplane of grape and A. niger followed by A. brasiliensis from carposphere and carpoplane of citrus). Other species of Aspergillus were recorded from citrus carposphere and carpoplane only, while others were isolated from grape only. In the juice, A. niger was common in both citrus and grape juices, while A. aculeatus was common only in grape juice and missed in citrus juice. Three species of Aspergillus were recorded from grape iuice only (Table 2-4).

Spores of black Aspergillus spp. are resistant to UV light (Rotem and Aust, 1991), which may account for their persistence in vineyards and on grape berries even after drying (King et al., 1981; Abdel-Sater and Saber, 1999; Abarca et al., 2003). A. aculeatus (Jarvis and Traquair, 1984) and A. carbonarius (Gupta, 1956) and several other species in this section have also been reported are in vineyards and are often associated with bunch rots (Amerine et al., 1980; Nair, 1985; Snowdon, 1990). A. niger and A. carbonarius have been isolated from grapes in France (Sage et al., 2002), Spain (Cabañes et al., 2002), Italy (Battilani et al., 2003; Tournas and Katsoundas, 2005), Portugal (Serra et al., 2003, 2005), Greece (Tjamos et al., 2004), South America (Da Rocha Rosa et al., 2002), and Chile (Díaz et al., 2009). The main fungal species isolated from grapes in Tunisian vineyards were Aspergillus spp. as A. niger aggregate (77%), Aspergillus carbonarius (15%) and Aspergillus flavus (8%) (Melki Ben Fredj et al., 2007). A. niger is the most common Aspergillus species responsible for postharvest decay of fresh fruit including grape (Magnoli et al., 2003) and A. carbonarius, A. niger, A. niveus, A. paradoxus, A. versicolor, A. wentii, and A. westerdijkiae were also identified on apparently healthy clusters of Chilean grapes (Díaz et al., 2009). Toxigenic strains of A. niger aggregate and A. carbonarius and ochratoxin A (Battilani et al., 2003; Serra et al., 2003) were often found associated with black rot of grapes (Logrieco et al., 2003). Strains of other ochratoxin A-producing species have been isolated from grapes less frequently, such as A. niger and A. ochraceus (Serra et al., 2005). A. niger was also isolated from citrus fruits (Moubasher et al., 1971), Sunkist lemon and grapes, USA (Tournas and Katsoudas, 2005), and lemon in Argentina (Maldonado et al., 2005). Aspergillus species were also frequently present in juice samples of Karachi, Pakistan (Anaissie et al., 2002; Nazim et al., 2008), canned fruit juices and beverages, Egypt (Abdel-Sater et al., 2001). A. niger was dominant in sugarcane juice with and without lemon in Karachi city (Ahmed et al., 2010). Unidentified Aspergillus species were also reported from fresh and pasteurized juices including orange juice, Bari, Italia (De-Donno et al., 1998).

*Petromyces (P. flavus* = anamorph: *Aspergillus flavus*) was isolated from fruits but was missed in both juices. Some strains of *P. flavus* are well known for the production of the naturally-occurring aflatoxins mainly B1 and B2 (**Logrieco** *et al.*, 2003).

*Neosartorya* (*N. fumigata* = anamorph: *Aspergillus fumigatus*) was recovered from all sources in grapevine and missed in citrus. *A. fumigatus* was isolated from sugarcane juice with and without lemon in Karachi city, Pakistan (Ahmed *et al.*, 2010).

*Cladosporium* yielded more percentage counts in the carposphere than those recorded in the carpoplane, also from grapevine than those from citrus however its propagules in citrus juice exceeded those in grape juice. Its peak in citrus was recorded in April while in grape in December. In grape carposphere, *C. cladosporioides* was the most common, contributing high percentages of total fungi followed by *C. sphaerospermum*, while *C. sphaerospermum* was the leading species in citrus carposphere in its count, but *C. herbarum* was recorded from grape carposphere and citrus juice only while *C. spongiosum* was isolated from citrus carposphere and grape juice only. In citrus juice *C. cladosporioides*, *C. sphaerospermum* and *C. oxysporum* were recorded in moderate frequency on both media while in grape juice *C. cladosporioides* was recorded in moderate frequency and *C. sphaerospermum* in low frequency (Table 2-4).

Cladasporium was one of the most frequently isolated genera associated with grapes in Tunisia (Melki Ben Fredj, 2007), in Argentina (Magnoli et al., 2003) and Spain (Bellí et al., 2006), one of the most common fungi in citrus fruits, grapes, strawberry, blueberry, raspberry and blackberry (Tournas and Katsoudas, 2005) and one of the moulds that able to cause spoilage of fruit juices and soft drinks (Stratford et al., 2000; Pitt and Hocking, 2009). C. herbarum was isolated from grapes and sun-dried grapes (Valero et al., 2007) and C. cladosporioides was one of the most dominant endogenous contaminant on the fruits of Sorbus domestica in Slovak Republic (Labuda et al., 2005; Kačániová and Fikselová, 2007).

*Alternaria* (3 species) was more prevalent in the carposphere and carpoplane of grape than their respectives in citrus, while it was recorded in low frequency in both juices. Two species (*A. chlamydospora* and/or *A. alternata*) were recovered from all sources, while *A. citri* from citrus only. *Alternaria* was one of the main fungal genera isolated from Tunisian grape berries (Melki Ben Fredj et al., 2007), Spanish grapes (Bau et al., 2005; Medina et al., 2005), citrus fruits (Moubasher et al., 1971; Splitstoesser, 1987; Tournas and Katsoundas, 2005), and passion fruits in Uganda (Ismail, 2006). *A. alternata* was also the most predominant species in grape samples in the south of Moravia, Czech Republic (Ostry, et al., 2007). *A. citri* and *A. alternata* were the incitants of serious plant diseases e.g. black rot of citrus (Whiteside, 1976; Logrieco et al., 2003).

*Fusarium* was infrequently isolated from the carposphere, carpoplane, and fruit juice of both plants. It contributed higher percentage counts in carposphere, and carpoplane of citrus than their respectives in grapevine. Of 8 *Fusarium* species recovered *F. semitectum* was the most frequent species in all sources of both plants. In fresh fruit juice, *F. solani* was recovered from the juice of both plants while *F. circinatum*, *F. chlamydosporum*, *F. semitectum* and *F. subglutinans* were isolated from grape juice only (Table 2-4). *Fusarium* was the most important fungus on wild fruits, Hong Kong (**Tang et al., 2003**) and grapefruit juice (**Tournas et al., 2006**). *F. oxysporum* and *F. moniliforme* (*F. verticillioides*) were isolated from tomato fruits in Maiduguri, northeastern Nigeria (**Akinmusire, 2011**), *F. solani* and *F. moniliforme* from decayed papaya fruits (**Bagwan, 2011**), *F. semitectum* and *F. chlamydosporum*, *F. moniliforme*, *F. moniliforme*, *F. acuminatum*, and *F. solani* from passion juice in Uganda (**Ismail, 2006**).

The genus Penicillium was one of the most common fungi in all sources of the two plants giving higher total counts in citrus compared with those of grapevine. Its peak was recorded in April (both carpospheres), December/April (citrus carpoplane), August/June (grape carpoplane and juice). The highest percentage of Penicillium propagules was recorded in citrus carpoplane (12.53 % - 15.63 %) and the highest species number was recorded in citrus carposphere (20 species). P. corylophilum, P. crustosum, P. duclauxii, P. P. citrinum, P. oxalicum, P. purpurogenum and P. roquefortii were recovered from both carpospheres while thirteen species were recorded from citrus carposphere only and one species was isolated from grape carposphere only. Only P. dauclauxii, P. griseofulvum, P. oxalicum, were infrequently isolated both carpoplanes while eleven species were recorded from citrus carpoplane only from which P. citrinum (1.62 % - 6.21 % of total fungi), P. digitatum (4.04 % - 2.82 %), and P. italicum (3.03 % - 3.77 %) contributed relatively large numbers, and three species were isolated from grapevine only. In the fresh juice, Penicillium was recorded in both fruits in high or moderate frequency. In citrus juice, its propagules exceeded those in grape juice. In the juice of citrus fruits P. digitatum, P. italicum, and P. purpurogenum, P. aurantiogriseum, P. glabrum, and P. viridicatum were recorded in moderate or low frequency while they were missed in grape juice. On the other hand, P. oxalicum, P. expansum and P. roquefortii were recorded in grape juice in moderate or low frequency, while they were missed in citrus juice (Tabl 2-4).

Penicillium spp. were found in grapes in Spain (Medina et al., 2005; Belli et al., 2006), Tunisia (Fredj et al., 2007), Portugal (Serra et al., 2003, 2005), Morocco (Selouane et al., 2009), Argentin (Magnoli et al., 2003), in wild fruits in Hong

Kong (Tang et al., 2003), citrus fruits (Tournas and Katsoudas, 2005), pineapple chunks (Tournas et al., 2006), passion fruits of pure origin in Uganda (Ismail, 2006). P. expansum was isolated from grapes in Potugal (Abrunhosa et al., 2001), P. expansum, P. aurantiogriseum, and P. spinulosum from grape samples in Czech Republic (Ostry et al., 2007), P. purpurogenum from all samples of the white Garnacha grape variety (Cabaňes et al. 2002), P. brevicompactum from rotting fruits of Madeira grapes, South America (Serra et al., 2006), P. brevicompactum, P. expansum, P. islandicum, and P. rugulosum from Japanese Quince (Chaenomeles japonica) fruits (Norin and Rumpunen, 2002), and P. digitatum and Penicillium sp. were also isolated in 38% of fresh juices (apricot, pineapple, orange, blood orange, banana, strawberry, tropical fruits, tangerine, apple, pear, peach, grapefruit, and pink grapefruit) and in 17% of pasteurised juices, Bari, Italia (De Donno et al., 1998), and from sugarcane juice in Karachi city (Ahmed et al., 2010).

Other fungal taxa of less frequency were recovered from the sources in one or both plants but in variable frequencies. *Botryodiplodia theobromae* was recorded from all sources of both plants. *B. theobromae* was recorded as causal agent of mango and banana fruit-rot (**El-Helaly** *et al.*, **1966**). *Setosphaeria rostrata* was isolated from all sources of both plants, except citrus carpoplane, while *Stemphylium* (*S. sarciniforme* and 2 unidentified) from all sources except citrus carposphere and *Quambalaria cyanescens* (*=Sporothrix cyanescens*) from all sources except citrus carposphere and carpoplane (Tab 2-4).

Cochliobolus (2 species), Emericella (5 species), Nigrospora oryzae, Phoma epicoccina and Rhizopus oryzae were isolated from carpospheres and carpoplanes but were missed in juices of both plants. The most prevalent species was C. lunatus followed by C. australiensis which was missed in citrus carposphere. Emericella variecolor was the most common species. Rhizopus oryzae was isolated from fresh Apricot, pineapple, orange, blood orange, banana, strawberry, tropical fruits, tangerine, apple, pear, peach, grapefruit pink grapefruit juices and pasteurized ones, Bari, Italia (De Donno et al., 1998), sugarcane juice with and without lemon in Karachi city, Pakistan (Ahmed et al., 2010).

Pleospora (P. allii, P. herbarum, and P. tarda, teleomorphs of Stemphylium vesicarium, S. herbarum and S. botryosum respectively) and Trichoderma (T. harzianum, T. reesei T. paracemosum and 1 unidentified) were isolated from all sources except grape juice. P. tarda was isolated from leaf and citrus fruit rind in Upper Egypt (Moubasher et al., 1971). T. harzianum was isolated from grapes and sun-dried grapes (Valero et al., 2007).

Acremonium potronii, Gibellulopsis nigrescens (=Verticillium nigrescens), Microdochium dimerum and Neurospora crassa were recorded in rare frequency from only grape carposphere, while Ramichloridium biverticillatum was recorded from grape carpoplane only, and Gliocladium virens, Pleurodesmospora sp. and Sagenomella diversispora were isolated only from grape carposphere and carpoplane.

Byssochlamyes spectabilis, Corynoascus sepedonium Drechslera biseptata, Memnoniella echinata, Pochonia sp., 2 Preussia species, Sarcopodium araliae, Scopulariopsis brumptii, Scytalidium infestans and Ulocladium botrytis were recorded in rare frequency from citrus carposphere only. Dichocladosporium chlorocephalum and Microascus brevicaulis were isolated infrequently recovered from citrus juice and carpopsphere only and Myrothecium (represented by M. verrucaria, M. roridum, and Myrothecium sp.) was recovered infrequently from citrus juice and carpoplane only. On he other hand, Clonostachys rosea and Apiospora montagnei were recovered in rare frequency from citrus carposphere and carpoplane while they were missed in grape fruits and juices from both fruits (Tab 2-4). Arthrinium sp. (anamorph of Apiospora montagnei) was recorded in dry leaves of Japanese quince plants in Sweden (Norin and Rumpunen, 2002).

Mucor (4 species) and Chaetomium globosum were recovered in rare frequency from both carpospheres and citrus carpoplane but was missed in both juices. M. circinelloides was the most common species followed by M. hiemalis in both plants. Stachybotrys (S. chartarum and a synnematous species) was recorded infrequently from both carposheres and grape carpoplane only, while Eurotium amstelodami and Fennellia nivea were recovered in rare frequency from both carpoplanes and citrus carposphere but not from fruit juice. Eurotium amstelodami was isolated from grapes and sun-dried grapes (Valero et al., 2007). Yeasts were recovered in high frequency from both fruit juices while they were less common in carpospheres and carpoplanes. They contributed their highest percentage counts in grape juice (99.14 % - 99.39 % of total fungi) and citrus juice (91.60 % - 95.42 %). The peak of yeast fungi were drawn in citrus in December (mature fruit) and in grape in October (mature fruit) for both carposhere and carpoplane while in Ocober (mature fruit) in both juices. Only 3 genera were recovered from all sources (Candida, Hanseniaspora and Issatchenkia) (Tab 2-4). According to Skinner et al. (1980) and Phaff (1990), the natural microbiota of fruits is commonly composed of yeasts and yeast-like organisms such as Aureobasidium, Rhodotorula, Sporobolomyces, Cryptococcus, Candida, Pichia, Kloeckera, Hanseniaspora, more rarely Saccharomyces and Schizosaccharomyces, and also the terrestrial species of Metschnikowia.

The genus *Candida* was recovered infrequently from different sources of both plants. Its highest percentage count was recorded from grape juice (71.41 % - 80.22 % of total fungi). Three species were recorded; *C. catenulata* and *C.* 

parapsilosis were recovered from citrus sources only and *C. prunicola* from grape sources only. *Candida* was the genus most frequently found in certain varieties of grapes in southern Spain (De la Torre et al., 1999) and in different angiosperm fruits, Southeastern Brazil (Prada and Pagnocca, 1997). *Candida* has also been reported as spoilage-causing organism in citrus juices (Hays, 1951; Grawmlich et al., 1986; Parish and Higgins, 1989; Teller and Parish, 1992) and in pasteurized fruit juices in Venezuela (Mendoza et al., 1982). *C. parapsilosis* was the dominant species in citrus juices (Hatcher et al., 2000), in fresh passion juice, Uganda (Ismail, 2006), and pasteurized and subsequently recontaminated single-strength orange juice, Florida (Arias et al., 2002).

Hanseniaspora (represented by H. occidentalis) and Issatchenkia (exemplified by I. orientalis) were recorded infrequently from carposphere, carpoplane, and juice of both plants. The highest percentage count of Hanseniaspora was recorded from grapevine carposphere (6.87 % - 9.21 % of total fungi) followed by citrus carpoplane (3.39 % - 3.64 %). Hanseniaspora species (anamorph Kloeckera) were common yeast constituents on grapes (Prakitchaiwattana et al., 2004; Phister et al., 2007), grapes and musts in Europe (Bioletti and Cruess, 1912), and in different angiosperm fruits, Southeastern Brazil (Prada and Pagnocca, 1997). Hanseniaspora was also commonly found in citrus juices (Hatcher et al., 2000), with H. occidentalis and H. uvarum being isolated from orange juice, Florida (Arias et al., 2002). H. uvarum is also associated with plants and fruits (Phaff and Starmer, 1987), on the pineapple fruit skins in Thailand and Australia (Chanprasartsuk et al., 2010).

*Issatchenkia* highest percentage count was recorded from grape juice (18.91 % - 71.41 % of total fungi) and citrus juice (26.60 % - 30.75 %) followed by citrus carposphere (23.01 % - 26.48 %). *I. orientalis* was the most frequent species recorded in *Parahancornia amapa* fruits in the Mocambo Forest, Salvaterra (Morais *et al.*, 1995), Thai fruits and vegetables, Thailand (Chanchaichaovivat *et al.*, 2007), and from pasteurized and subsequently recontaminated single-strength orange juice, Florida (Arias *et al.*, 2002).

*Cryptococccus* (4 species) was recovered infrequently from carpospheres of citrus and grape and grape juice (*C. albidus* and *C. laurentii*) or grape carposphere (*C. carnescens* and *C. magnus*), grape carpoplane and citrus juice (*C. laurentii*). *Cryptococcus* was prevalent in pineapple fruit in Rio de Janeiro, Brazil (Robbs et al., 1989), angiosperm fruits, Southeastern Brazil (Prada and Pagnocca, 1997). *C. albidus* and *C. laurentii* were isolated from soft grapes and peach, El-Minia city, Egypt (Haridy, 1994) and *C. albidus* was part of the natural microbiota of certain varieties of grapes in southern Spain (De la Torre et al., 1999).

The genus *Debaryomyces* (2 species) was recovered infrequently from different sources of both plants, except grapevine carpoplane. Its highest percentage count was recorded from citrus carpoplane (4.65 % - 4.89 % of total fungi). Both *D. hansenii* and *D. pseudopolymorphus* were recovered from both citrus carposphere and carpoplane while only *D. pseudopolymorphus* was recovered from grape carposphere and boh fresh juices. *Debaryomyces polymorphus* was the most common yeast species found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, Washington (**Tournas** *et al.*, **2006**).

*Geotrichum* (represented by *G. citri-aurantii*) was recovered infrequently from only citrus carposphere, carpoplane and juice. Similar observation was made by **Tournas** *et al.* (2006) when they reported that *Geotrichum* spp. were common in grapefruit juice in Washington and *G. citri-aurantii* was isolated from pasteurized orange juice, Florida (Arias *et al.*, 2002).

*Klyuveromyces marxianus* was recorded in rare frequency from grape carposphere only, while *Pseudozyma* was recorded in rare frequency from citrus carposphere only, and *Kodemaea ohmeri* was isolated in rare frequency from citrus carposphere and carpoplane only. *K. marxianus* was isolated from soft apples, grapes, dates, and strawberries, El-Minia city, Egypt (Haridy, 1994).

The genus Pichia was recovered infrequently from all sources of both plantations except grape carpoplane and juice. Its highest percentage count was recorded from citrus juice (56.42 % -57.01 % of total fungi) followed by citrus carpoplane (1.82 % - 4.52 %). Three species were collected of which P. fermentans was recovered from both carpospheres and citrus carpoplane and juice, P. guilliermondii (anamorph: Candida guilliermondii) from grape carposphere, and P. caribaea from citrus carposphere and juice. Pichia spp. were the most common yeasts found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, Washington (Tournas et al., 2006), different angiosperm fruits, southeastern Brazil (Prada and Pagnocca, 1997), pasteurized fruit juices in Venezuela (Mendoza et al., 1982), and from citrus juices (Hatcher et al., 2000). P. guilliermondii was isolated from soft apricot fruits, El-Minia city, Egypt (Haridy, 1994), fruits of Anacardium giganteum at the Mocambo Forest, Salvaterra (Morais et al., 1995), Thai fruits (Chanchaichaovivat et al., 2007), and pineapple fruit skins and fresh pineapple juice in Thailand and Australia (Chanprasartsuk et al., 2010) and the orange, apple, lemon, and grapefruit juices in Zagreb, Croatia (Uhitil et al., 2009). P. guilliermondii and P. fermentans were the most common yeast species from the fresh sugarcane juice (El-Tabey Shehata, 1960), and P. fermentans from fresh-squeezed single-strength orange juice, Florida (Arias et al., 2002), and from orange fruit and juice in a spontaneous fermentation (Las Heras-Vazquez et al., 2003).

Rhodotorula (2 species) was isolated infrequently from carposphere and carpoplane of both plants but in high frequency from grape juice. *R. glutinis* was recovered from grape carposphere and juice and *R. mucilaginosa* from both carpospheres and grape carpoplane and juice. In this respect, *Rhodotorula* spp. were the most common yeasts found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, Washington (Tournas et al., 2006), and pineapple fruit of in Rio de Janeiro, Brazil (Robbs et al., 1989) as well as from citrus juices (Hatcher et al., 2000) and pasteurized fruit juices in Venezuela (Mendoza et al., 1982). *R. mucilaginosa* was isolated

from orange fruit and juice in a spontaneous fermentation (Las Heras-Vazquez et al., 2003) and from pasteurized grapefruit juice, Florida (Arias et al., 2002). Sporidiobolus (S. ruineniae and S. pararoseus) were isolated in low frequency from grape juice only, while a black yeast species from grape carpoplane and Rhodosporidium (R. paludigenum and R. diobovatum) and Sporobolomyces (S. roseus) was isolated infrequently from grape carpophere, carpoplane, and juice and was missing in all cirus sources. Black yeast isolates were prevalent in pineapple fruit in Rio de Janeiro, Brazil (Robbs et al., 1989).

AUMC number	Isolation source	Accession GenBank number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
Filamentou	us fungi (Basidiom	iycota, Ustilagin	omycetes, Qu	ambalariaceae)			
6294	Citrus juice	JQ425382	661	AJ535500 = IMI 298177 $DQ317622 = CBS357.73^{T}$	99	Quambalaria cyanescens	de Beer et al. 2006
Ascomyce	teous yeast strains						
7754	Citrus fruit	JQ083433	374	EU131181 = GcaCC015 AF411060	99	Geotrichum citri-auriantii	Arias et al. 2002
7748	Citrus fruit	JQ425350	728	$GU246263 = CBS 5367^{T}$	98	Kodamaea ohmeri	Groenewald & Smith 2010
7765	Grape fruit	JQ083432	497	FM199972 = H7S6K11 FM199958 = H4S5K11	98	Issatchenkia orientalis (=Pichia kudriavzevii )	Daniel et al. 2009
7767	Grape fruit	JQ083434	432	EU343809 = CBS 8848 <sup>T</sup>	93	Candida prunicola	Kurtzman 2001
7766	Grape fruit	JQ425352	516	FJ515204 = UM5 AY939808 = CBS 5147 <sup>T</sup>	96 95	Issatchenkia orientalis (=Pichia kudriavzevii)	Leinberger et al. 2005
7769	Grape juice	JQ425351	487	FM199972 = H7S6K11 EU798698 = NN2573	100	Issatchenkia orientalis (=Pichia kudriavzevii)	Daniel et al. 2009
7768	Grape juice	JQ425355	437	$EU343809 = CBS 8848^{T}$	93	Candida prunicola	Kurtzman 2001
7764	Grape uice	JQ425401	416	EF199745 = szty2w $GU246263 = CBS 5367^{T}$	99 98	Kodamaea ohmeri	Groenewald & Smith 2010
Basidiomy	ceteous yeast strai	ns					
7777	Grape juice	JQ425364	623	AF444635 = CBS 9070 $AF444541 = CBS 316^{T}$	99 98	Rhodotorula mucilaginosa	Scorzetti et al. 2002
7796	Grape juice	JQ425366	606	AF444635 = CBS 9070 $AF444541 = CBS 316^{T}$	99	Rhodotorula mucilaginosa	Scorzetti et al. 2002
7248	Citrus fruit	JQ425393	628	AF444635 = CBS 9070 $AF444541 = CBS 316^{T}$	99	Rhodotorula mucilaginosa	Scorzetti et al. 2002
7246	Grape fruit	JQ425371	661	EU871517 = S22814 AF190008= CBS 140 <sup>T</sup>	99	Cryptococcus magnus	Fell et al. 2000

Table 2 Percentage counts calculated to total fungi and frequency of occurrence of carposphere fungi recovered bimonthly from the citrus and grape on DYM and DRBC agar media during the period from April 2008- February 2009 (counts of CFU calculated per g fresh fruit rind (citrus) or fresh fruit (grape) in each sample, collectively in 17 samples in case of citrus and 14 samples in grape).

		Citrus	carposphere	Grape carposphere				
Taxa	D	YМ	Ι	ORBC	]	DYM	Ľ	RBC
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O
Filamentous fungi	62.51	16H	74.31	17H	82.05	14 H	80.92	14 H
Acremonium potronii							0.03	1 R
Alternaria	4.29	4 L	2.01	4L	5.44	7 H	5.65	7 H
A. alternata	3.97	3L	1.97	4L	5.19	7 H	5.05	7 H
A. chlamydospora	0.14	2R	0.04	1R	0.06	1 R	0.17	3 L
A. citri	0.18	1R						
Alternaria sp.					0.17	1 R	0.44	2 R
Apiospora montagonii	0.03	1 R						
Aspergillus	0.73	11H	0.97	14H	18.29	13 H	17.25	14 H
A. aculeatinus							0.07	3 L
A. aculeatus	0.01	1R			3.58	5 M	4.26	7 H
A. brasiliensis	0.08	3 L	0.21	5M	5.29	4 L	4.92	3 L
A. campestris			0.01	1R				
A. clavatus			0.01	1R				
A. dimorphicus	0.01	1 R						
A. flavus var. columnaris	0.01	1 R	0.03	2R				
A. japonicus							0.03	1 R
A. lacticoffeatus	0.04	1 R	0.07	1R				
A. niger	0.44	6M	0.47	10H	9.07	10 H	6.90	9 H
A. ochraceus	0.04	3 L	0.06	3L	0.31	4 L	0.28	4 L
A. petrakii	0.02	1 R						
A. proliferans			0.03	1R	0.03	1 R		
A. speleneus	0.04	1 R	0.07	1R				
A. sydowii	0.02	1 R						
A. terreus	0.01	1 R			0.01	1 R	0.23	3 L
A. tubingensis							0.56	2 R
A. versicolor			0.03	1R				
Botryodiplodia theobromae	0.11	2 R	0.01	1R				
Byssochlamys spectabilis	0.09	1 R	0.06	2R				
Chaetomium globosum	0.16	2 R	0.03	1R			0.03	1 R

		Citrus	carposphere		Grape carposphere				
Гаха		УM	Ľ	RBC	DYM DRBC				
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O	
Cladosporium	41.89	8 M	59.82	8M	52.31	9 H	53.65	7 H	
C. cladosporioides	4.95	6 M	8.63	8M	41.50	8 H	41.59	7 H	
C. herbarum	0.41	2 R	0.33	3L	0.02 2.59	1 R 4 L	2.00	3 L	
C. oxysporum	36.49	2 K 7 M	50.69	3L 7M			3.09 8.97	3 L 5 M	
C. sphaerospermum	0.04		0.16	2R	8.19	6 M	8.97	5 M	
C. spongiosum		1 R							
Clonostachys rosea	0.02	1 R	0.01	1R	0.76	4 1	0.04	2.0	
Cochliobolus			0.03	1R	0.76	4 L	0.04 0.02	2 R	
C. australiensis			0.02	1D	0.75	3 L		1 R	
C. lunatus	0.02	1 D	0.03	1R	0.01	1 R	0.02	1 R	
Dreschlera biseptata	0.02	1 R	0.02	10					
Dichocladosporium chlorocephalum	0.07	1 R	0.03	1R	0.41	2.1	0.27	21	
Emericella	0.01	1 R	0.01	1R	0.41	3 L	0.27	3 L	
E. heterothallica					0.06	1 R	0.10	1.0	
E. nidulans					0.08	1 R	0.10	1 R	
E. quadrilineata	0.01	1.5	0.01	15	0.05		0.06	1 R	
E. variecolor	0.01	1 R	0.01	1R	0.27	3 L	0.10	2 R	
Eurotium amstelodami	0.04	1 R	0.06	2R					
Fennellia nivea	0.01	1 R	- 10	~	1.0-		0.05		
Fusarium	10.99	3 L	5.12	3L	1.05	6 M	0.86	6 M	
F. chlamydosporum					0.02	1 R			
F. proliferatum					-		0.02	1 R	
F. semitectum	10.32	3 L	5.09	2R	0.84	5 M	0.64	3 L	
F. solani	0.53	1 R							
F. verticillioides	0.14	1 R	0.03	1 <b>R</b>	0.19	2 R	0.19	3 L	
Gibellulopsis nigrescens					0.02	1 R	0.10	1 R	
Gliocladium virens					0.04	1 R			
Aicroascus brevicaulis	0.04	1 R							
Aichrodochium dimerum					0.01	1 R			
Aucor	0.05	1 R	0.01	1R	0.04	1 R			
1. circinelloides	0.02	1 R	0.01	1R	0.04	1 R			
1. hiemalis var. luteus	0.04	1 R							
Veosartorya fumigata					0.03	1 R	0.03	1 R	
Veurospora crassa					0.02	1 R	0.02	1 R	
Vigrospora crussa Vigrospora oryzae	0.09	2 R	0.07	3L	0.01	1 R	0.01	1 R	
Penicillium	1.57	11 H	5.19	11H	0.45	9 H	1.05	8 H	
P. aurantiogriseum	0.04	1 R	1.47	3L	0.45	711	1.05	011	
P. brevicompactum	0.04	1 R	0.03	1R					
P. citrinum	0.58	3 L	2.97	2R	0.01	1 R	0.06	2 R	
	0.04	1 R	0.01	1R	0.01		0.00	2 K	
P. corylophilum	0.04	IK				1 R	0.02	1.D	
P. crustosum	0.10	2.0	0.03	1R	0.04	1 R	0.02	1 R	
P. digitatum	0.19	2 R	0.25	3L			0.02	1.0	
P. duclauxii	0.14	4 L					0.02	1 R	
P. expansum	0.04	1 R							
P. fellutanum			0.01	1R					
P. glabrum			0.03	1R					
P. hirsutum			0.05	1R					
P. implicatum	0.01	1 R							
P. italicum	0.12	2 R	0.15	3L					
P. olsonii	0.02	1 R	0.07	3L					
P. oxalicum	0.09	2 R	0.05	2R	0.25	8 H	0.64	7 H	
P. puberulum	0.19	2 R	0.04	2R					
P. purpurogenum			0.01	1R	0.11	5 M	0.19	3 L	
P. raistrickii	0.02	1 R							
P. roquefortii			0.04	1R			0.05	1 R	
P. viridicatum	0.07	1 R							
P. waksmanii							0.06	1 R	
Petromyces flavus	0.19	5 M	0.15	6M	0.06	3 L	0.06	2 R	
Phoma epicoccina	1.85	5 M	0.64	4L	1.32	6 M	0.75	4 L	
Pleospora	0.16	3 L	0.01	1R	0.90	5 M	0.39	3 L	
P. herbarum	0.02	1 R			0.11	1 R			
2. tarda	0.02	2 R	0.01	1 <b>R</b>	0.79	5 M	0.39	3 L	
leurodesmospora sp.	0.17	2 M	0.01	111	0.02	1 R	0.07	21	
reussia	0.04	2 R			0.02	1 11			
P. minima	0.04	1 R							
	0.04	1 R 1 R							
Preussia sp.	0.01	1 K	0.01	1 D					
Pseudonectria pachysandricola			0.01	1R	0.45	2.0	0.49	2.1	
Quambalaria cyanescens	0.04	1 D			0.45	2 R	0.48	3 L	
Chizopus oryzae	0.04	1 R			0.26	5 M	0.02	1 R	
agenomella diversispora				2 <sup>1</sup> 1000			0.05	2 R	
copulariopsis brumptii			0.03	1R					
Scytalidium infestans Setosphearia rostrata	0.01	1 R	0.01	1R	0.05	2 R			

			carposphere			carposphere		
Taxa		YM		DRBC		DYM		ORBC
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O
Stachybotrys	0.02	1 R			0.04	1 R	0.03	1 R
S. chartarum	0.02	1 R						
Stachybotrys sp.					0.04	1 R	0.03	1 R
Stemphylium					0.03	2 R	0.12	2 R
S. sarciniforme					0.03	2 R	0.06	1 R
Stemphylium sp. 157							0.06	1 R
Trichoderma			0.01	1R			0.04	1 R
T. reesei			0.01	1R				
Trichoderma sp.							0.04	1 R
Ulocladium botrytis			0.01	1R				
Yeasts	37.49	5 M	25.69	8M	17.95	9 H	19.08	8 H
Candida	7.40	3 L	0.68	3 L	5.35	2 R	6.72	3 L
C. catenulata	7.40	3 L	0.67	2 R				
C. parapsilosis			0.01	1 R				
C. prunicola					5.35	2 R	6.72	3 L
Cryprococcus	0.02	1 R	0.08	4 L	0.13	2 R	1.56	4 L
C. albidus			0.04	2 R	0.21	3 L	0.92	4 L
C. carnescens					0.02	1 R	0.17	3 L
C. laurentii	0.02	1 R	0.04	3 L	0.06	2 R	0.46	3 L
C. magnus	0102		0101	02	0.000	210	0.02	1 R
Debaryomyces	0.60	2 R	0.09	1 R	0.02	1 R	0.02	
D. hansenii	0.60	2 R 2 R	0.05	1 R	0.02	110		
D. pseudopolymorphus	0.00	2 K	0.03	1 R	0.02	1 R		
Geotrichum citri-aurantii	0.09	1 R	0.04	2 R	0.02	1 K		
Hanseniaspora occidentalis	2.33	2 R	1.18	2 R 2 R	9.21	4 L	6.87	3 L
Issachenkia orientalis	2.55	3 L	23.01	3 L	2.29	4 L 4 L	2.94	3 L 3 L
Kluvveromyces marixianus	20.40	31	23.01	31	0.06	2 R	0.01	1 R
Kuyveromyces marixianus Kodemaea ohmeri	0.02	1 R	0.01	1 R	0.00	2 K	0.01	IK
Pichia	0.02	3 L	0.01	2 R	0.05	2 R	0.39	3 L
		1 R			0.03	2 <b>R</b>	0.39	31
P. caribaea	0.01		0.01	1 R	0.01	1 D		
P. fermentans	0.52	3 L	0.56	2 R	0.01	1 R	0.20	2.1
P. guillieromondii	0.02	1.0			0.04	1 R	0.39	3 L
Pseudozyma sp.	0.02	1 R			0.12	2.0	0.11	2.1
Rhodosporidium					0.12	2 R	0.11 0.06	3 L
R. diobovatum					0.11	1 R		1 R
R. paludigenum			0.01	1 D	0.01	1 R	0.05	2 R
Rhodotorula			0.01	1 R	0.34	5 M	0.16	5 M
R. glutinis			0.01	1.5	0.06	2 R	0.02	1 R
R. muclaginosa			0.01	1 R	0.28	4 L	0.13	4 L
Sporobolomyces roseus					0.19	3 L	0.31	3 L
Total CFUs (%)	22568	17 H	30296	17H	18616	14 H	19299	
	(100)		(100)		(100)		(100)	14 H
No. of genera (54)	34		32		33			31
No. of species $(120 + 2)$	67+1		64+1		58			59

\*F = Frequency of occurrence out of 17 samples of citrus fruits or 14 of grapevine fruits. \*OR = Occurrence remarks: for citrus samples; H = high, 9 - 17; M = moderate, 5-8; L = Low, 3 - 4; R = rare, 1 or 2 samples, and for grapevine: H, 7-14; M, 5-6; L, 3-4; R = 1-2 samples.

Table 3 Percentage counts calculated to total fungi and frequency of occurrence of carpoplane fungi recovered from citrus and grape on DYM and DRBC agar media bimonthly during the period from April 2008- February 2009 (counts of CFU calculated per 20 fresh fruit rind pieces (citrus) or fresh fruit pieces (grape) in each sample, collectively in 17 samples in case of citrus and 14 samples in grape).

		Citrus o	carpoplane	Grape carpoplane				
Таха	D	YM	DI	RBC	D	YM	DRBC	
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O
Filamentous fungi	69.29	17 H	64.78	17 H	79.44	14 H	76.92	13 H
Alternaria	8.08	3 L	6.78	3 L	7.84	7 H	13.87	8 H
A. alternata	7.07	2 R	5.84	2 R	7.25	7 H	13.05	8 H
A. chlamydospora	1.01	2 R	0.75	3 L	0.59	2 R	0.82	2 R
A. citri			0.19	1 R				
Apiospora montagonii	0.20	1 R						
Aspergillus	21.41	14 H	21.66	16 H	42.31	14 H	40.11	13 H
A. aculeatinus					1.92	5 M	0.69	2 R
A. aculeatus					14.35	9 H	7.42	7 M
A. auricomus							0.14	1 R
A. brasiliensis	11.31	7 M	6.59	6 M	0.74	2 R		
A. carneus			0.19	1 R				
A. dimorphicus			0.19	1 R				
A. flavus var. columnaris			1.32	4 L				
A. lacticoffeatus			0.38	1 R				
A. niger	8.69	12 H	10.92	13 H	25.0	11 H	30.77	11 H
A. ochraceus	0.20	1 R	0.75	4 L	0.15	1 R	0.41	2 R
A. oryzae			0.19	1 R				
A. ostianus	0.20	1 R						
A. robustus	0.20	1 R						
A. sclerotiorum	0.20	1 R	0.19	1 R				

Таха	Citrus carpoplane DYM DRBC				Grape carpoplane C DYM DRBC					
	%CFU	YM F&O	%CFU	F&O	%CFU	YM F&O	%CFU	F&O		
A. speleneus	0.40		0.56	1 R						
A. sulphureus	0.40	2 R								
A. sydowii	0.20	1 R			0.15	1 D				
A. terreus A. tubingensis			0.38	1 R	0.15	1 R	0.69	2 R		
A. tubingensis Botryodiplodia theobromae	2.02	3 L	1.51	4 L	0.59	1 R	0.89	2 R 1 R		
Cladosporium	2.63	3 L 3 L	2.64	4 L 5 M	6.66	5 M	6.59	6 M		
Ciadosporium C. cladosporioides	1.21	2 R	0.38	1 R	6.21	5 M	6.59	6 M		
C. cuaosporioiaes C. oxysporum	0.20	2 R 1 R	0.38	1 R	0.21	5 M	0.39	0 101		
C. sphaerospermum	1.21	2 R	2.07	4 L	0.44	1 R				
Chaetomium globosum	0.20	1 R	2.07	4 L	0.44	ΪK				
Clonostachys rosea	0.20	IK	0.19	1 R						
Cochliobolus	0.81	3 L	0.19	2 R	0.15	1 R	0.14	1 R		
C. australiensis	0.20	1 R	0.50	2 K	0.15	ΪK	0.14	1 R		
C. lunatus	0.61	2 R	0.38	2 R	0.15	1 R	0.14	IK		
Corynoascus sepedonium	0.20	1 R	0.38	1 R	0.15	1 K				
Emericella	0.40	2 R	0.38	2 R						
E. dentata	0.20	1 R	0.50	2 K						
E. nidulans	0.20	1 R								
E. quadrilineata	0.20	ΪK	0.19	1 R						
E. variecolor			0.19	1 R						
E. variecolor Eurotium amstelodami	0.20	1 R	0.19	2 R			0.14	1 R		
Fennellia nivea	0.20	1 K	0.19	1 R	0.29	1 R	0.14	1 IX		
Fennenia nivea Fusarium	9.89	5 M	8.47	3 L	0.29	2 R	1.37	5 M		
Fusarium F. lactis	0.61	1 R	1.13	1 R	0.44	2 K	1.37	J 1 <b>VI</b>		
F. proliferatum	0.61	1 R 1 R	0.56	1 R 1 R						
F. semitectum	8.48	4 L	6.78	3 L	0.29	2 R	1.37	5 M		
F. solani	0.20	4 L 1 R	0.70	<u>э</u> Е	0.29	2 K	1.37	J 1 <b>VI</b>		
F. verticillioides	0.20	1 K			0.15	1 R				
Gliocladium virens					0.15	1 R				
Haptocillium sp.					0.13	1 K	0.14	1 R		
Memmnoniella echinata			0.19	1 R			0.14	1 K		
	0.20	1 R	0.19	1 K						
Mucor circinelloides	0.20	1 K	0.29	1 D						
Myrothecium sp.			0.38	1 R	0.15	1 D				
Neosartorya fumigata					0.13	1 R 1 R	0.14	1 D		
Neurospora crassa Nigrospora oryzae	0.20	1 R			0.29	1 K	0.14	1 R 1 R		
	12.53	9 H	15.62	10 11	1.18	5 M		8 H		
Penicillium	12.55	2 R	<u>15.63</u> 2.07	10 H 2 R	1.18	5 M	1.51	δH		
P. aurantiogriseum			2.07	2 K						
P. bilaii	0.20	1 R								
P. brevicompactum	0.40	1 R 1 R	6.21	4 L						
P. citrinum	1.62		0.21	4 L						
P. corylophilum	0.20	1 R	0.38	1 D						
P. crustosum		1 R		1 R						
P. digitatum	4.04	2 R	2.82	4 L	0.15	1.D	0.14	1 D		
P. duclauxii	0.20	1 D	0.19	1 R	0.15	1 R	0.14	1 R		
P. griseofulvum	0.20	1 R			0.15	1 D	0.27	1 R		
P. humuli	2.02	2 1	2 77	21	0.15	1 R				
P. italicum	3.03	3 L	3.77	3 L			0.07	<i>(</i> ) <i>(</i> )		
P. oxalicum	0.61	1 R	0.19	1 R	0.15	1 D	0.96	6 M		
P. pinophilum					0.15	1 R	0.14	1.5		
P. purpurogenum	0.40	1 D			0.44	2 R	0.14	1 R		
P. restrictum	0.40	1 R								
P. roquefortii	0.20	1 R								
P. viridicatum	0.40	1 R	1		0 = 1		0.0.5			
Petromyces flavus	2.63	6 M	1.88	5 M	0.74	5 M	0.96	3 L		
Phoma epicoccina	4.85	2 R	1.88	1 R	7.99	5 M	5.08	5 M		
Pleospora	0.20	1 R	0.38	2 R	0.44	3 L	0.55	4 L		
P. allii		. –			0.15	1 R				
P. tarda	0.20	1 R	0.38	2 R	0.29	2 R	0.55	4 L		
Pleurodesmospora sp.					0.15	1 R				
Pochonia sp.	0.40	1 R								
Quambalaria cyanescens							0.96	2 R		
Ramichloridium biverticillatum					0.15	1 R				
Rhizopus oryzae	1.82	3 L	0.75	2 R	7.25	6 M	2.88	7 H		
Sagenomella diversispora							0.27	1 R		
Sarcopodium araliae			0.19	1 R						
Setosphaeria rosrata					2.07	4 L	0.41	3 L		
Stachybotrys sp.							0.14	1 R		
Stemphylium			0.19	1 R			0.27	2 R		
S. sarciniforme							0.14	1 R		
Stemphylium sp. 533			0.19	1 R						
							0.14	1 R		

		Citrus o	carpoplane	Grape carpoplane				
Таха	DYM		DI	RBC	DYM		DI	RBC
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O
Trichoderma	0.40	1 R	0.38	1 R	0.15	1 R		
T. paraceramosum					0.15	1 R		
T. reesei	0.40	1 R	0.38	1 R				
Yeasts	30.71	5 M	35.22	6 M	20.56	4 L	23.08	7 H
Candida	10.30	2 R	11.68	2 R	6.51	1 R	7.97	2 R
C. catenulata	10.30	2 R	11.68	2 R				
C. prunicola					6.51	1 R	7.97	2 R
Cryptococcus laurentii							0.14	1 R
Debaryomyces	4.65	3 L	4.89	3 L				
D. hansenii	0.61	2 R	0.38	2 R				
D. pseudopolymorphus	4.04	3 L	4.52	1 R				
Geotrichum citri-aurantii	0.61	1 R	0.94	2 R				
Hanseniaspora occidentalis	3.64	1 R	3.39	1 R	2.07	1 R	1.10	2 R
Issachenkia orientalis	9.49	3 L	9.42	3 L	10.95	2 R	12.23	3 L
Kodemaea ohmeri	0.20	1 R	0.38	1 R				
Pichia fermentans	1.82	1 R	4.52	1 R				
Rhodosporidium paludigenum					0.29	1 R		
Rhodotorula muclaginosa							0.69	2 R
Sporobolomyces roseus					0.29	1 R	0.69	2 R
Yeast sp. (black)					0.44	1 R	0.27	1 R
Total CFUs (%)	495	17 H	531	17 H	676	14 H	728	14 H
	(100)		(100)		(100)		(100)	
No. of genera (48)	27		28		26		27	
No. of species $(99 + 1)$	55		51+1		39		37	

\*F = Frequency of occurrence out of 17 samples of citrus and 14 of grapevine.

\*O = Occurrence remarks for citrus: H = high, 9-17; M = moderate, 5-8; L = Low, 3-4; R = rare, 1-2 samples; = For grapevine: H, 7-14; M, 5-6; L, 3-4; R = 1-2 samples.

**Table 4** Percentage counts calculated to total fungi and frequency of occurrence of fungi recovered from citrus and grapevine juices on DYM and DRBC agar media bimonthly during the period from April 2008- February 2009 (counts of CFU calculated per ml juice in each sample, collectively in 8 samples in case of citrus and 6 samples in grapevine).

			rus juice		Grape juice				
Таха		DYM		DRC		ΥM	DRBC		
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O	
Filamentous fungi	4.58	7 H	8.40	7 H	0.61	6 H	0.86	6 H	
Alternaria alternata	0.05	1 L	0.13	1 L	0.0004	1 L			
Aspergillus	1.24	6 H	4.54	5 H	0.25	5 H	0.82	6 H	
A. aculeatinus					0.12	2 M	0.24	2 M	
A. aculeatus					0.005	3 H	0.01	1 L	
A. brasiliensis	0.18	1 L	0.34	1 L	0.06	1 L	0.19	2 M	
A. japonicus					0.002	1 L			
A. niger	1.01	5 H	4.12	4 H	0.07	2 M	0.38	5 H	
A. ochraceus	0.05	1 L	0.08	2 M	0.0004	1 L	0.001	1 L	
Botryodiplodia theobromae	0.14	2 M	0.04	1 L					
Cladosporium	2.25	4 H	2.89	3 M	0.33	2 M	0.03	1 L	
C. cladosporioides	1.05	2 M	1.23	2 M	0.32	2 M	0.03	1 L	
C. herbarum	0.18	2 M	0.08	2 M					
C. oxysporum			1.53	2 M					
C. sphaerospermum	1.01	3 M	0.04	1 L	0.01	1 L	0.002	1 L	
C. spongiosum					0.001	1 L			
Dichocladosporium chlorocephalum	0.09	1 L							
Fusarium			0.04	1 L	0.002	2 M	0.002	1 L	
F. circinatum					0.001	1 L	0.001	1 L	
F. chlamydosporum					0.0004	1 L			
F. semitectum					0.0004	1 L			
F. solani			0.04	1 L	0.0004	1 L			
F. subglutinans							0.001	1 L	
Microascus brevicaulis	0.05	1 L							
Myrothecium roridum			0.04	1 L					
Neosartorya fumigata							0.001	1 L	
Penicillium	0.73	5 H	0.51	3 M	0.002	3 H	0.004	2 M	
P. aurantiogriseum			0.08	1 L					
P. digitatum	0.32	2 M	0.17	1 L					
P. expansum	0.012	2111	0117		0.0004	1 L			
P. glabrum	0.05	1 L							
P. italicum	0.09	2 M	0.13	2 M					
P. oxalicum	0.07	2	0.10	2	0.002	2 M	0.003	2 M	
P. purpurogenum	0.23	1 L	0.13	1 L	0.002		0.000	2.01	
P. roquefortii	0.20		0.10				0.001	1 L	
P. viridicatum	0.05	1 L					0.001	10	
Pleospora herbarum	0.02		0.04	1 L					
Quambalaria cyanescens	0.05	1 L	0.01		0.02	2 M	0.01	2 M	
Setosphearia rostrata	0.05		0.04	1 L	0.001	2 M	0.01	171 -	
Stemphylium			0.04	1 L 1 L	0.0004	1 L			

		Cit	rus juice	Grape juice				
Taxa	Ľ	DYM	I	DRC	DY	ΥM	DR	BC
	%CFU	F&O	%CFU	F&O	%CFU	F&O	%CFU	F&O
S. sarciniforme			0.04	1 L				
Stemphylium sp.157					0.0004	1 L		
Trichoderma harzianum			0.08	1 L				
Yeasts	95.42	5 H	91.60	5 H	99.39	6 H	99.14	6 H
Candida	3.44	2 M	4.16	2 M	80.22	3 H	71.41	2 M
C. cateulata	3.44	2 M	4.16	2 M				
C. prunicola					80.22	3 H	71.41	2 M
Cryprococcus	0.046	1 L			0.001	1 L	0.001	1 L
C. albidus							0.001	1 L
C. laurentii	0.046	1 L			0.001	1 L		
Debaryomyces pseudopolymorphus	2.29	2 M	0.76	2 M	0.001	1 L		
Geotrichum citri-aurantii	0.46	1 L	0.93	2 M				
Hanseniaspora occidentalis	1.42	2 M	0.64	2 M	0.23	2 M	0.49	2 M
Issachenkia orientalis	30.75	2 M	26.60	2 M	18.91	3 H	27.21	2 M
Pichia	57.01	3 M	56.42	4 H				
P. caribaea			0.13	1 R				
P. fermentans	57.01	3 M	56.26	4 H				
Rhodosporidium paludigenum					0.0004	1 L	0.001	1 L
Rhodotorula					0.03	3 H	0.01	3 H
R. glutinis					0.001	1 L		
R. muclaginosa					0.03	3 H	0.01	3 H
Sporidiobolus					0.0004	1 L	0.003	2 M
S. pararoseus							0.001	1 L
S. ruineniae					0.0004	1 L	0.002	1 L
Sporobolomyces roseus					0.002	1 L	0.01	1 L
Total CFUs (%)	436.4	8 H	471.4	8 H	50283.4	6 H	21931.2	6 H
	(100)		(100)		(100)		(100)	
No. of genera (27)	15		17		17		14	
No. of species (54)	23		26		29		22	

\*F = Frequency of occurrence out of 8 samples for citrus juice and 6 samples for grapevine juice.

\*O = Occurrence remarks for citrus juice: H = high, 4-8; M = moderate, 2-3; L = Low, 1 samples = For grapevine juice: H, 3-6; M, 2; L = 1 sample.

# CONCLUSION

The present study reveals a positive correlation between abundance of certain groups of fungi and the substrates. Yeasts were dominant in both fruit juices with the leading species are Pichia fermentans in citrus juice, Candida prunicola in grape juice and Issachenkia orientalis in both. However, filamentous fungi predominated in both carposhere and carpoplane with Cladosporium predominating in both carposphere and Aspergillus section Nigri species in grape fruit carpoplane. This observation could be attributed to presence of sugars or sugar metabolites in the substrates that favours their establishment. Maure stage was more prone to fungal attack in both fruits, where the peaks of many probably pathogenic carpoplane fungi of both fruits were recorded, e. g. species of Alternaria, Aspergillus secion Nigri, Fusarium, Phoma, Penicillium and yeasts.

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