Pak. J. Bot., 42(4): 2955-2962, 2010.

MYCOFLORA ASSOCIATED WITH SUGAR CANE JUICE IN KARACHI CITY

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Abstract

The study was conducted for investigating the quality of 28 sugar cane juice samples collected from different localities of Karachi. pH value of sugarcane juice samples with lemon ranged from 4.60-6.56, whereas the sugarcane juice samples without lemon ranged from 5.0-6.85. Mycoflora of samples was studied by using direct plate and serial dilution techniques. Twenty eight samples of sugarcane juice with and without lemon were tested and 18 different species belonging to 11 different genera of fungi isolated by direct plating method were *Absidia corymbifera, Acremonium* sp., *Aspergillus flavus, A. fumigatus, A. niger, A. sulphureus, A. terreus, A. wentii, Fusarium semitectum, F. sporotrichoides, Humicola grisea, Gilmanieila humicola, Curvularia lunata, Monilia* sp., *Rhizopus stolonifer, R. oryzae, Penicillium* sp., and yeast (*Saccharomyces* spp.) whereas *Aspergillus candidus, A. subolivaceous, A. erythrocephalus* and *A. tamarii* were isolated in addition to these by serial dilution techniques. The highest number of fungi were isolated by serial dilution technique and *A. niger* appeared as a dominant fungus of sugarcane juice with and without lemon by both of the techniques. The addition of lemon juice reduced the occurrence of *A. corymbifera, C. lunata* and *A. erythrocephalus* by serial dilution technique.

Introduction

Sugarcane (*Saccharum officinarum*) is an important cash crop of Pakistan which is mainly grown for sugar and sugary production. Cultivation of sugarcane provides an important source of income and employment for the farming community of the country. Apart from its edible uses, it is also used for making chip board, paper, barrages, confectionery, uses in chemicals, plastics, paints, synthetics, fibre, insecticides and detergents. Pakistan occupies an important position in cane producing countries of the world. Sugarcane production in Pakistan has increased overtime. Area of sugarcane for the year 2007-2008 were 308.8 hectares and production 18793.9 tones with an average yield of 60.9 kg/hectare in Sindh whereas in overall Pakistan the area was 1241.3 hectares and the production was 63920.0 tones (Anon., 2008).

Sucrose, monosaccharides, some polysaccharides and glycoproteins are associated with sugarcane juice (De Armas *et al*., 1999). Some sellers in sugarcane juice also add the lemon, ginger and mint for enhancing the taste. Lemon is not used just for enhancing the flavor but it also act as disinfectant. Ohio University reveals that lemon oil aroma does not influence the human immune system but may enhance mood. The inclusion of the lime juice during the main meal of the day was determined to have been protective against the contraction of cholera (Rodrigus & Strom, 2000). But the stored lemons are subjected to the growth of molds (Morton & Miami, 1987).

The occurrence of mold from sugarcane and sugarcane juice are also common. According to Sanguino & Tokeski (1980), *Cladosporium, Curvularia, Fusarium, Helminthosporium, Pestalotia* and *Phoma* are found in sugarcane caryopses. In sugarcane juice, the microbial contamination found were mainly yeast (Milintawisamai *et al*., 2009). The distribution of *Aspergillus flavus* and *A.parasiticus* in sugarcane field soils and on harvested sugarcane stems were studied by Takahashi *et al*., (2004) who found

that aflatoxin production were ca. 89% in 146 of 164 and of all the isolates ca. 69% were *A. flavus* isolates. Aflatoxin G was produced by 40 % of *A. flavus* isolates. *Aspergillus niger* and *A. flavus* are the common allergens and may cause opportunistic invasive infections (De hoog *et al*., 2000; Denning, 1998; Mau *et al*., 2002). *Aspergillus fumigatus* from and its dense population in sugarcane bagasse seemed to suggest a special association of the fungus with this substrate (Sandhu *et al*., 1997). *A. fumigatus* is an ubiquitous mold and the most common cause of invasive aspergillosis in immune compromised patients (Oricuolo *et al*., 2007). *A. fumigatus* also produced a kojic acid mycotoxin that can cause cardiovascular and brain disorders. Yeasts (*Saccharomyces* spp.) have been used as an alternative to filamentous fungi to produce citric acid (Burden & Eveleigh, 1990). A species of yeast (*Saccharomyces boulardii*) have been shown to reduce the symptoms of acute diarrhoea in children (Kurugol & Koturoglu, 2005; Sauri & Sierra, 1994) and prevent the re-infection of *Clostridium difficile* (McFarland *et al*., 1994).The growth of the yeast is inhibited by organic acids and toxin produced by bacterial activity (Oliva-Neto & Yokoyava, 1997).

The purpose of this study was to acquire the knowledge on quality of sugarcane juice which is selling in Karachi at different places and to generate data that can be used to provide awareness to the community and also help to improve the conditions of sugarcane juice making.

Materials and Methods

Collection of samples: Twenty eight sugarcane juice samples with and without lemon were collected from different places of Karachi including Samanabad (2), Alnoor-society (1), Saddar (4), Orangi town (1), Tariq road (1), Shah-faisal colony (1), Garden (1), Boat basin (1), Haroon bahria (1), Numaish chowrangi (1), Gulistan-e-Johar (2), Airport (1),Nipa (1), Kiran Jahangir colony (1), University campus (1), Sohrab goth (3), Maskan (1), Water-pump(3) and from Ranchorline (1).

pH of samples: Small quantity of sugarcane juice sample was taken and pH determined by using the pH meter (Brady, 1990).

Isolation of fungal flora: In direct plating method 0.01 ml of sugarcane juice was dispersed in a sterilized Petri plate and approximately 10-15 ml of molten cooled PDA was poured containing @ 20,000 units/l penicillin and @ 200 mg/l streptomycin and then after slightly rotating the Petri plate was left for solidification (Warcup, 1950). For the observation of colony forming unit of fungal flora, 2 ml sugarcane juice sample was suspended in sterilized test tube containing 18 ml of sterilized distilled water was shaken well which gave dilution of 1:10. 2 ml of suspension from 1: 10 was transferred to second test tube which gave 1:100 dilutions. Similarly 1:1000 and 1:10000 dilutions were made. There were three replicates for 1:1000 and 1:10000 dilutions. 1 ml aliquot from 1:1000 and 1:10000 dilutions were transferred to the sterilized Petri plates and 10-15 ml of molten cooled agar poured containing 20,000 units/liters penicillin and 200 mg/liters streptomycin. Petri dishes were incubated at 28±2^{o}C for 5-7 days. Procedure was replicated thrice for both lemon containing sample and non-lemon containing samples (Waksman & Fred, 1922).

Identification of flora: Mycoflora of sugarcane juice samples were identified by using standard mycological literature (Barnett, 1960; Domsch *et al*., 1980; Ellis, 1971; Nelson *et al*., 1983; Raper *et al*., 1965).

Results

pH of samples: pH of sugarcane juice with lemon ranged from 4.60-6.56 whereas the pH of sugarcane juice without lemon was acidic and ranged from 5.0-6.85 (Fig. 1).

Isolation of fungi: Results of direct plating method showed that about 18 different species of fungi belonging to 11 genera viz., *Aspergillus flavus* Link ex Gray, *A. fumigatus* Fres., *A. niger* Van Tiegham, *A. sulphureus* Thom & Church, *A. terreus* Thom, *A. wentii* Wehmer, *Fusarium semitectum* Berk & Rav, *F. sporotrichoides* Sherb, *Absidia corymbifera* (Cohnn) Sacc and Trotter, *Acremonium* sp. Link ex Fr., *Curvularia lunata* (Wakker) Boedijin, *Gilmanieila humicola* Barron, *Monilia* sp., Pers ex Fr., *Penicillium* sp., Link ex Fr., *Rhizopus oryzae* Went & Prisensen Geerlings, *R. stolonifer* (Ehrente ex Link) Lind, *Humicola grisea* Traaen., and yeast (*Saccharomyces* spp.) were isolated from sugarcane juice without lemon. *A. niger* was predominant and showed highest frequency of occurrence (71.42± 3.39) while the 9 different species showed the same frequency of occurrence with the lowest infection % viz., *Absidia corymbifera* (3.57±0.06*), A. sulphureus* (3.57±0.06), *A. wentii* (3.57±2.69), *C. lunata* (3.57±1.85), *G. humicola* (3.57±2.69), *F. semitectum* (3.57±1.40*), Monilia* sp., (3.57±0.61), *Penicillium* sp (3.57±0.43) followed by *A. niger, A. flavus* and *A. fumigatus* which showed high frequency of occurrence with the infection % of 53.5 ± 3.87 and 46.42±1.73 respectively. *Acremonium* sp., *A. terreus* and *F. sporotrichoides* were also fairly abundant with the infection % (32.14±3.53), (32±2.12) and (25±2.67) respectively. 71% sugarcane juice samples without lemon and 82 % samples with lemon were found to be contaminated with *A. niger.* In addition to this, 17 different species of fungi with different frequency of occurrence were isolated from sugarcane juice with lemon (Table 1).

A total of 19 different species of fungi belonging to 10 genera were isolated by serial dilution technique with and without lemon. *A. niger* was predominant fungus of sugarcane juice without lemon and showed the highest frequency of occurence (98.02 in 10^4 dilutions). Other isolated fungi include *Absidia corymbifera, Acremonium* sp., *Aspergillus flavus, A. fumigatus, A. candidus, A. erythrocephalus, A. subolivaceous, A. sulphureus, A. tamari, A. terreus, A. wentii, Curvularia lunata, Gilmanieila humicola, Monilia* sp, *Fusarium sporotrichoides, Rhizopus stolonifer, Penicillium* sp and yeast. *A. flavus* (68.77 in 10^4 dilution) and *A. fumigatus* (68.77 in 10^4 dilution) were fairly abundant in the samples (Table 2).

The species which were isolated through serial dilution technique from the sugarcane juice with lemon were same as isolated from the sugarcane juice without lemon.. However, addition of lemon in sugarcane juice reduced the occurrence of *Absidia corymbifera, Curvularia lunata* and *A. erythrocephalus* (Table 2).

Discussion

The main objective of the study was to determine the pH values and frequency of mycoflora associated with sugarcane juice. The prevalence of fungi was observed in both the sugarcane juice samples i.e., with and without lemon, which were collected from different areas of Karachi city. Mostly the sugarcane juice hawkers are situated at the road side so the number of pollutants and micro organisms in the air may add to the juice. Yusof *et al.*, (2000) reported that fresh sugarcane juice became spoiled at 5±1^{o}C and 1 day stored at 27±1^{o}C.

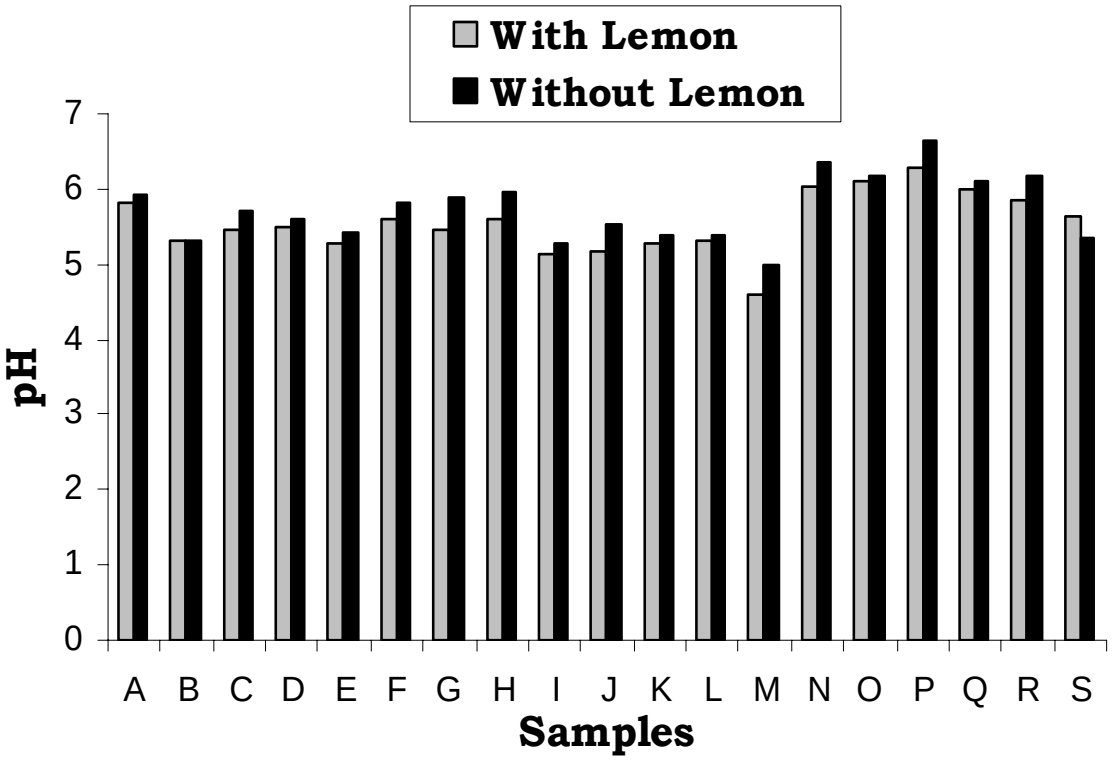

Fig. 1. pH of the samples of sugarcane juice with and without lemon
A= Samanabad, B= Alnoor-society, C= Saddar, D= Orangi town, E= Tariq road, F= Shah-faisal colony, G= Garden, H= Boat basin, I= Haroon bahria, J= Numaish chowrangi, K= Gulistan-e-Johar, L= Airport, M= Nipa, N= Kiran Jahangir colony, O= University campus, P= Sohrab goth, Q= Maskan, R= Water-pump, S= Ranchorline.

Table 1. Isolation of fungi from sugar cane juice with and without lemon by direct plate method.

Name of fungi	Without lemon			With lemon		
	NSI	I % ± S.D.	S.E.	NSI	I % ± S.D.	S.E.
Absidia corymbifera	1	3.57 ± 0.06	0.01	1	3.57 ± 0.06	0.01
Acremonium sp.	9	32.14 ± 3.53	0.66	11	39.28 ± 2.45	0.46
Aspergillus flavus	15	53.5 ± 3.87	0.73	16	57.14 ± 2.06	0.38
A.fumigatus	13	46.42 ± 1.73	0.326	13	46.42 ± 1.69	0.31
A.niger	20	71.42 ± 3.39	0.640	23	82.14 ± 4.38	0.82
A.sulphureus	1	3.57 ± 0.06	0.01	2	7.14 ± 0.08	0.01
A.terreus	9	32 ± 2.12	0.40	5	17.85 ± 0.98	0.18
A.wentii	1	3.57 ± 2.69	0.50	3	10.71 ± 3.06	0.57
Curvularia lunata	1	3.57 ± 1.85	0.34	2	7.14 ± 1.88	0.35
Fusarium semitectum	1	3.57 ± 1.40	0.26	-	-	-
F. sporotrichoides	7	25 ± 2.67	0.50	5	17.85 ± 2.89	0.54
Gilmanieila humicola	1	3.57 ± 2.69	0.50	1	3.57 ± 1.91	0.36
Humicola grisea	1	3.57 ± 1.34	0.25	-	-	-
Monilia sp.	1	3.57 ± 0.61	0.11	2	7.14 ± 0.43	0.08
Penicillium sp.	1	3.57 ± 0.43	0.08	2	7.14 ± 0.08	0.01
Rhizopus oryzae	2	7.14 ± 0.25	0.04	2	7.14 ± 0.48	0.09
R. stolonifer	4	14.28 ± 0.49	0.09	6	21.4 ± 0.36	0.06
Yeast (*Saccharomyces* spp.)	2	7.14 ± 0.84	0.15	5	17.85 ± 1.61	0.30

NSI = Number of samples infected
S.D = Standard deviation
S.E. = Standard error

Table 2. Isolation of fungi from sugarcane juice without and with lemon by serial dilution method.

Name of fungi	Dilution factor			
	Without lemon		With lemon	
	NSI	CFU/ml 10^4	NSI	CFU/ml 10^4
Absidia corymbifera	1	0.33	2	1.33
Acremonium sp.	7	21.65	8	28.98
Aspergillus candidus	1	0.66	2	0.66
A.erythrocephalus	1	0.66	1	0.66
A. flavus	20	68.77	20	45.6
A. fumigatus	21	77.25	22	60.27
A. niger	21	98.02	20	100
A. subolivaceous	1	13.33	00	00
A. sulphureus	2	3.66	1	1.33
A. tamarii	1	1.66	3	2.98
A. terreus	10	17.97	10	22.97
A. wentii	2	0.99	2	4.66
Curvularia lunata	2	3.33	3	6.66
Fusarium sporotrichoides	7	7.47	6	15.31
Gilmanieila humicola	2	12.16	2	11.33
Monilia sp.	1	3.0	2	2
Penicillium sp.	4	9.64	4	5.65
Rhizopus stolonifer	4	3.66	-	-
Yeast (*Saccharomyces* spp.)	7	33.15	6	22.65

NSI = Number of samples infected.
CFU = Colony forming units.

Our present studies showed that the mycobiota was dominated by the species of *Aspergillus, Acremonium* and Yeast (*Saccharomyces* spp.). Among *Aspergillus* the most frequent species were *Aspergillus niger* followed by *A. flavus*, *A. fumigatus* and *A. terreus*. In addition to these *Curvularia lunata*, *Gilmanieila humicola*, *Fusarium sporotrichoides, Monilia* sp., and species of *Rhizopus* were also isolated. *Aspergillus* species were also frequently present in drinking water as well as in juice samples (Nazim *et al*., 2008; Anaissie *et al*., 2001). Kumeda *et al*., (2003) reported that isolates of FP-1 which is distributed throughout sugarcane field soil in the Southern-most Island of Japan, is belonging to section Flavi, and were able to produce aflatoxin B and G. *A. niger* was reported to cause most serious disease of crown rot of peanut (Gibson, 1953a & 1953b) whereas *A. flavus*, which is considered as the important mycotoxins producer can cause severe damage to the liver, kidneys and nervous system of man even at a low dosage (Rodricks, 1976). A. *niger* and *A. flavus* are common allergens and may cause opportunistic invasive infections (De Hoog *et al*., 2000; Denning, 1998; Mau *et al*., 2002). Two different species of *Fusarium* viz., *F. semitectum* and *F. sporotrichiodes* which were isolated through direct plate method but only *F. sporotrichiodes* was isolated from serial dilution technique. *Fusarium* was ranking on the seventh number among the total fungal species which showed the highest frequency of occurrence in serial dilution technique. *Fusraium* which produce mycotoxin can have a role in human esophageal carcinogenesis (Hsia *et al*., 1983). *Fusarium* species only causes superficial and subcutaneous infections such as onychomycosis and keratomycosis in human (Nelson *et al.*, 1994). *Fusarium* are now considered as third most common fungal genus isolated from systemic infections in the bone marrow transplantation patients (Morrison *et al*., 1993). *Fusarium* and *Drechslera* are included in potentially pathogenic species

(Gravesen *et al*., 1994; Samson & Pitt, 1990). Yeast (*Saccharomyces* spp.) was also significantly isolated through the serial dilution technique in both type of sugarcane juices with and without lemon. De Azeredo *et al*., (1998) isolated *Cryptococcus laurentii*, *Cryptococcus albidus*, *Rhodotorula mucilaginosa* and *Debaryomyces hansenii* from sugar cane juice. The implification of *Penicillium* sp., in allergy, asthma or other respiratory problems has been a subject of several studies worldwide (Schwab & Straus, 2004). Strong association between *Penicillium* sp., and health problems were also reported by Cooley *et al*., (1998). The genus also includes common contaminants of food and beverages (Samson & Pitt, 1990; Pitt & Hocking, 1999).

The results obtained from the present investigation indicates that a wide variety of fungal species were present in sugarcane juice samples all over Karachi. A number of harmful fungi were isolated on several occasions which produce number of mycotoxins and caused potential health hazard to human. There is need to determine the kind and amount of mycotoxin present in sugarcane juice and also improve the quality of sugarcane juice in order to save the human health.

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(Received for publication 30 December 2009)