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FRUIT BORNE MYCOFLORA OF AMLA (*PHYLLANTHUS EMBLICA* L.)

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Abstract

Fungal species composition on retail Amla fruits obtained from local market of three localities of Hyderabad, Hala and Tando Allah Yar was studied. Forty-eight fruit samples were randomly picked from each locality. Using two media types, a total of 19 genera and 42 fungal species were isolated from surface sterilized and non-sterilized fruits. From fruits of all three localities, species in the genera *Aspergillus* (5 species), *Penicillium* (4 species), *Fusarium* (4 species), *Alternaria* (4 species), *Cladosporium* (5 species) and *Curvularia* (3 species) were isolated most frequently. The fungal frequency of Tando Allah Yar samples was higher. *A. niger* and *A. flavus* were the most common fungi isolated from fruits of all three localities. The fungi isolated do not appear to be recorded from fruits of Amla in Pakistan.

Introduction

Over the past 40 years, fungi in foods have received special attention because of their ability to produce toxic metabolites. Although some fungi such as *Claviceps purpurea* have been known for centuries because of their high and acute toxicity, it was only after the discovery in 1960 of the aflatoxins, carcinogenic metabolites of *Aspegillus flavus*, that a large number of species were found as mycotoxin producers (Northolt & Soentoro, 1988). The production of mycotoxins, a group of secondary fungal metabolites, is reportedly dependant on the physic-chemical environment where the mould develops (Jimenez *et al.*, 1991). Under favourable temperature and humidity these fungi grow on certain foodstuffs, most commonly ground nuts, dried fruits, tree nuts, spices and a range of cereals. Morton (1987) reported that emblics preserved in the market have been found contaminated with yeast, molds and bacteria and in storage were subjected to blue mold and rotting caused by *Penicillium islandicum.* Infection by fungi and bacteria may occur during the growing season, at harvest time, during handling, storage, transport and marketing, or even after purchase by the consumer (Dennis, 1988). These fruits of Amla (*Phyllanthus emblica* L.) which are frequently used in making pickles, jellies and preserves are probably the richest known natural source of vitamin C. Its mineral and vitamin contents include calcium, iron, phosphorus, carotene, thiamine, riboflavin, niacin, vitamin C (one fruit contains as much as 24 oranges) and tannins. They are also used in making quality inks, ordinary dyes and shampoos and in tanning industry. In addition, dried Amla fruit is used in Ayurvedic and Unani system of medicine for various ailments like fever, liver disorder, indigestion, anemia, heart complaints and urinary problems (Bhattacharjee, 2004). The economic loss resulting from fungal and mycotoxin contamination of food stuffs is difficult to estimate. However, judging from the widespread occurrence of fungal and mycotoxin contamination and the large number of

food stuffs affected, one can assume that such losses must be large (Stinson, 1981). These losses result in human illness, reduced food supply, poorer quality, economic hardships for growers and processes and ultimately higher price.

Taking into consideration the economic and medicinal value of Amla, a survey was conducted in retailer market of three different localities viz., Hyderabad, Hala and Tando Allah Yar to examine the samples of Amla for the presence of fungal species.

Materials and Methods

Fruit samples of Amla were collected from retailer market of Hyderabad, Hala and Tando Allah Yar. The fruits were randomly picked and were surface sterilized with a 2% aqueous solution of Sodium hypochlorite (NaOCl) for two minutes followed by rinsing with sterile distilled water (Kulik, 1981). Another set of untreated fruits was also used. The dried shell of fruits were then cut into 4 pieces with a sterile scalpel and plated together equispaced from each on PDA and Czapek's Dox Agar. Two of the pieces had their inner surfaces turned up and remaining two had their outer surface turned down. Plates were incubated at 28°C for 7 days during which the number of pieces that yielded colonies was noted, enumerated and sub cultured for identification. The fungi were identified after reference to Thom & Raper, (1945), Gilman (1957), Ellis (1971, 1976), Ellis & Pamella (1985), Booth (1971), Domsch *et al*., (1980). The total number of fungal species was calculated on percent basis to find out the difference in nature and number of fungi arising out between treated and non-treated fruits and the difference of two media used.

Results and Discussion

a. Hyderabad: Out of 48 samples, collected from retail market of Hyderabad, 15 species belonging to 9 genera viz, *Aspergillus candidus, Acremoniella velata, Acrospeira mirabilis, Aspergillus flavus, A. niger, A. ochraceous, Ceratocystis moniliformis, Cladosporium resinae, Curvularia protruberata, Fusarium poe, F. semitectum, F. sporotrichioides, F. ventricosum, Paeclomyces variotii* and *Veronica pervispora* were isolated and identified. Surface sterilized fruits yielded higher number of fungi than from fruits without sterilization. The highest number of fungal species was obtained on Potato Dextrose Agar with and without treatment (Table 1). Of the fungi isolated *Aspergillus niger, A. flavus, Ceratocystis moniliformis, Fusarium sporotrichioides* and *Paecelomyces variotti* were few predominant species.

b. Hala: Out of 48 samples collected from retail market of Hala, 14 species belonging to 7 genera viz, *Aspergillus granulosus, A. niger, A. ochraceous, A. flavus, Cladosporum spherospermum, Penicillium cyano-fulvum, P. digitatum, P. islandicum, P. paxilli, Periconia cambrensis, Scytalidium lignicola, Stachylidium bicolor, Torulla graminis, Torulla ndjilensis* were isolated and identified. Of the fungi isolated *A. niger, Penicillium islandicum* and *A. flavus* were found to be predominant. Here also surface sterilized fruits yielded higher number of fungi than fruits without sterilization. But in contrast to Hyderabad samples, highest number of fungal species was obtained on Czapek Dextrose agar with and without treatment (Table 1).

Table 1. Occurrence (%) of fungi from *Phyllanthus emblica* L. (Amla fruits).

Location / Name of fungi	Surface disinfected		Non disinfected	
	PDA	Czapek	PDA	Czapek
Hyderabad				
Acremoniella velata	2.82	…	…	…
Acrospeira mirabilis	…	…	2.64	…
Aspergillus candidus	1.22	…	…	…
Aspergillus flavus	1.22	4.66	6.90	7.51
Aspergillus niger	11.16	…	9.53	11.16
Aspergillus ochraceus	…	…	3.04	
Ceratocystis moniliformis	…	…	…	7.91
Cladosporium resinae	1.83	…	…	…
Curvularia protruberata	1.42	…	…	…
Fusarium poe	…	…	2.23	…
Fusarium semitectum	…	…	…	4.06
Fusarium sporotrichioides	…	…	7.30	…
Fusarium ventricosum	2.03	…	3.85	…
Paeclomyces variotii	6.49	…	…	…
Veronica pervispora	1.01	…	…	…
Hala				
Aspergillus flavus	…	…	6.57	5.54
Aspergillus granulosus	…	…	3.9	…
Aspergillus niger	5.13	1.85	13.55	9.24
Aspergillus ochraceous	…	4.52	…	…
Cladosporium spherospermum	1.23	…	…	…
Penicillium cyano-fulvum	…	6.16	…	…
Penicillium digitatum	…	…	…	5.13
Penicillium islandicum	14.58	6.78	…	…
Penicillium paxilli	…	3.08	…	…
Periconia cambrensis	…	4.11	…	…
Scytalidium lignicola	…	…	…	2.46
Stachylidium bicolor	…	2.05	…	…
Torulla graminis	…	…	…	2.26
Torulla ndjilensis	…	…	1.85	…
Tando Allah Yar				
Aspergillus flavus	…	3.31	…	7.62
Aspergillus niger	11.42	15.40	17.22	14.57
Alternaria brassicola	2.8	…	…	…
Alternaria citri	3.15	…	…	…
Alternaria dennissi	…	…	1.82	…
Alternaria raphani	…	…	2.65	…
Canoplea mangenetii	…	0.50	…	…
Cladosporium cladosporioides	2.48	1.49	…	…
Cladosporium gallicola	…	2.48	…	…
Cladosporium orchidearum	1.32	…	…	…
Curvularia geniculata	1.66	…	…	…
Curvularia pallescens	…	2.98	…	…
Dennisioidiscus presinus	…	0.33	…	…
Dreschlera australiensis	…	…	1.32	…
Drechslera hawaiensis	0.99	…	…	…
Drechslera ravenatii	0.66	…	…	…
Periconia britanica	0.50	2.48	…	…
Polypaecilum insolitum	…	…	0.83	…

c. Tando Allah Yar: Out of 48 samples, collected from retail market of Hala, 18 species belonging to 9 genera viz., *Aspergillus niger, A. flavus, Alternaria brassicola, A. citri, A. dennissi, A. raphani, Canoplea mangenetii, Cladosporium orchidearum, C. cladosporioides, C. gallicola, Curvularia geniculata, C. pallescens, Dennisioidiscus presinus, Drechslera australiensis, D. hawaiiensis, D. ravenatii, Periconia britanica*, and *Polypaecilum insolitum* were isolated and identified. Of these, *A. niger, A. flavus, Cladosporium cladosporioides, Alternaria citri, Curvularia pallescens, Periconia britanica, Alternaria brassicola* and *A. raphani* were found to be predominant. From these samples, surface sterilized fruits yielded higher number and variety of species on both media (Table 1).

A difference in species composition in fruit samples of three locations studied was apparent. The samples of Tando Allah Yar yielded quantitatively as well as qualitatively more fungi than the samples of Hyderabad and Hala. The fungi isolated do not appear to be recorded from fruits of Amla in Pakistan (Sultan *et al*., 1997). It is interesting to note that *A. niger* and *A. flavus* were most frequent and recorded from 70.14% and 24.75% fruits samples respectively, whereas, *P. islandicum* was isolated with incidence of 58.33% only from the samples of Hala. This result is similar with the observation of Morton (1987) who found rotting of Amla fruits caused by *P.islandicum* in storage. *Aspergillus* and *Cladosporium* were the common genera on two types of media used. *Alternaria* and *Periconia* were common on PDA only whereas all *Penicillium* species were recorded in Czapek medium except *P. islandicum*, which was isolated from both media (Table 1).

It is interesting to note that in Pakistan and India people are used to grind Amla fruits into a fine powder and use it in various ways for the cure of various ailments. For example, to maintain the cholesterol level, to treat diabetes, conjunctive, glaucoma and acidity. And most commonly, it is being used for promoting hair growth and in prevention of premature graying of hair by majority of ladies. (Anon., 2008).

However, present study put a big question mark on the efficacy of this "gift of nature", which is here reported to be rich in fungal population. Especially, *Aspergillus, Penicillium* and *Fusarium* associated with Amla fruits are known to have strains that produce toxic metabolites. This observation strengthens the need for close monitoring of the mycological quality of retail Amla fruit samples to protect the public health. Furthermore, it is strongly recommended that the dominant flora of Amla fruits in other areas should also be determined. Correct species identification will indicate the mycotoxins which may be present in given samples under given environmental conditions and indicate ways to prevent mycotoxin production.

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