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Occurrence of Mycobiota Associated With *Ficus carica* L.

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Abstract:
Fungal contamination of various agricultural commodities and foodstuffs (like dry fruits), is a major problem in the developing countries like India. Fungi play a significant role in deteriorating the aesthetic and nutritive value of stored food commodity. Therefore, the aim of this study was to evaluate the mycoflora associated with figs.

Ten samples of dried figs were collected from local shops of Amravati region during 2016-17. Samples were analyzed for the moisture contents and the presence of fungi by adopting direct plating and dilution plating methods. Altogether 15 fungal species were isolated from figs *viz. Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus versicolor*, *Eurotium chevalieri*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Fusarium solani*, *Penicillium granulatum*, *Penicillium nigricans*, *Penicillium oxalicum* and *Rhizopus stolonifer*. Among all the fungi, genus *Aspergillus* was the most predominant isolate with 6 different species. Two species from *Aspergillus,* Section *Flavi*-*A. Flaus* and *A. parasiticus* are known to produce the toxic and carcinogenic compounds aflatoxins (AFs) which are hazardous to animal and human health.

Therefore, the occurrence of contamination with spoilage and toxigenic fungi in dried figs could be avoided or at least diminished if good agricultural (harvesting and handling), manufacturing (sorting and packaging) and proper storage practices will be applied.

Keywords: Dried figs, postharvest, ecological factors, mycoflora, afiatoxins (AFs).

Introduction

Fig (*Ficus carica* L.) family -Moraceae is a native to south west Asia and spread to Mediterranean by human (Tous and Ferguson, 1996). Fungal infections to figs may occur on the tree during ripening stages, after falling from the tree, during drying process, storage, transportation and handling (Ozay *et. al.*, 1995; Heperkan *et. al.*, 2012). Both the skin and inner cavity of fig fruits can be contaminated by fungi.

Thirty-one species assigned to 14 genera were isolated from dried figs. *Aspergillus* was represented by maximum 12 species, *Penicillium* was second by 5 species. Three teleomorphic ascomycetes namely, *Emericella nidulans, E. quadrilineata* and *Eurotium amstelodami* also detected. (Sadullah and Abdullah, 2015). Several environmental factors like humidity and temperature during storage influence the infestation by fungi and aflotoxin production (Drusch and Ragab, 2003).

Natural occurrence of fungal contamination of dried fruits and spices have been investigated in many parts of the world by different authors (Zohri and Abdel- Gawad, 1993; Ozay *et. al.,* 1995; Abdel-Sater and Saber, 1999; MacDonald *et. al.*, 1999; Bayman *et. al.*, 2002; Möller and Nyberg, 2003; Aksoy *et. al.*, 2007; Juan *et. al.*, 2007; Zinedine *et. al.*, 2007; Musaiger *et. al.*, 2008; Ozay and Özer, 2008; Bircan, 2009; Hedawoo and Chakranarayan, 2011).

Figs infection by toxigenic fungi has been reported in a number of studies and revealed a high risk due to contamination with mycotoxins (Bircan, 2009; Heperkan *et. al.*, 2012). Moreover, fungi contaminated dry figs caused considerable changes of all the biochemical contents (total Carbohydrates, Sugar, Proteins, Fat and dietary fibers) as well as affecting quality (Embaby *et. al.*,2012).

Materials and Methods:-

a) Sample collection:-

Ten samples of dried figs were purchased from local markets of Amravati region. The collected samples were put in paper bags and brought into laboratory for isolation of fungi.

b) Moisture content:-

The moisture content of dried figs was determined using the International Organization for Standardization (ISO) method (Hamid and Lopez,2000).

c) Mycological analysis:-

i) Direct plating method- Direct plating is considered to be the more effective technique for mycological examinations of particulate foods. The dried fig pieces were surface disinfected with 2% Sodium hypochlorite solution for 2 min. then rinsed with sterile distilled water. Seven pieces

were placed in each petri plates containing PDA medium. The plates were incubated at 27^0 C for 7 days (Pitt and Hocking, 1999).

ii) Dilution plating method- The dilution plating method is the most commonly used technique for the examination of food and feedstuff (Jarvis *et. al.*, 1985). According to International Commission on Microbiological Specifications for fruits (ANON, 1989), sample suspension were prepared by adding 40gm of sample in 200ml sterile distilled water for 2 – 4 hours. Then shake well using a mechanical shaker for 20-30 minutes.Serial dilutions were prepared from 10^{-1} to 10^{-5}ml under aseptic condition, fungal spores sediment more quickly, so it is important to draw aliquots for dilution or plating as soon as possible (Beuchat,1992). One ml of appropriate dilution was transferred into petri plates contains PDA medium by sterile pipette, for each sample three replicates used, then plates were incubated at 27^{o}C for 7days (Akerstrand, 1995).

d) Identification of fungi:-

All the fungi were identified on the basis of their colony morphology and spore characteristics (Rajankar *et.al.*, 2007). All species identifications were according to the keys, manuals and descriptions provided by Raper and Thom (1949); Raper and Fennel (1965); Gilman (2001); Subramaniyan (1971); Nagmani *et.al.*, (2006)

e) Percent occurrence:-

For calculating the percent occurrence, following formula was used -

$$\% \text{ occurrence of fungus} = \frac{\text{No. of colonies of a particular fungal species in all plates}}{\text{Total no. of colonies of all the fungal colonies in all the plates}} \times 100$$

TABLE- 1 :- Optimum temperature, atmospheric humidity and moisture %

Month	Temperature ^{0}C		Average humidity (%)	Moisture content in figs (%)
	Min.	Max.		
December- 2016	8	32	56	10.15
January- 2017	10	35	54	
February- 2017	14	38	42	

(**Source:-** Weather report in Amravati, India https://www.timeanddate.com)

TABLE- 2 :- Isolated mycoflora & their percent occurrence on dried figs

Sr. No.	Fungi Isolated	Direct plating method	Dilution plating method	% Occurrence
1.	*Alternaria alternata*	-	+	1.29
2.	*Aspergillus candidus*	+	+	2.23
3.	*Aspergillus flavus*	+	+	1.92
4.	*Aspergillus fumigatus*	-	+	1.28
5.	*Aspergillus niger*	+	+	2.5
6.	*Aspergillus parasiticus*	-	+	2.04
7.	*Aspergillus versicolor*	-	+	27.18
8.	*Cladosporium cladosporioides*	-	+	8.8
9.	*Cladosporium herbarum*	-	+	0.94
10.	*Eurotium chevalieri*	+	+	52.72
11.	*Fusarium solani*	+	+	5.402
12.	*Penicillium granulatum*	-	+	1.304
13.	*Penicillium nigricans*	+	+	13.382
14.	*Penicillium oxalicum*	-	+	5.312
15.	*Rhizopus stolonifer*	+	+	2.04

(+) Fungus present, (-) Fungus absent

Results and Discussions

The experiments were carried out during winter season in the month of Dec.16, Jan.17 and Feb.17. In that period the minimum temperature was recorded as- 8^{0}C, 10^{0}C & 14^{0}C and maximum temperature was 32^{0}C, 35^{0}C & 38^{0}C in the respective months. Also the average humidity percentage was recorded as- 56%, 54% and 42% respectively. (**Table-1**). It was noticed that, the growth of fungi is directly proportional to optimum temperature. As the temperature increases fungal growth also increases. Similarly, growth of fungi is also directly proportional to

humidity. As humidity increases, fungal growth also increases as supported by Pal (2015).

In the beginning of experiment, moisture content in the dried figs was measured as 10.15 % (**Table-1**). This range showed appropriate moisture content of the fig samples which allow the growth of xerophilic fungi. Our results agree with Beatriz *et. al.*, (2006) which states that, high sugar concentration and low water activity in dried fruits assist the development of xerophillic fungi like *Aspergilli* and *Penicilli* especially *A. niger* (Toma and Rajab, 2014).

For mycological analysis, dried figs were plated aseptically in direct plating or indirect plating (Serial dilution plating). In direct plating technique, total 7 fungi were noticed viz. *Aspergillus candidus, Eurotium chevalieri, A. flavus, A. niger, Fusarium solani, Penicillium nigricans* and *Rhizopus stolonifer*. Fifteen fungal species representing seven genera were isolated by serial dilution technique. Their percent occurrence (contamination) is presented in **Table-2**.

Most of the recovered fungi were previously reported from dried figs in many parts of the world (Zohri and Abdel-Gawd, 1993; Bayman *et. al.*, 2002; Alghalibi and Shater, 2004; Iamanaka *et. al.*, 2007; Senyave *et. al.*, 2008; Embaby *et. al.*; 2012; Heperkan *et. al.*, 2012; Saadulllah and Abdullah, 2015; Javanmard, 2010; Doster *et. al.*, 1996; Toma and Rajab, 2014, Tournas *et. al.*, 2015).

Three species were isolated with high frequency namely- *Eurotium chevalieri* (52.72%), *Aspergillus* versicolor (27.18%) and *Penicillium nigricans* (13.38%); followed by *Cladosporium cladosporioides* (8.8%), *Fusarium solani* (5.40%) and *Penicillium* oxalicum (5.31%). While *Cladosporium herbarum* showed the least frequency i.e. (0.94%). Our results are in line with the report of Saadulllah and Abdullah (2015), where the fungal contamination is associated with figs in the Kurdistan region of Iraq.

Aspergillus was represented by 6 species and showed the widest diversity among all isolated fungi *viz. A. candidus, A. flavus, A. fumigatus, A. niger, A. parasiticus* and *A. versicolor* followed by *Penicillium* (3) and *Cladosporium* (2) species. These species were found common to soil, different agricultural and food commodities in India (Srivastava *et. al.*, 2014).

A.flavus (1.92%) and *A. parasiticus* (2.04%) and to less extent some species in the genus *Fusarium* (5.40%) are the most important species contaminating dried figs because of their potential to produced mycotoxins (Heperkan *et. al.*, 2012).

Due to the contamination of aflatoxins, the fig is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide; but in India the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conductive for fungal growth, proliferation and aflatoxin contamination (Reddy *et. al.*, 2011).

Conclusion

The present study revealed that dried figs are highly contaminated with several mycotoxigenic fungi such as *A. flavus, A. parasiticus* and others. Therefore, strict hygiene mycological measured should be done during harvest, storage and drying to minimize contamination with such fungi. Therefore, the authorities should take the lead in the efforts to establish mandatory regulations in fig farming to decrease contamination risk to toxigenic fungi. These would lead to enhanced food safety, enhanced international trade efforts and improved public health. Development of efficient pre- and post-harvest hygienic practices must be considered as components to be integrated into fig production processing. Nowadays, research and practical directions for establishment of such good agricultural practices as hygienic harvesting (using nets under trees for fig collection), drying (mesh apparatus with about 1 meter height instead of drying on the cement flooring) and processing plants are being provided by authorities.

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