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Fungal Contamination of Some Common Spices

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A B S T R A C T

Spices form an integral part of our daily diet, medicine, religious rituals, cosmetics and perfumery. Ajowan, black pepper and fennel are extensively used spices in India. Spices are exposed to microbial contamination during pre, post-harvest and storage period. Seed Mycoflora not only deteriorate the quality of spices but also increase the mycotoxin contamination. Tropical countries like India suffer the losses mainly due to storage fungi. The present investigation deals with the isolation and identification of fungi associated with the ajowan, black pepper and fennel. Seed borne fungi was isolated by employing agar plate technique from both unsterilized and surface sterilized test seed samples. Total 26 fungal species were isolated from the test spices. Seven fungal species viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvalaria lunta, Mucorsp, Rhizopus nigricans* and *Penicillium chrysogenum* were commonly isolated from all the three spice samples. *Aspergillus* constitute a dominant storage fungi represented by 9 species.

Introduction

Spices constitute an important group of horticultural commodities which play a significant role in national economy. They are the plant substances from indigenous or exotic origin. According to the International organization for Standardization (ISO) the term 'Spices and condiments' refer to an aromatic or pungent vegetable substances used for flavouring and also have several commercial uses. Spices includes leaves (coriander, mint), flower (clove), leaf bases (garlic, onion), fruit (red chilli, black pepper), stem bark (cinnamon), rhizome (ginger, turmeric) and other plant parts (Ram Kumar and Paranay jain, 2010). They have been used as an integral part of our daily diet, medicine, religious rituals, cosmetics and perfumery. Spices are used as a raw material in folk medicine, as an ingredient in drug preparations of traditional medical systems, in pharmaceuticals and as a supplement for dietetic products especially for self medication (Weiser *et al.,* 1971). The therapeutic activity of spices is due to the presence of tartaric acid, acetic acid, citric acid, succinic acid, gums, pectin, sugars, tannins, alkaloids, flaviniods, glycosides and sesquiterpenes (Algohary *et al.,* 1994; Mohamedian *et al.,* 1996)

Aida oils, oleoresins, ground spices like curry powder, paprika and as a variety of spice mixtures. From last few decades, as there is a definite shift towards traditional/ ethnic medicines and spices form a part of many of the medicinal preparations. The demand for the good quality of the spices has been increased, but the quality of spices is far from being satisfactory due to the postharvest losses. Shelar *et al.,* (2008) stated seed deterioration is one of the basic reasons for the low productivity with annual losses of 25% of the harvested crop. The stored grains deteriorate rapidly and marked changes in quality and quantity are due to invasion of microorganisms and losses caused by them are referred to as biodeterioration (Christensen, 1957).

Spices are exposed to microbial contamination during pre and postharvest period (Hashem and Alamri, 2010). Contamination may also occur during processing, storage, distribution, sale and usage of spices (McKee, 1995). In spices, most of the microbial populations are probably regarded as commensal residents on the plant and survived drying and storage phases (Farid *et al.,* 2013). According to Kneifel and Berger, (1994) soil and air are the main inoculums source for causing contamination in crude spices in field and reported that fungi are the most predominant contaminants of spices. Krishnaswamy *et al.,* (1971) reported that mycoflora of spices like paprika, black pepper, white pepper and ginger was dominated by airborne fungi. Spices are heavily contaminated with xerophilic storage moulds such as *Aspergillus, Penicillium* (Skrinjar and Harvot, 1995; Dimic *et al.,* 2000; Santos Garcia *et al.,* 2001; Ramagnoli *et al.,* 2007; Abou-donia, 2008). Improper storage conditions provide ideal environment for the rapid colonization by storage fungi and drying on bare ground may results in toxic moulds contamination and mycotoxins production.

Materials and Methods

In the present work is aimed to throw light on the investigation of detailed survey of mycoflora of three spices viz Ajowon (*Trachyspermum ammi* L.) Black pepper (*Piper nigrum* L.) and Fennel (*Foeniculum vulgare* Mill.). Seed samples were collected from the local markets and malls in loose and pack form respectively from the Hyderabad. All the spice samples (12) were packed in transparent polythene bags and transported to laboratory. In the present study agar plate method has been used to isolate seed mycoflora.

Isolation of fungi was done from the unsterilized seeds (untreated) and surface sterilized seeds (treated) with 2% sodium hypochlorite solution by employing Agar plate technique (Muskett, 1948). Potato dextrose medium was used for the isolation of fungi. Inoculated petriplate were incubated at 27^0C. Examination of plates were done from the 4^{th} day up to 10^{th} day. Fungi appearing on the seeds and around it were isolated and monosporic cultures were raised for specific identification was done by referring relevant literature (Nagamani *et al.,* 2006). Incidence of fungal species is calculated by percentage relative frequency (Marasas *et al.,* 1988; Giridhar and Reddy, 1997).

Results and Discussion

Data given in the table, a total 26 fungal species were isolated from the test species viz *Alternaria alternata, Alternaria humicola, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus sydowii, Aspergillus tamarii, Aspergillus terreus, Chaetomium sp, Cladosporium sp, Cochiliobolus spicifer, Curvularia lunata, Drechslera hawaiiensis, Fusarium moniliforme, Fusraium oxysporum, Mucorsp, Penicillium chrysogenum, Rhizopus arrihizus, Rhizopus nigricans, Rhizopus stolonifera, Trichoderma longibrachiatum, Trichoderma viride*. A high percentage frequency of fungi was isolated from unsterilized seeds when compared to surface sterilized seeds. Fungi screened from the test samples belong to ascomycetes, zygomycetes and mitosporic fungi.

On individual basis 15, 17 and 19 fungal species were isolated from ajowon, black pepper and fennel.

Seven fungal species viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvalaria lunta, Mucorsp, Rhizopus nigricans* and *Penicillium chrysogenum* were commonly isolated from all the three spice samples. Maximum fungal diversity was detected in fennel (12 genera) followed by ajowan and minimum fungal diversity was found in black pepper. It was observed that *Aspergillus* constitute a dominant storage fungi represented by a 9 species.

It was observed that *Aspergillus niger* (28%) showed the maximum percentage of incidence and minimum of *Trichoderma longibrachiatum* (2%) on ajowan seeds. *Aspergillus flavus* was recorded at high percentage on black pepper (26%) and fennel (38%), where as *Fusarium oxysporum* (3%) and *Trichoderma viride* (2%) on black pepper and fennel seeds respectively. Spices constitute an important fraction of human diet due to its high nutritive value and they also act as good substrates for the growth of toxigenic fungi. Spices are exposed to a wide range of microbial contamination due to poor collection conditions, unpretentious production process, extended drying times and improper storage.

Table.1 Percentage frequencies of fungi isolated from three spices seed samples by agar plate method

S.No	Name of the fungi	Percentage (%) incidence of different species of fungi on spices.					
		Trachyspermum ammi		***Piper nigrum***		***Foeniculum vulgare***	
		US	S	US	S	US	S
1.	*Alternaria alternata*	20	17	20	12	27	18
2.	*Alternaria humicola*	-	-	22	16	27	12
3.	*Alternaria tenuissima*	18	12	-	-	-	-
4.	*Aspergillus candidus*	-	-	12	6	15	10
5.	*Aspergillus flavus*	18	15.5	28	26	40	38
6.	*Aspergillus fumigatus*	23.2	20	16	15	30	26
7	*Aspergillus niger*	30	28	19	17	22	20
8.	*Aspergillus nidulans*	14	6	8	6	8	6
9.	*Aspergillus ochraceus*	22	16	12	8	10	10
10.	*Aspergillus sydowii*	-	-	10	8	-	-
11.	*Aspergillus tamarii*	-	-	-	-	20	15
12.	*Aspergillus terreus*	16	10	20	10	30	20
13.	*Chaetomium sp*	15	08	15	12	20	12
14.	*Cladosporium sp*	16	6	-	-	-	-
15.	*Cochiliobolus spicifer*	-	-	-	-	32	26
16.	*Curvularia lunata*	-	-	18	16	36	24
17.	*Drechslera hawaiiensis*	10	8	9	6	12	10
18.	*Fusarium moniliforme*	-	-	-	-	-	-
19.	*Fusraium oxysporum*	8	6	8	3	-	-
20	*Mucorsp*	22	20	20	15	36	34
21..	*Penicillium chrysogenum*	18	16	12	10	40	35
22.	*Rhizopus arrihizus*	-	-	22	18	-	-
23.	*Rhizopus nigricans*	-	-	-	-	18	14
24.	*Rhizopus stolonifera*	-	-	-	-	28	18
25.	*Trichoderma longibrachiatum*	10	2	-	-	-	-
26.	*Trichoderma viride*	-	-	-	-	6	2

In addition, spices can be contaminated through dust, wastewater and animal human excreta in unpacked spices, which are sold in markets and bazaars (Banerjee and Sarkar, 2003; Schweiggert *et al.*, 2005).

In the present study 26 fungal species belonging to 12 genera were isolated from the three test samples. Improper harvest, drying, package practices, post-harvest and storage conditions also cause for wide spectrum of fungi. It shows that fungi are predominant contaminants of spices. The unsterilized seed harboured more number of fungi than surface sterilized seed.

The results obtained reveals that maximum fungal species were isolated from fennel, where as Moharram *et al.*, 1989 reported 25 species of *Aspergillus* from *Foeniculum vulgare* and *Pimpinella anisum* seeds collected from local markets of Egypt. Ath-Har *et al.*, (1988) studied the mycoflora of *Piper nigrum, Coriandrum sativum, Capsicum frutescens, Cuminum cyminum, Foeniculum vulgare, Trigonella foenumgraecum* and *Brassica nigra. Aspergillus flavus, A. niger, A. nidulans, A. sydowii, A. ochraceus, Penicillium* and *Rhizopus* spp. were most frequently isolated from the above spices.

The most predominant genus encountered was *Aspergillus* represented by 9 species in the test samples. Similar observations were made by Moharram *et al.*, (1989); El-Kady *et al.*, (1995); Abdul kadir *et al.*, (2003) reported *Aspergillus flavus* and *Aspergillus niger* were dominant in their survey of seed mycoflora from common spices collected from the markets of sultanate of Onam. Climatic conditions of tropical and subtropical countries favour the growth of *Aspergilli* as stated by Pitt (1980).

The results indicates that though the spices like black pepper, fennel and ajwon are known for their antimicrobial properties and used in therapeutic uses were heavily contaminated by fungi. Seed mycoflora not only deteriorate the quality of spices but also increase the mycotoxin contamination. Improving the storage, post-harvest and processing conditions may help in low risk of fungal contamination.

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