



## Original Research Article

### Mycoflora Associated with Spices

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#### ABSTRACT

##### Keywords

Spices,  
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samples.

The mycoflora association of 3 spices (Coriander, Cumin and Fennel) have been studied using mycological techniques. The presence of moulds was compared on loose and packed samples on PDA and SDA at 27±1°C. The species of fungi that were isolated belonged to fourteen (14) genera (*Alternaria*, *Aspergillus*, *Candida*, *Cunninghamella*, *curvularia*, *Emericlla*, *Fusarium*, *Helminthosporium*, *Microascus*, *Mucor*, *Nigrospora*, *Penicillium*, *Rhizopus*, *Trichoderma*). *Aspergillus* species (*A.flavus* and *A.niger*) were more predominant species isolated, followed by *Fusarium oxysporum*. *A.flavus* was most frequently isolated fungal species in all the samples of spices in loose and packed form. The level of mycoflora associated in spices suggest a need for better control in all aspects of the production, processing, and usage of these spices from spoilage and food-borne diseases due to contamination of spices and future work recommended.

## Introduction

Spices have been defined as the plant substance from exotic or indigenous origin, with strong taste and aroma. They are essentially flavouring agents used in small amounts and are reported to have beneficial and antimicrobial properties (Atanda et al., 2006). Spices such as Coriander, Cumin and Fennel are used for flavouring and medication and are highly valuable due to their preservative and antioxidant properties.

*Coriandrum sativum* is used externally to

treat ulcer and rheumatism and several other medicinal uses (Hegi, 1926). Both leave and seed contain antioxidant property (Wangensteen, 2004). *Cuminum cyminum* is used as preservative and stimulant, antispasmodic and antimicrobial agent (Romeilan, 2010). Mainly used in seasoning, curry powder, pickle and chutney (Farell, 1985). *Foeniculum vulgare* belong to family Apiaceae is used in folk medicine as stimulant, diuretic, carminative and sedative (Charles, 1993). It is also considered as spices due to

terpenic compound isolated from its seeds volatile oil (Masada, 1967) whose main constituent are (E)-anethole, fenchone and methyl chavicol which have antifungal property (Dukic, 2003).

Spices become associated by fungi in the field, during storage, transport, processing and handling (Elshafie et al., 2002). Spices are raw agricultural materials and if the moisture content is too high, toxigenic moulds, like *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. May grow (Abou-donia, 2008) though spices are generally resistant to microbial spoilage.

Fungi are normal component of food mycoflora and cause spoilage and mycotoxin production. It is well known fact that several fungi causes considerable damage to spices under storage condition, and also decrease in germination ability, discoloration of parts, loss in weight and production of toxin and it depends upon the type of fungi present, the composition of food, storage and handling (Mandeel, 2005). The most frequent fungal pathogen of spices is from genera *Aspergillus* and *Penicillium* (Koci et al., 2007).

In the present work, detailed and comprehensive survey of fungi from spices viz. *Coriandrum sativum*, *Cuminum cyminum* and *Foeniculum vulgare* was undertaken. The present work was conducted on spices collected from Agra region of U.P, India during the period of August 2012- March 2013.

## Materials and Methods

The three spices used in this study were obtained from the local market and malls in loose and pack form respectively from Agra region. These were *Coriandrum sativum* (Coriander), *Cuminum cyminum* (Cumin) and *Foeniculum vulgare* (Fennel).

All the spice samples (18) were packed in transparent polythene bags and transported to laboratory. Moisture content is measured and mean moisture percentage is calculated (Essono et al., 2007).

Two nutritive media were chosen: Potato dextrose Agar (PDA) with antibiotic and Sabouaud Dextrose Agar (SDA) which is used to isolate the fungal species (colony). Mycological analyses were done by Serial dilution method.

For isolation of seed mycoflora, Agar plate method was implemented (ISTA, 1966). Inoculated petriplate were incubated at  $27\pm 1^\circ\text{C}$ . Results were determined after 5 to 7 days because of the later growth of other fungi.

Identification of isolated fungal species was performed (Barnet, 1960; Smith, 1969; Subrahmanian, 1971; Gilman, 1975) on the basis of morphological and cultural basis (Ananthanarayan and Paniker, 1999). Incidence of fungal species is calculated by percentage relative frequency (Marasas et al., 1988; Giridhar and Reddy, 1997).

$$\% \text{ of frequency} = \frac{\text{No. of observation in which a species appeared}}{\text{total no. of observations}} \times 100$$

## Results and Discussion

Data revealed in table, a total 26 fungal species were isolated on the test spices viz. *Alternaria alternata*, *A. brassicae*, *Aspergillus flavus*, *A.niger*, *A. candidus*, *A. fumigatus*, *Candida* sp., *Cunninghamella* sp., *Curvularia lunata*, *Emericella nidulans*, *Fusarium oxysporum*, *F. solani*, *Helminthosporium gramineum*, *Microascus cinereus*, *Mucor* sp., *Nigrospora oryzae*, *Penicillium chrysogenum*, *P. citrinum*, *P. janthinellum*, *P. notatum*, *Penicillium* sp., *Rhizopus arrhizus*, *R. nigricans*, *R. stolonifer*, *Trichoderma viride*).

**Table.1** List of isolated fungi and their incidence percentage on the seeds of different spices

S.No	Fungal Species	Percentage incidence of different species of fungi on spices - Absent; all values are in %.		
		SPICES		
		<i>Coriandrum sativum</i>	<i>Cuminum cyminum</i>	<i>Foeniculum vulgare</i>
1.	<i>Alternaria alternata</i>	-	3.03	3.70
2.	<i>Alternaria brassicae</i>	-	6.06	-
3.	<i>Aspergillus candidus</i>	-	9.09	3.70
4.	<i>Aspergillus flavus</i>	14.70	12.12	18.51
5.	<i>Aspergillus fumigatus</i>	-	-	7.40
6.	<i>Aspergillus niger</i>	11.76	18.18	7.40
7.	<i>Aspergillus terreus</i>	5.88	6.06	-
8.	<i>Candida sp.</i>	-	6.06	-
9.	<i>Cunninghamella sp.</i>	-	6.06	-
10.	<i>Curvularia lunata</i>	11.76	-	11.11
11.	<i>Emericella nidulans</i>	-	3.03	-
12.	<i>Fusarium oxysporum</i>	8.82	3.03	14.81
13.	<i>Fusarium solani</i>	5.88	-	-
14.	<i>Helminthosporium gramineum</i>	2.94	-	-
15.	<i>Microascus cinereus</i>	2.94	-	3.70
16.	<i>Mucor sp.</i>	2.94	6.06	7.40
17.	<i>Nigrospora oryzae</i>	2.94	-	-
18.	<i>Penicillium chrysogenum</i>	-	3.03	3.70
19.	<i>Penicillium citrinum</i>	-	3.03	-
20.	<i>Penicillium janthinellum</i>	-	+	7.40
21.	<i>Penicillium notatum</i>	5.88	3.03	3.70
22.	<i>Penicillium sp.</i>	5.88	-	-
23.	<i>Rhizopus arrhizus</i>	2.94	3.03	-
24.	<i>Rhizopus nigricans</i>	2.94	-	3.70
25.	<i>Rhizopus stolonifer</i>	-	3.03	-
26.	<i>Trichoderma viride</i>	11.76	6.06	3.70

On individual basis 15, 17 and 14 fungal species were reported on Coriander, Cumin and fennel respectively. Six fungal species viz. *Aspergillus flavus*, *A.niger*, *Fusarium oxysporum*, *penicillium notatum* and *Trichoderma viride* were found common fungal species on all three spice seeds.

It was observed that *Aspergillus flavus* showed the highest percent of incidence on all the three spices viz. 14.70%, 12.12 % and 18.51%.The maximum (14.70%) *Aspergillus flavus* and lowest (2.94%) on *Helminthosporium gramineum*, *Microascus cinereus*, *Mucor sp.*, *Nigrospora oryzae*, *Rhizopus arrhizus*

and *R.nigricans* was recorded on Coriander seeds.

On Cumin seeds maximum percentage of incidence was recorded of *Aspergillus niger* (18.18%) and minimum of (3.03%) on *Alternaria alternata*, *Emericella nidulans*, *Fusarium oxysporum*, *penicillium chrysogenum*, *P. citrinum*, *P. notatum*, *Rhizopus arrhizus* and *R. stolonifer*.

On Fennel seeds maximum percentage of incidence was counted of *Aspergillus flavus* (18.51%) and minimum (3.70%) of *Alternaria alternata*, *Aspergillus candidus*, *microascus cinereus*, *Penicillium chrysogenum*, *P.notatum*, *Rhizopus nigrican* and *Trichoderma viride*. Total twenty six fungal species belonging to fourteen genera were isolated from three test spices. It shows the fungal efficiency in developing association with broad spectrum of seeds, irrespective of their types. Similar results regarding the incidence of fungi have been given by Sumanth et al., (2010), on seeds of Indian spices. *Aspergillus niger*, *A. flavus* and *A.fumigatus* have been recorded as most dominant fungi. During the course of study, the genus *Aspergillus* constituted a major group (Shah, Rakesh and Jain, 1993). Grewal and Mahendra, (1965), reported such observation. Takatori et al., (1977) and Ayres et al., (1986) found that *Aspergillus* sp. and *penicillium* sp. the main components of fennel, Coriander, Cumin, Cardomom and Cinnamon.

Misra, (1981) and Roy et al., (1988) isolated *A.flavus*, *A. niger*, *A.fumigatus*, *A.ochraceus*, *A.candidus*, *A.sydwii*, *Chaetomium dolicholrichum*, *Fusarium*, *Alternaria*, *Curvularia* and *Rhizopus* from seeds of Coriander, Cumin and Fennel, Amomum, Pepper and bark of Acacia. Ath-Har et al., (1988) reported that

*A.flavus*, *A.niger*, *A.nidulans*, *A.sydwii*, *A. ochraceus*, *Penicillium* and *Rhizopus* sp. were most frequently isolated from spices.

Due to lack of proper post harvest preservation techniques, large portion of annual yield gets damaged by fungal action (Seema and Basu, 2003). The spices could be subjected for contamination with fungi mainly during spice processing, storage and transport (Dimic et al., 2008).

Improving the conditions of spices under processing, storage and transport and continuous mycological and mycotoxicological control prior to food processing is necessary to lower the risks from incompatibility of seeds in order to efficiently protect human health

Future studies will look at the combination of isolation with mycotoxin examination and make it applicable in the food industry.

## References

- AbouDonia, M.A. 2008. Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. *Global Veterinaria*. 2(4): 175- 181.
- Ananthanarayan and Paniker, *A text Book of Practical Microbiology*. 1999. 6<sup>th</sup> Edition University Press, London.
- Atanda, O.O., Akpan, I. and Oluwafemi, F., 2006. The potential of some spice essential oils in the control of *A.parasiticus* CFR 223 and aflatoxin production. *Elsevier*, 1-7.
- Ath-Har, M.A., Prakash, H.S. and Shetty, H.S. 1988. Mycoflora of Indian spices with special reference to aflatoxin producing isolates of *Aspergillus flavus*. *IJM*, 28(12): 125-

- Ayres, G.I., Mund, T.I. and Sondin, E.W., 1980. Microbiology of Food Spices and Condiments. *A Series of Books in Food and Nutrition*. Schmeigert, 249.
- Barnett, U.L., Illustrate genera of imperfect fungi. 1960. *IInd ed. Minneapolis burgess publ.co.*
- Charles, D.J., Morales, M.R. and Simon, J.E., 1993. Essential oil content and chemical composition of finocchio fennel. In: *Janick, J. and J.E. Simon(eds), New crops. Wiley, New York, 570-573.*
- Dimic, G.R., Kocic-Tanackov, S.D., Tepic, A.N., Vujicic, B.L. and Sumic, Z.M., 2008 Mycopopulation of spices, *BIBLID*, 39: 1-9.
- Dukic, N.M., Kujundzic, S., Sokovic, M. and Couladis, M. 2003. Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill obtained by different distillation conditions. *Phytotherapy Research*, 17(4): 368-371.
- Elashafie, A.E., Al-Rashdi, T.A., Al-Bahry, S.N., Bakheit, C.S., 2002. Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia*, 155(3): 155-160.
- Essonon, G., Ayodele, M., Akoa, A., Foko, J., Olembo, S. and Gockwski, J., 2007. *Aspergillus* species on cassava chips in storage in rural areas of southern. Cameroon: their relationship with storage duration, moisture content and processing methods. *African Journal of Microbiology*. 001-008(s).
- Farell, K.T., In: Spices, Condiments and Seasonings. 1985. *The AVI Publishing Co., Inc., Westport, Connecticut*, 98-100.
- Gilman, J.C., 1975. A manual of soil fungi. *Oxford and IBH Publishing Corporation, New Delhi, Bombay, Calcutta.*
- Giridhar, P. and Ready, S.M., 1997. Incidence of mycotoxin producers on spices from Andhra Pradesh. *J. Indian Bot. Soc.* 76: 161-164.
- Hegi, G., *Illustrierte Flora von Mitteleuropa. J. F.1926. LehmannsVerlag, München.* 5(2): 1071-1074.
- International Seed Testing Association, International rules for seed testing. 1988; Proc. Inter. Assoc., 63:1-102.
- Koci-Tanackov, S.D., Dimi, G.R. and Karali, D. 2007. Contamination of spices with moulds potential producers of sterigmatocystine. *APTEFF* 38: 1-190.
- Mandeel, Q.A. 2004. Fungal contamination of some imported spices. *Mycopathologia*, 159: 291-298.
- Marasas, W.F.O., Burgess, L.W., Anelich, R.Y., Lamprecht, S.C. and Van Schalkwyk, D.J., 1988. Survey of *Fusarium* species associated with plant debris in South African soils. *South African Journal of Botany*, 54: 63-710.
- Masada, Y., 1976. Analysis of essential oils by gas chromatography and mass spectrometry. *Copyright by the Hirokawa Publishing company, INC printed in Japan.*
- Misra, N., 1981. Influence of temperature and relative humidity on fungal flora of some spices in storage. *Z LebensmUntersForsch.* 172(1): 30-31.
- Romeilah, R.M., Fayed, S.A. and Mahmoud, G.I. 2010. Chemical compositions, antiviral and antioxidant activities of seven essential oils. *J. Appl. Sci. Res.* 6(1): 50-62.
- Roy, A.K., Sinha, K.K. and Chourasia, H.K., 1988. Aflatoxin contamination

- of some common drug plants. *Appl. Environ. Microbiol.*, 54: 842–843
- Seema, K and Basu, M. 2003 Evaluation of aflatoxin contamination in different spices. *Ind. Phytopath.* 56(4): 457-459.
- Smith, G., 1969. An introduction to industrial mycology. *Eward Arnold Ltd. London.*, 390.
- Subrahmanian, C.V., 1930 'Hyphomycetes' 1971; ICAR, New Delhi.
- Sumanth, G.T., Waghmare, B.M. and Shinde, S.R., 2010. Incidence of mycoflora from the seeds of Indian main spices. *AJAR*, 5(22):3122-3125.
- Takatori, K., Watanabe, K., Udagawa, S. and Kurata, H. 1977. Mycoflora of imported spices and inhibitory effects of the spices on the growth of some fungi. *Proc. Jpn. Assoc. Mycotoxins*, 9: 36–38.
- Wangensteen, H.; Samuelsen, A. B.; Malterud, K. E. 2004. Antioxidant activity in extracts from coriander. *Food Chemistry*. 88(2): 293.