RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF ASPERGILLUS SPS. FROM REGULARLY USED CONDIMENTS AND SPICES

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ABSTRACT

The mycoflora associated with spices not only deteriorate the quality of spices and condiments but also increase the chance of consuming toxic elements through harmful fungi like Aspergilii. *Aspergillus* species associated with 12 different spices such as Clove, Turmeric, Cardamom, Fennel, Bishop's weed, Cumin seeds, Coriander seeds, Cinnamon, Sesame seeds, Mustard seed, Black pepper and Capsicum were studied by using Petridish method, following Direct Plate Method as suggested by International Standard for Seed Testing (ISTA).In the present study, it was found that all the spices and condiments are heavily infected by fungi. Numbers of *Aspergillus* species were recorded along with *Alternaria, Penicillium, Fusarium, Curvularia, Rhizopus, Mucor, Cladosporium* from the sample of 12 spices during the period of investigation. *Aspergillus flavus* (17.74%), *Aspergillus niger* (17.12%), *Aspergillus versicolor* (14.51%), *Aspergillus fumigatus* (11.29%) were found associated in very high concentration as major contaminants in the spice samples. Total eleven species of *Aspergillus* were found associated with different spices. Two species were associated with Mustard seeds and Clove; three species were associated with *Curcuma*, four with Cardamom and Black pepper, five with Cumin, sesame seeds and with Bishop's weed, six with Cinnamon and Fennel, seven with Coriander seeds. Capsicum showed highest fungal infestation with nine different species of *Aspergillus*.

Keywords : *Cladosporium*, Spices, Fungi, *Aspergillus*, Infestations, etc.

INTRODUCTION

Spices and herbs are valued for their distinctive flavors, colors and aromas and are among the most versatile and widely used ingredient in food preparation and processing throughout the world (Ayres et al., 1980). They are widely used as raw materials for pharmaceutical preparations (Galenic products) and as a supplement for dietetic products, especially for "self medications" in public (Weiser et al., 1971). Spices themselves have little or no nutritional value as mere collections of carbohydrates, fats and proteins of potential interest; it is the fact that spices contain no calories. Most species owe much of their flavoring properties to volatile oils, but in some cases the flavor is due to fixed oil. These include the alcohols, esters, terpens, phenols and their derivatives, organic acids, alkaloids and resins.

Since spices harbor many kinds of organisms, including those associated with food spoilage. Depending on chemical nature, different organic substances harbor different microorganisms. These may spoil the quality of the substrate by discoloration, formation of foul odour, change of chemical makeup or production of toxic substances.

Spices are consumed daily in India and hence microbiological examination of spices is important from the point of view of their quality which can be affected by contaminating microorganisms during storage. Contamination of various toxigenic moulds in spices is also known, but little data is available on spices from central India. Hence it was thought necessary to study the seed mycoflora especially of *Aspergillus* species from the spices. The present investigation deals with the *Aspergillus* species associated with twelve different spices such as Clove, *Curcuma*, Cardamom, fennel, Cumin seeds, Coriander

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seeds, Cinnamom, Sesame seeds, Mustard seeds, Black pepper, Bishop's weed and Capsicum.

Field fungi capable of attacking and infecting the growing product may cause varying degrees of decomposition and damage. The damage from invading fungi and molds may be manifested as leaf spot diseases, dry rot, decomposed and discolored tissue of stems and roots, or decay in seeds and fruits. Storage fungi (which can grow under limited moisture conditions) may cause moldiness in some products stored under conditions of temperature and relative humidity favorable to their growth.

Pockets of moist product can arise in a dried and otherwise normal product through roof leaks, insect activity, and moisture translocation when temperature gradients develop within the product mass. These pockets can promote the rapid growth of molds in the stored product. Moldiness can range in appearance from mycelium-matted leafy spices and surface mold on cassia bark, to internal molds in nutmegs and capsicum pods.

MATERIAL AND METHODS

Different samples of the spices were collected in the sterile polythene bags from two different kitchens of different localities in the Mumbra city. The samples were brought to the laboratory for further study. Measured quantity of samples i.e. five grams were plated on sterilized Malt extract medium and Rose Bengal Streptomycin media in Petri dishes. The media used were sterilized by autoclaving at 120°C for 20 minutes and also following Direct Plate Method as suggested by ISTA (1966). Measured amount of each samples of the same quantity were placed on moist sterilized blotting paper in pertridish. After plating the samples the plates were incubated at room temperature and observed regularly after 3 days. The fungal growth appearing over the surface of seeds or on the media adjacent to plated seeds was picked up and transferred to the fresh culture media for pure isolates. The fungi were examined under microscope by preparing the slides in lacto phenol cotton blue mounting media. The fungal colonies were identified using standard methodologies. *Aspergillus* species were identified with the help of standard published literature.

RESULTS & DISCUSSION:

During the present survey, a total of 11 *Aspergillus* species were recorded along with *Alternaria, Penicillium, Fusarium, Curvularia, Rhizopus, Mucor* and *Chaetomium* from the samples of all the spices studied. Aspergillus spp. was found to be associated with all the spices in all the months and every time during the period of investigations. *Aspergillus flavus, A.fumigatus* and *A.niger* were found associated in very high concentration with every spice in every season of the year.

Table 1. Seasonal distribution of *Aspergillus* species associated with different spices

Names of spices	Names of spices	Summer	Monsoon	winter
(Botanical name)	(Common name)			
Curcuma longa	Curcuma (Haldi)	-	++	+
Cumin cyminum	Cumin seeds (Zira)	++	+++	++
Sesamum indicum	Sesame seeds (Til)	+++	++	+
Capsicum annum	Capsicum (Mirch)	++	+++	+++
Brassica compestries	Mustard (Sarson)	+++	++	++
Coriandrum sativum	Coriander (Dhania)	+	+++	++
Cinnamonum beylanicum	Cinnamon (Dalchini)	+	++	-
Elettaria cardamomum	Cardamom (Elaichi)	-	++	+
Eugenia caryophyllata	Clove (loung)	-	+	-
Piper nigrum	pepper (Kali Mirch)	-	+	+
Carum opticum	Bishop's weed (Ajwain)	+	++	++
Foeniculum vulgare	Fennel seeds (Sonff)	+	++	+++

(- No infestation of *Aspergillus*, + *Aspergillus* infestation, ++ High infestation, +++ Severe infestation)

Table 2. Percent of individual *Aspergillus* species associated with spices

Sr. No.	Names of Fungi	No. of spices	Percentage
1	*Aspergillus niger*	11	17.74%
2	*Aspergillus flavus*	11	17.74%
3	*Aspergillus fumigates*	7	11.29%
4	*Aspergillus ochraceus*	3	04.83%
5	*Aspergillus candidus*	6	09.96%
6	*Aspergillus tamari*	2	03.22%
7	*Aspergillus terreus*	3	04.83%
8	*Aspergillus sydowi*	6	09.67%
9	*Aspergillus versicolor*	9	14.51%
10	*Aspergillus repens*	2	03.22%
11	*Aspergillus solani*	2	03.22%

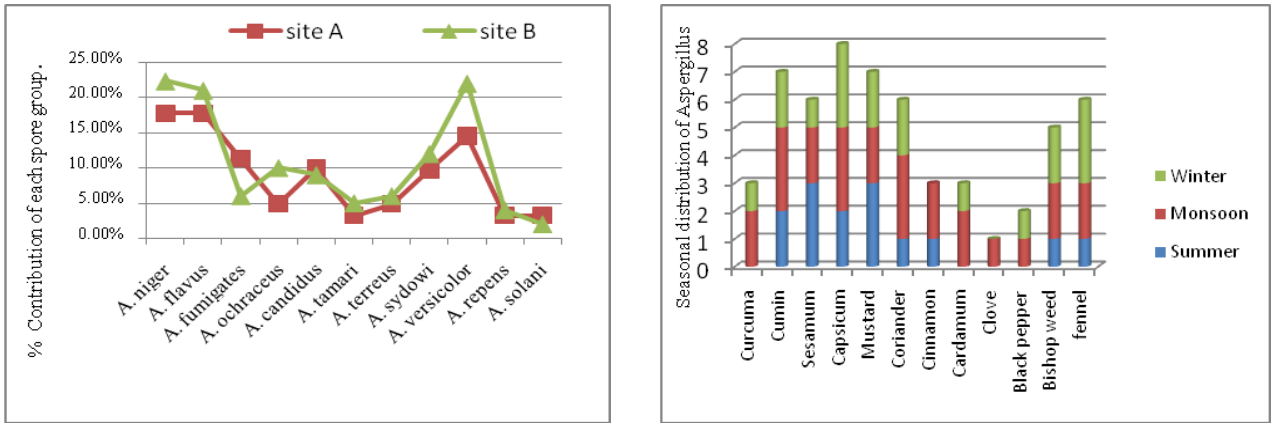

Fig 1: % Contribution of individual *Aspergillus* species associated with spices (Site-A: Indoor & Site-B: Outdoor), Fig 2. Seasonal distribution of *Aspergillus* sps. on various spices.

Results are tabulated in Table 1-2 along with graphs Fig1.- % Contrbution of individual *Aspergillus* species associated with spices (Site-A: Indoor & Site-B: Outdoor), Fig 2.-Seasonal distribution of *Aspergillus* sp. on various spices. Capsicum showed highest fungal infection among the spices studied. About 15.51% of *Aspergillus* species found associated with this spice. *Aspergillus* species also have been reported to be most frequent occurrence on seeds of some spices and condiments. Other than *Aspergillus* species *Alternaria* and *Penicillium* were found to be associated in high concentration. The rhizome of *Curcuma longa* also showed fungal contamination of about 5.17% of *Aspergillus* species. Three species of *Aspergillus* found to be associated during the present study. Clove showed fungal infestation especially of *Aspergillus* genus about 3.44%.Two species of this genus found to be associated. The fungus showed its appearance during monsoon season. Four species of *Aspergillus* found to be associated with Cardamom. They found predominant in monsoon and winter seasons. Black pepper showed about 8.62% of fungal contamination of *Aspergillus.* About 5 spp. were found to be associated. The moisture content also affects the presence of fungal spores in spices. Sesame seeds showed fungal infestation in winter, summer as well as in monsoon i.e. fungal contamination present throughout the year of about 8.62% of *Aspergillus* found to be associated with this spice. Mustard seeds showed high concentration of fungal infection, out of which *Aspergillus* genus showed poor degree of contamination of about 3.44%.. Bishop's weed also showed 8.62% of fungal infection especially of genus *Aspergillus*, of about seven different spp. of *Aspergillus* found to be associated with this spice.

CONCLUSION:

During the study period, it was found that all the spices and condiments are heavily infected by fungus All the spices showed a heavy contamination of fungi however, the extent of infestation varied with the spices. Such variation may be may be assigned to the difference in their physiochemical qualities and chemical content. The fungal infestation of species of *Aspergillus* and *Penicillium* along with spices is of significance since they are known to produce toxic metabolites to cause various changes in seed constituents and reduction in germination power. Although the spices found in India may be pure in the sense that they may not be grossly adulterated with foreign matter, they are far from pure microbiologically. In view of these observations the question of health hazard due to the consumption of these spices deserves careful attention.

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