RESEARCH ARTICLE

Incidence of mycoflora associated with some spices

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ABSTRACT

The spices including black pepper, fennel and cumin are extensively utilized by majority of world's polulation in response to multifold used as food, medicine, flavouring and colouring agent. In storage, seed borne contaminants not only deteriorate the seeds but also alter the physio-chemical properties of seeds leading to increase the chances of consuming toxic elements, produced by storage mycoflora. In the present investigation, composite samples of black pepper, fennel and cumin were mycologicaly analyzed by standard agar plating technique revealed the prevalence of altogether 15 fungal seed borne pathogens representing 12 genera with maximum four species of *Aspergillus*. A population of 12 fungal species associated with seeds of black pepper; 11 isolates existed on fennel while eight on cumin. The fungal isolates, *Aspergillus flavus, A. fumigatus, A. niger, Rhizopus nigricans* and *Cladosporium cladosporioides* were predominant on all three test spices. *Chaetomium globosum* restricted only to black pepper; *Syncephalastrum recemosum* to cumin while *Torula herbarum* was existed only on fennel. *Aspergillus niger* was predominant with greatest 23% incidence on cumin, while excluding *Chaetomium globosum,* other fungal isolates had moderate to low percent incidence on spices.

Key words- *Spices, mycoflora, percent incidence, isolates, predominant.*

INTRODUCTION

The spices are among the globally demanded, expensive and valued products in response to their distinctive flavour, colour and aroma, utilized throughout the globe concerning to multifold uses including medicinal, religious, ritual, cosmetics and perfume production or as a vegetable, and widely used as ingredient in food preservation and food processing. A small quantity of spices are consumed in daily diet for good health due to strong flavour and high content of protein, carbohydrates, minerals, microelements, essential oil and antioxidants such as flavonoids (Wikipedia, 2014). These antioxidants also act as natural preservatives, preventing spoilage of food, leading to a higher nutritional content in stored food. India is leading producer of spices in the world, contributing 73% of global spice production. About 63 different spices are cultivated in the country (Divakara and Sharma, 2001). Aside from being used as food, spices have great demand in pharmaceutical industries for medicine formulation due to antimicrobial property and antioxidant activity. *Piper nigrum* L. (Black pepper) is proved to cure illness such as constipation, diarrhoea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problem, lung disease, oral abscesses, sunburn, tooth decay and toothaches (Ahmed *et al*., 2012).

Foeniculum vulgare Mill (Fennel) is rich source of aromatic compounds with various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, estrogenic, diuretic etc, hence its various preparations are used to cure chronic couth, to improve eyesight, to improve milk supply of breastfeeding mother due to phytoestrogen content, hypertension (Rahimi and Ardekani, 2013; Annies Remedy, 2014). *Cuminum cyminum* L. (Cumin) is recommended to treat paleness of the face, to relieve indigestion and digestive gas, and to relieve minor aches and pains, the oil helps to heal wounds, cuts, and scrapes (Wisegeekhealth, 2014).

The spices are prone to attack by several fungal pathogens that cause enormous losses both to pre- and post-harvest crop as well as seed deterioration by successive seed contaminants under supportive storage climate resulting in seed abortion, seed rot, seed necrosis, loss of seed nutrients, alteration of physiochemical properties of seeds, loss the seed weight, seed viability & vigour, medicinal properties, aesthetic changes including discoloration, heating & mustiness, cracking and abnormal odour contributing the losses of spices to the extent of 30% and also development of diseases at later stages of plant growth due to transmission of pathogen from seed to seedling during germination of infested seeds (Wikipedia, 2014).

Prevalence of mycoflora of spices concerning to black pepper, fennel and cumin has been highlighted by Bokhari (2007), Sumanth *et al*., (2010), Pant (2011), Hedawoo and Chakranarayan (2011) and Ramesh and Jayagoudar (2013). Srivastava and Chandra (1985) isolated mycobiota of coriander, cumin, fennel and fenugreek in India and reported dominant nature of *Aspergilli* and *Fusaria.* Hashmi (1988) has reported 46 fungal seed borne isolates representing 20 genera on seeds of spices in Pakistan. Literature review suggest that a little is known from Amravati District concerning to incidence of fungal flora adhering to spices, it seemed to be worthwhile to undertake a more comprehensive and systematic study of stored samples of spices from this region.

MATERIALS AND METHODS

Samples of *Piper nigrum L.* (Black pepper), *Foeniculum vulgare Mill.* (Fennel) and *Cuminum cyminum L.* (Cumin) have been collected from markets and stockists of Amravati District and brought to the laboratory. Samples of each spice was mixed together to obtained composite sample for individual spice. A composite sample of each spice was screened for prevalence of fungal pathogens adhering to spices employing standard agar plate method as recommended by ISTA (2012).

The randomly selected 200 seeds from composite sample of each spice were transferred to 10 petri plates containing sterile jelly nutrient Potato Dextrose Agar (PDA) medium composed of peeled potato (200 gm^{-1}), Dextrose (200 gm^{-1}) and agar (20 gm^{-1}) in a litre of water. After incubation for seven days at laboratory temperature ranged between 25^0C to 28^0C, all these petri plates containing seeds were examined under stereo-zoom microscope for appearance of fungal growth on seed surface. The fungal pathogens appeared on seeds were isolated and identified referring available literature, standard mycological books and manuals after sub-culturing on nutrient medium (Neergaard, 1977; Gilman, 2001; Mokadam *et al*., 2006). Fungal count and infestation level have been recorded as a percentage of infested seeds in a sample following a technique reported earlier (CMI, 2010). Purified fungal isolates were propagated and maintained on Czapek's Dox agar nutrient medium in sterile slants.

RESULTS AND DISCUSSION

The mycological analysis of composite samples of black pepper, fennel and cumin was carried out by standard agar plating technique and results on the incidence of mycoflora are presented in table I. The data revealed the prevalence of population of altogether 15 fungal seed borne pathogens representing 12 genera, of these, *Ascomycota* and *Deuteromycota* contributed higher count of 6 isolates each, while *Zygomycota* had 3 fungal isolates. The isolates confined to seed samples of spices in varying magnitude of incidence included *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. nidulans, A. niger, Chaetomium globosum, Cladosporium cladosporioides, Curvularia lunata, Fusarium moniliformae, Helminthosporium tetramera, Mucor variance, Penicillium chrysogenum, Rhizopus nigricans, Syncephalastrum recemosum* and *Torula herbarum.*

A population of altogether 12 seed borne fungal isolates was confined to seeds of *Piper nigrum* L. (Black Pepper) belonging to 9 genera. *Ascomycota* and *Deuteromycota* contributed higher count of 5 isolates each, while *Zygomycota* had two isolates viz., *Mucor variance* and *Rhizopus nigricans*. The species of *Aspergillu*s viz., *A. flavus, A. fumigatus* and *A. niger* were predominant, exhibiting higher fungal incidence ranged between 11.9 to 19.4%. The isolates, *Alternaria alternata* and *Chaetomium globosum* had low level of incidence. Remaining isolates exhibited moderate level of incidence ranged between 5.5 to 10.0% (Table 1).

Foeniculum vulgare Mill (Fennel) samples exhibited prevalence of total 11 fungal species representing 8 genera. Of the total 5 fungal isolates each categorized under *Ascomycota* and *Deuteromycota* while only *Rhizopus nigricans* represents *Zygomycota*. Greatest fungal incidence was recorded for *Aspergillus flavus*. Significant level of incidence, 14.5% and 10.8% was detected for *A.niger* and *Aspergillus fumigatus* respectively. *Curvularia lunata* had least incidence. Remaining isolates were recorded with moderate level of incidence ranged from 4.8% to 7.2 % (Table 1).

Altogether 8 isolates of seed borne nature was confined to seeds of *Cuminum cyminum* L. (Cumin) belonging to 6 genera. *Ascomycota* and *Zygomycota* contributed highest count of 3 isolates each, while *Deuteromycota* represented by two isolates, *Cladosporium cladosporioides* and *Helminthosporium tetramera*. An ascomycetous isolate, *Aspergillus niger* exhibited higher, 23% incidence followed by *Syncephalastrum recemosum* with 21.3% incidence. Moderate level of incidence, ranged between 5.4 – 12.0 % was recorded for *Rhizopus nigricans, Mucor variance, Aspergillus fumigatus* and *A. flavus* while others had least incidence (Table 1).

The isolates, *Aspergillus flavus, A. fumigatus, A. niger, Cladosporium cladosporioides* and *Rhizopus nigricans* were detected from all the three test spices while *Alternaria alternata, Aspergillus nidulans, Fusarium moniliformae* confined to seeds of black pepper. *Chaetomium globosum* isolated only from black pepper, while *Penicillium chrysogenum* and *Torula herbarum* restricted to fennel.

Table 1: List of isolated fungi and their incidence on seeds of different spices

Sr. No.	Fungi isolated	Black pepper	Fennel	Cumin
		% incidence		
1	*Alternaria alternata*	3.3	6.6	-
2	*Aspergillus flavus*	14.9	20.5	5.4
3	*A. fumigatus*	11.9	10.8	8.1
4	*A. nidulans*	5.3	6.1	-
5	*A. niger*	19.4	14.5	23.0
6	*Chaetomium globosum*	3.0	-	-
7	*Cladosporium cladosporioidis*	6.4	7.2	4.2
8	*Curvularia lunata*	6.0	3.9	-
9	*Fusarium moniliforme*	6.0	4.8	-
10	*Helminthosporium tetramera*	4.1	-	4.1
11	*Mucor variance*	7.5	-	8.2
12	*Penicillium chrysogenum*	-	4.8	-
13	*Rhizopus nigricans*	10.0	12.5	12.0
14	*Syncephalastrum racemosum*	-	-	21.3
15	*Torula herbarum*	-	7.2	-

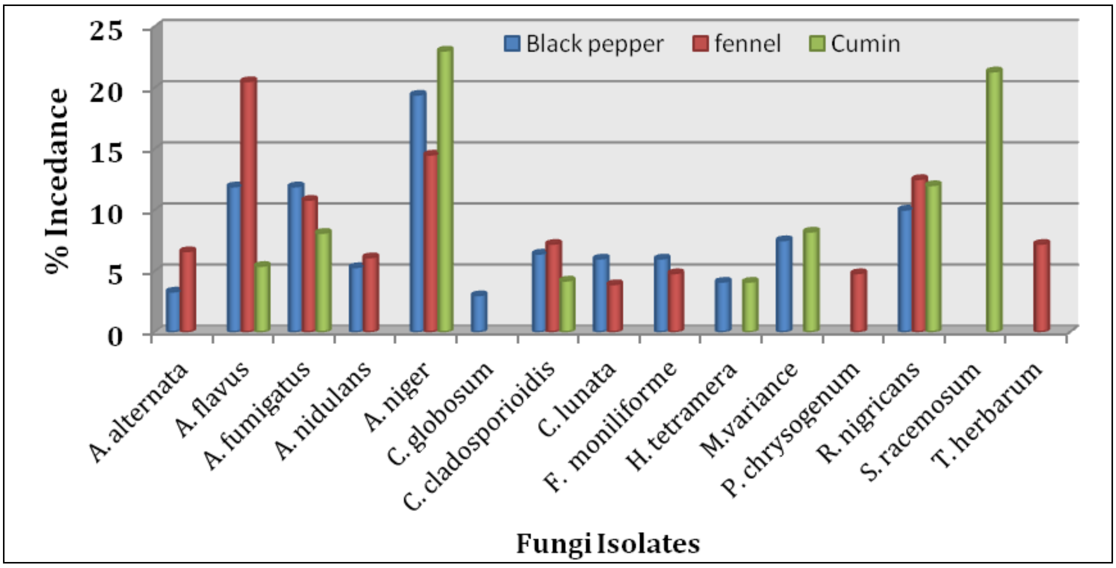

Fig. 1 Percent incidence of mycoflora associated with spices

Mycological analysis revealed that *Ascomycota* and *Deuteromycota* contributed equal count of isolates followed by *Zygomycota*. The fungal isolate belong to genera, *Aspergilli* of *Ascomycota* and *Alternaria alternata, Curvularia lunata, Fusarium moniliformae* and *Cladosporium cladosporioides* of *Deuteromycota* contributed as major components of all three spices understudy represented a group of taxa of cosmopolitan fungal organisms that can exploit virtually any organic substrate provided favourable storage environment of oxygen, temperature & relative humidity and accumulates toxic secondary metabolites (Bhajbhuje, 2013). *Ascomycota* and *Deuteromycota* had equal count of isolates but *Ascomycot*a had comparatively higher level of incidence over *Deuteromycota* and *Zygomycota* (Fig. 1). It may seem possibly due to prevalence of equal count of fungal flora associated with seed coat with heavy infestation. Moreover, most members of *Ascomycota* are known facultative parasites on crop plants as well as involved as saprophyte in biodegradation of seeds, and debris of plant and animal origin (Ramesh and Jayagoudar, 2013). Members of *Deuteromycota* complete their life cycle asexually producing numerous resistant, thick walled conidia which may remain viable for longer duration in adverse climatic environment (Bhajbhuje, 2013). It was interesting to record members of *Oomycota* and *Basidiomycota* did not persist on test spices may be possibly attributed to mode of nutrition as majority of fungal organisms of these groups are obligate parasites of other crop plants.

The prevalence of maximum fungal isolates on all the spices exhibited heavy infestation by fungal pathogens. Maximum appearance of saprophytes on unsterilized seeds reduced the appearance of some deep seated fungal pathogens. The occurrence of fifteen fungal pathogens on the three test spices indicates the ability of fungal pathogens in developing association with broad spectrum of seeds, irrespective of their types. This report is in confirmation to earlier findings (Bokhari, 2007; Sumanth *et al*., 2010). Pant (2011) reported common occurrence of species of *Alternaria, Aspergillus, Curvularia, Rhizopus* and *Mucor* on coriander seeds. Sumanth *et al*. (2010) detected higher level of incidence of *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium cladosporioides, Curvularia lunata, Helminthosporium tetramera* on some Indian spices including ammi, caraway, cardamom, coriander and cumin in higher frequency on agar plates. This report confirmed the seed borne nature of the fungal organisms recorded from all three spices.

The report of the present study revealed that *Aspergillus niger* was the highly predominant followed by *A. fumigatus* and *A. flavus* on all three spices. *Aspergilli* are among the most abundant and widely distributed organisms on the globe (Ramesh and Jayagoudar, 2013). Members of the genus *Aspergillus* are known obligate saprophyte and survive in the environment in the wide range of temperature varying from 18^0C – 32^0C (CMI, 2010). *Aspergillus niger* has potential to produce *ochratoxin-A*. *Aspergillus flavus* secretes aflatoxin B_1, B_2, G_1 & G_2 and other toxic compounds including *strigmatocystin, cyclopiazonic acid, kojic acid, β-nitropropionic acid, aspertoxin, aflatrem, gliotoxin* and *aspergillic acid* (Brakhage , Schroeckh, 2011). *Penicillium* have been reported as a common opportunistic pathogen, secretes penicillic acid, causing systemic penicilliosis in AIDS patients in Southern Asia and proved to be nephrotoxic in pigs and broilers may cause tremors, coagulopathy and enteritis (Seema and Basu, 2003)). Members of *Helminthosporium* have been reported to produce *Helminthosporin*; *Curvularia lunata* produces 2-methyl-(5-hydroxy methyl) furan-2 carboxylate. *Fusarium moniliformae* contributed with 6.0% incidence on black pepper, secretes a diverse range of mycotoxins includes *trichothecenes*, *zearalenone* and *fumonisins* that have been suspected of causing toxicity in human. *F. moniliformae* were reported to cause *keratitis* and also associated with wound and infections of the eyes and fingernails (Seema and Basu, 2003). Several species of *Alternaria* are reported to secrete *Altersolarol-A* and *alternaric acid dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III* (Brakhage , Schroeckh, 2011).

CONCLUSION

Spices are most important agriculture commodities, because of their aroma and taste with high contents of antioxidants. They are widely used in food preparations, medicines, as preservative throughout the world. The improper and poor storage condition, as well as traditional agricultural practices resulted contamination of spices from field to fork level. In conductive storage climate, diverse fungal pathogens existing on spices proliferates and secrete several harmful products including toxins, digestive enzymes and secondary metabolites. It leads to loss spices quality, aroma, and makes the spice unfit for the consumption and germination. The report of present survey revealed prevalence of heavy load of fungal contaminants on spices understudy. The magnitude of infestation varied with the spices may be attributed to their variable seed contents and physiochemical qualities. Thus, it is necessary to find the ways and means to reduce heavy load of the fungal contamination of spices by improving modern agriculture and safe storage practices.

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