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Mycoflora of Some Spices from Dharwad, India

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Abstract

Spices are most important agricultural commodities, commonly used to flavor the food preparations. Due to poor agriculture and storage practices spices get contaminated from field to fork level, among the contaminants fungi are the most predominated once, when they get the conducible atmosphere for their growth they grow and alters the spice quality and taste. The spices contaminated by fungi might cause the health hazards for humans. The fungal growth is mainly influenced by factors like Temperature and Relative Humidity. In the present investigation a total of 118 fungal species belongs to 44 genera were isolated out of which 18 species belonging to 08 genera of Zygomycetes, 07 species belonging to 02 genera of Ascomycets and 93 species belonging to 34 genera of Mitosporic fungi in the present investigation no Basidiomycetes were encountered. Among the isolates majority of the fungi belongs to the group Mitosporic fungi, than Zygomycetes and Ascomycetes group respectively, Genera like Rhizopus, Mucor and Absidia were dominant in Zygomycetes, Genus Chaetomium in Ascomycetes and in Mitosporic fungi genera like Aspergillus, Cladosporium, Curvularia, Penicillium, Alternaria and Fusarium are most predominant.

Keywords: Mycoflora, contamination, spices, percentage of occurrence.

Introduction

Spices are important agricultural commodities, because of their taste and aroma they are commonly used to flavor the food preparations. Spices occupy a prominent place in the traditional and culinary practices all over the world[1,2], coming to the history of spices, India is known as home of spices and one of the largest producer of spices due to poor agricultural and storage practices which affects the production and quality of spices[3,4]. Fungi are predominant contaminants of spices but most such mycopopulations are probably regarded as commensal residents on the plant that survived may be responsible for spoilage and degradation of spice quality by producing enzymes, mycotoxins and other secondary metabolites. Fungal growth of stored spices is mainly influenced by Temperature and Relative Humidity[5]. Prevalence of seed spice mycoflora depending upon the field and storage conditions[6].

The spices contaminated by fungi might cause the health hazards for humans[7]. The spoilage of spice quality and mycotoxin production mainly influenced by type of fungi, food composition and also handling and storage practices. Several other reports have shown fungal contamination of different spices and other agricultural commodities. Francisco das Chagas O. et al[8] reported a wide range of field and storage fungi from Black Pepper, White Pepper and Brazil nuts. Studies on spoilage mould and mycotoxigenic fungi from imported raw spices was screened by Qaher A. Mandeel[9]. Farid M. Toma and Nareen Q. Faqi Abdulla[10] Investigated of microbial status of some spices and crude herbal drugs. Analysis of commodity wise mycoflora, contamination status and mycotoxin magnitude in commercially important agricultural products reported by Vinod Kumar et al [11].

In the present investigation a detailed survey of mycoflora of five selected spices from Dharwad region was under taken Viz; *Pepper nigrum* L. (Pepper), *Coriandrum sativum* L. (Coriander), *Cuminum cyminum* L. (Cumin), *Elettaria cardomomum* Maton.(Cardamom) and *Cinnamomum tamela* T. Nees and C.H. Eberm (Indian cassia). The screening of the mycoflora of spices from Dharwad region was done during the period of November 2009 to October 2012.

Material and Methods

Study area: Dharwad is selected as the study area for screening spice mycoflora, the area is located at $15^0 44'$ North latitude $74^0 99'$ East longitude in Karnataka state (India), because of the rich vegetation and conducive atmosphere for fungal growth it harbors different groups of fungi. The sites selected for the present investigation is Market Area and in and around Dharwad.

Sample collections: The five spice samples Viz; *Pepper nigrum* L. (Pepper), *Coriandrum sativum* L. (Coriander), *Cuminum cyminum* L. (Cumin), *Elettaria cardomomum* Maton. (Cardamom) and *Cinnamomum tamela* T. Nees & C. H. Eberm (Indian cassia) were collected from Local market area Kirana merchant shops and spice sailors etc. on regularly monthly basis, from the chosen sites from during the period of November 2009 to October 2012. The selected spice samples of unknown variety was collected in sterile polyethylene bags separately from the chosen sites, during the collection field data

Viz; collection area, weight, type of sample, date of collection etc was carefully noted in the field note book. Collected samples were brought to the laboratory and used for further mycoflora analysis.

Isolation of fungi from spices: Mycoflora of selected spices was isolated by using different isolation methods Viz. Serial Dilution Method[12], Agar Plate Method[13] and Standard Blotter Method[14] for the isolation different media were used Viz. Potato Dextrose Agar (PDA), Czapek Dox Agar (CZA), Malt Extract Agar (MEA) Yeast Extract Agar (YEA) and Sabouraud Dextrose Agar (SDA), Chloramphenicol antibiotic is used to inhibit the Bacterial growth.

Counting of Fungal Colonies: Fungal colonies start appearing on the inoculated plates after 24 hours white mycelium start to appear and in a couple of days, a full growth of the fungus takes place to form a colony. But in some cases, appearance of fungal colony takes many days as noticed in slow growing fungi. At the same time number of fungal colonies, color of the colony from both the sides and textures of the colony were noted.

The incubated plates were observed under stereo-binocular microscope. To avoid loss of fungi which developed late, and which might be covered by fast growing forms, all plates in a series were checked frequently under stereo-binocular microscope for a period of two weeks. All plates observed for one month before discarding.

Slide preparation, Photomicrography and Identification: Lactophenol with cotton blue stains was used for the slide preparation; the prepared slides were sealed with DPX mountant. The fungal illustrations was done based on Erma Camera Lucida drawings along with micrometric measurements by using 4X, 5X, 10X and 40X objectives 10X eye piece was used depending upon the fungal morphology and necessity.

Photo micrographs of the prepared slides were taken by Carl Zeiss Imager M2 Model Microscope with Jenoptic Prog. Res. C5 attached Camera using 10X and 40X objectives and 10X eye piece.

Identification of different fungi from five selected spices was done with help of slides prepared by direct mount from the culture; prepared slides were made permanent, with help of Erma Camera Lucida drawings along with micrometric measurements, Photomicrography and by referring fungal monographs and manuals such as Thom C. and Raper K.B.[15], Raper K.B. and Thom C.[16], Tandon R.N.[17], Subramanian C.V.[18], Barnett H.L. and Hunter B.B.[19], Domsch K.H. and Gams W.[20], Ainsworth G.C., Sparrow F.K. and Sussman A.S.[21], Ellis M.B.[22], Ellis M. B.[23], Ellis M.B. and Ellis J.P.[24], Raper K.B. and Fennell D.I.[25], Bilgrami K.S., Jamaluddin S. and Rizwi M.A.[26], Gilman J.C.[27], Nagamani A. Kunwar I.K. and C. Manoharachary[28], Pande Alaka[29] also research articles and other related literature fungal Identification and illustrations were made up to the Genera and Species level.

All identified specimens are deposited, under the code number MASD (Mycoflora Analysis of Spices from Dharwad), in the Mycology Laboratory, Department of Botany, Karnatak University, Dharwad, Karnataka State, India.

Presentation of data: In the presentation of data the terms "Percentage of fungal occurrence" were used, The term percentage of fungal occurrence is used to denote,

$$\% \text{ of fungal occurance} = \frac{\text{Total Number of individual fungal occurrence on all the samples in 36 months}}{\text{Total number of fungal occurrence on all the sample in 36 months}} \times 100$$

Results and Discussion

The present study reveals the isolation of 118 fungal species belongs to 44 genera, out of which 18 species belonging to 08 genera of Zygomycetes, 07 species belonging to 02 genera of Ascomycets and 93 species belonging to 34 genera of Mitosporic fungi. Among the isolates majority of the fungi belongs to the Mitosporic fungi group, than Zygomycetes and Ascomycetes group respectively (table 1).

Table-1
Different groups of fungi isolated from selected Spices

Groups	Genera	Species
Zygomycetes	08	18
Ascomycetes	02	07
Mitosporic fungi	34	93
Total	44	118

The predominant fungal genera were *Aspergillus, Cladosporium, Curvularia, Penicillium, Alternaria* and *Fusarium* in Mitosporic fungi, in Ascomycets genus *Chaetomium* is dominant and in Zygomycetes genera like *Rhizopus, Mucor* and *Absidia* were dominant. Among the five selected spices samples Cumin samples shows highest number of fungal species isolates belongs to different groups Viz. Zygomycetes (14), Ascomycets (05), Mitosporic fungi (65); from Coriander Zygomycetes (10), Ascomycets (05), Mitosporic fungi (61) isolates; Pepper Zygomycetes (11), Ascomycets (04), Mitosporic fungi (45); Cardamom shows Zygomycetes (09), Ascomycets (01), Mitosporic fungi (38) and Indian cassia samples shows the least number of fungal isolates Zygomycetes (06), Ascomycets (04), Mitosporic fungi (25) respectively (table 2 and figure 1).

Isolation of Fungi with respect to the different isolation methods Serial dilution method shows highest number of fungal species isolates belongs to different fungal groups viz. Zygomycetes (16), Ascomycets (05), Mitosporic fungi (78) species from Agar Plate Method Zygomycetes (14), Ascomycets (04), Mitosporic fungi (63) species and Standard Blotter Method Zygomycetes (10), Ascomycets (07), Mitosporic fungi (40) species were isolated respectively (figure 2). Among the fungal media PDA media shows highest number of isolates in total (84) species isolates; MEA shows total (66) species isolates; SDA shows

total (58) species isolates; CZA shows total (29) species isolates and YEA shows total (16) species isolates respectively (figure 3).

The percentage of fungal occurrence reveals predominantly occurring fungal genera like *Rhizopus* (2.799%), *Mucor* (1.806%) and *Absidia* (1.320%) in Zygomycetes, Genus *Chaetomium* shows (2.849%) occurrence in Ascomycetes and in Mitosporic fungi genera like *Aspergillus* (25.434%), *Cladosporium* (8.825%), *Curvularia* (8.756%), *Penicillium* (7.157%), *Alternaria* (5.976%) and *Fusarium* (5.906%) are most predominant genera. The highest percentage of occurrence was recorded in the genus *Aspergillus* (25.434%) and the lowest percentage of occurrence was observed in the genus *Rhynchophoma* (0.138%) and other genera shows percentage of occurrence ranges between (0.138% to 25.434%) (figure 4).

The other workers screened mycobiota of different spices and reported the predominant fungal genera viz. *Aspergillus, Eurotium* and *Fusarium* from Capsicum powder samples by Santos L et al[30]. Abdulkadir E. Elshafie et al[31] isolated twenty fungal species out of which species like *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* Spp., *Rhizopus* spp. and *Syncephalastrum* Spp. were predominately isolated ; Abou Donia M. A [32]screened 303 samples representing different types of spices and medicinal plants from Egypt and reported genera like *Aspergillus, Fusarium, Cladosporium, Penicillium, Mucor Absidia* and *Rhizoctonia* were dominant among the isolates. Hashem Mohamed et al[33] examined fifteen spices for their mould profile and isolated a total of 57 species. I. A. E- Kady et al[34] isolated 81 species belongs to 38 genera of fungi from 24 different of spices, collected from Egypt. Ahene, R.E. et al[35] screened fungal and bacterial contaminants of six spices and spice products in Ghana. Maetrese Arianne J. Beley et al[36] isolated fungal species from soy milk products and also analyzed the presence of aflatoxin level by using Enzyme-Linked Immunosorbent Assay (ELISA) method. Abedi-Tizaki Mostafa et al[37] reviewed rapid detection methods for analysis of Fungi and Mycotoxins in agriculture products.

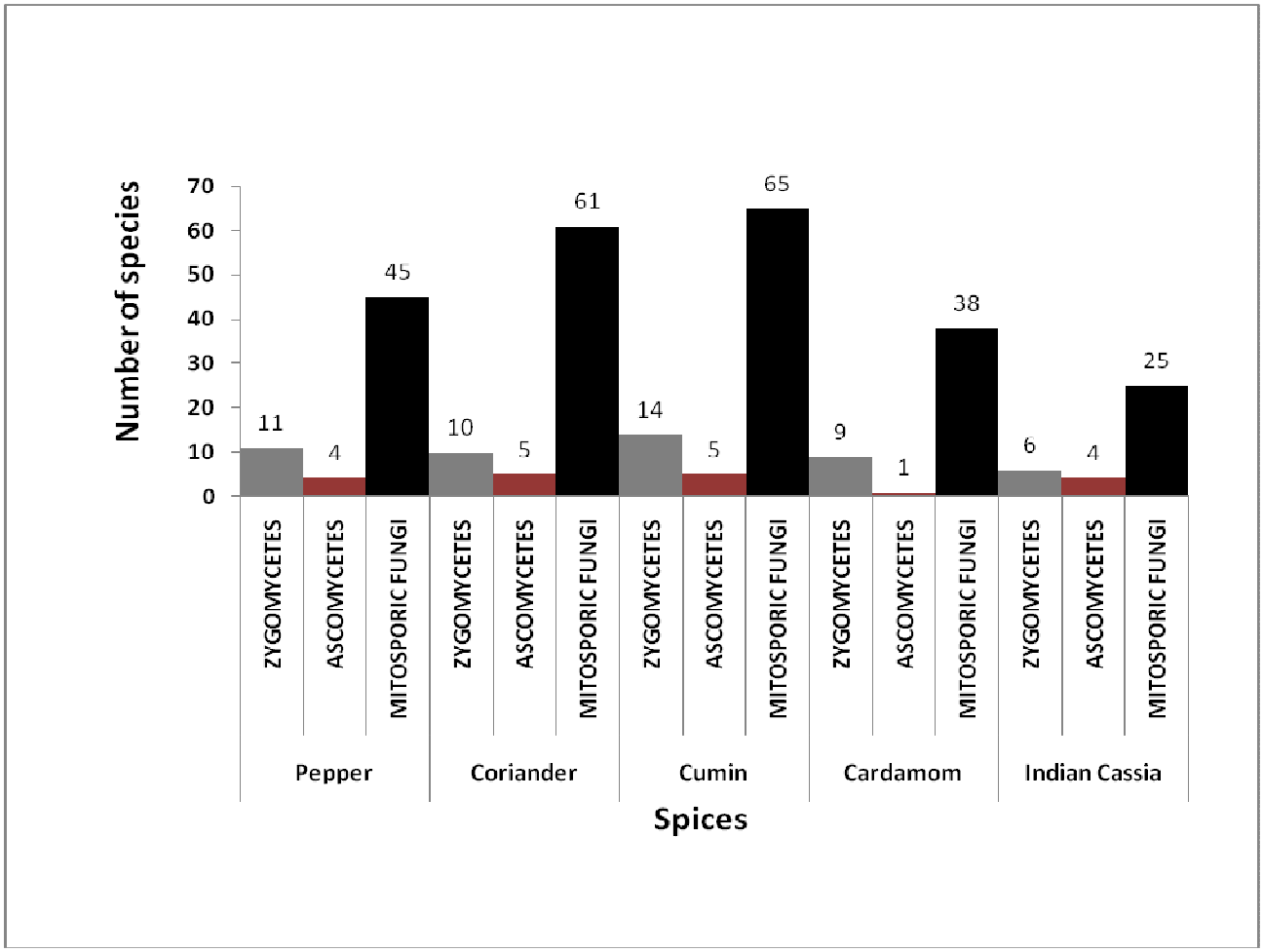

Figure-1
Different species of fungi isolated from five selected spices

Table-2
Different fungal species isolated from five Spices from Dharwad

Sl. No.	Name of the fungi	**Spices**				
	ZYGOMYCETES	**Pep.**	**Cori.**	**Cumin**	**Cardo.**	**In. Cassia**
1	*Absidia butleri* Lendner	+	+	+	-	-
2	*Absidia lichtheinsmi* Lendner	+	-	+	+	-
3	*Choanephora cucurbitarum* Thaxter	-	+	+	+	-
4	*Circinella simplex* Tiegh.	+	+	-	-	+
5	*Cunninghamella elegans* Lendner	+	+	+	-	-
6	*Mucor bacilliformis* Hesselt.	-	+	+	+	-
7	*Mucor fragalis* Bainier	+	-	+	+	+
8	*Mucor hiemalis* Wehmer	+	+	+	-	-
9	*Mucor javanicus* Wehmer	-	-	+	+	+
10	*Mucor rouxianus* Wehmer	+	+	-	-	-
11	*Mucor varians* Povah	+	-	-	+	-
12	*Mycotypha indica* P.M. Kirk & Benny	-	+	+	-	-
13	*Rhizopus arrhizus* Fisher	+	+	+	-	-
14	*Rhizopus nigricans* Ehrenb	-	-	+	+	-
15	*Rhizopus nigricans* Ehrenb var. minutus Chaudhuri	-	-	+	+	+
16	*Rhizopus nodosus* Namyslowski	+	-	-	+	-
17	*Rhizopus oryzae* Went et Gerlings	-	+	+	-	+
18	*Syncephalastrum racemosum* Cohn ex J. Schrot.	+	-	+	-	+
	ASCOMYCETES					
19	*Chaetomium amberpetense* Rama Rao & Ram Reddy	-	+	+	-	+
20	*Chaetomium atrobrunneum* Udagawa & Takada	-	+	-	+	-
21	*Chaetomium caprophilum* Narendra & VG Rao	+	-	+	-	+
22	*Chaetomium mollicellum* L. M. Ames.	+	-	+	-	+
23	*Chaetomium spirale* Zopf.	+	+	-	-	+
24	*Chaetomium subterraneum* Swift & Povah	-	+	+	-	-
25	*Emericella nidulans* Vuill.	+	+	+	-	-
	MITOSPORIC FUNGI					
26	*Aegerita candida* Pers.	-	+	+	+	-
27	*Alternaria amaranthi* van Hook	+	+	-	+	-
28	*Alternaria burnsii* Uppal, Patel & Kamat	-	+	+	-	+
29	*Alternaria carthami* Chowdhury	+	-	+	-	+
30	*Alternaria Citri* Ellis & Pierce	+	-	+	+	-
31	*Alternaria cyamopsidis* Rangaswami & Venkatarao	-	+	-	+	-
32	*Alternaria gomphernae* Togashi	-	+	+	+	-
33	*Alternaria longipes* Mason	-	-	+	+	+
34	*Alternaria palandui* Ayyangar	+	+	+	-	-
35	*Alternaria ricini* Hansford	-	+	+	+	-
36	*Alternaria tenuissima* Wiltshire	+	-	+	-	-
37	*Aspergillus alliaceus* Thom & Church	-	+	+	-	+
38	*Aspergillus ambiguous* Sappa.	+	+	+	-	-
39	*Aspergillus awamori* Nakazawa	-	+	-	+	+
40	*Aspergillus caespitopsis* Raper &Thom	+	-	+	-	+
41	*Aspergillus candidus* Link.	+	+	+	-	-
42	*Aspergillus chevalieri* Thom & Church	-	+	-	+	+
43	*Aspergillus deflectus* Fenell & Raper	+	-	+	-	+
44	*Aspergillus flavus* Link.	+	+	-	-	-
45	*Aspergillus funiculosis* Smith	-	-	-	+	+
46	*Aspergillus heteromorphus* Batista & Maia	+	+	+	-	-

47	*Aspergillus kanagawaensis* Nehira	-	+	-	+	+
48	*Aspergillus microcysticus* Sappa.	-	+	+	+	-
49	*Aspergillus montevidensis* Talice & Mackinnon	+	+	-	+	-
50	*Aspergillus ochraceus* Wilhelm	+	+	+	-	-
51	*Aspergillus parasiticus* Speare	-	-	+	+	-
52	*Aspergillus petrakii* Voros	+	-	-	+	+
53	*Aspergillus puniceus* Kwon & Fenell	-	+	+	-	-
54	*Aspergillus repens* De Bary	+	-	+	+	-
55	*Aspergillus speluneus* Raper & Fennell	+	-	-	-	+
56	*Aspergillus spinuloses* Warcup	+	-	+	+	-
57	*Aspergillus tericola var. indicus* N. Comb.	-	+	-	+	+
58	*Aspergillus terricola* Marchal	-	+	+	+	-
59	*Aspergillus ustus* Thom & Church	+	+	-	+	-
60	*Aspergillus versicolor* Tiraboschi	+	-	+	-	+
61	*Aurobasidium olae* Subram. Comb. nov.	-	+	+	-	-
62	*Bipolaris tetramera* Shoemaker	-	+	+	+	-
63	*Cladosporium cladosporioides* de Vries	+	+	-	-	+
64	*Cladosporium fulvum* Cooke.	-	-	+	+	-
65	*Cladosporium herbarum* Link ex Fries	+	+	+	-	-
66	*Cladosporium oxysporum* Berk. & M.A. Curtis	+	+	+	+	-
67	*Cladosporium variabile* de Vries	+	+	+	-	-
68	*Cochliobolus australiensis* Alcorn	-	+	+	-	-
69	*Curvularia inaequalis* Boedijn	+	-	+	-	-
70	*Curvularia indica* Subram.	+	+	-	+	-
71	*Curvularia lunata* Boedijn	-	-	+	+	+
72	*Curvularia maculans* Boedijn	+	-	+	-	-
73	*Curvularia pallescens* Boedijn	-	+	+	-	-
74	*Curvularia prasadii* Mathur R.L. &B. L.	-	+	-	-	+
75	*Curvularia senegalensis* Subram.	+	-	+	-	+
76	*Exosporina laricis* Oudem.	+	-	+	-	-
77	*Fusariella intermedia* Mouchacca & Nicot	-	+	+	-	-
78	*Fusarium merismoids* Corda	+	+	-	-	-
79	*Fusarium nivale* Cesati	-	+	+	+	-
80	*Fusarium oxysporum* Schl. ex Fries	+	-	+	-	+
81	*Fusarium poae* Wr.	+	+	-	-	-
82	*Fusarium udum* Butler	-	+	+	-	-
83	*Fusicladiella melaena* Hughes	-	+	+	-	-
84	*Fusidium aureum* Link.	-	+	-	+	-
85	*Geotrichum candidum* Link. ex. Sacc.	+	-	+	-	-
86	*Gliocladium penicillioides* Corda	-	+	+	+	-
87	*Gliocladium roseum* Bainier	+	-	+	-	-
88	*Memnoniella echinata* Galloway	-	+	+	-	-
89	*Memnoniella levispora* Subram.	+	+	-	-	-
90	*Monodictys fluctuate* M. B. Ellis	-	+	+	-	-
91	*Monosporium acuminatum* var. *terrestre* Sacc.	-	+	+	-	-
92	*Nigrospora oryzae* Petch	-	-	+	-	+
93	*Nigrospora sphaerica* Mason	+	-	+	-	-
94	*Paecilomyces varioti* Baineir	-	+	-	+	-
95	*Papulaspora irregularis* Hotson	-	+	+	-	-
96	*Penicillium brefeldianum* Dodge	+	-	+	-	-
97	*Penicillium chermesinum* Biourge	+	-	-	+	-
98	*Penicillium cyaneum* Biourge	-	+	-	-	+
99	*Penicillium decumbens* Thom	+	+	+	-	-

100	*Penicillium janthinellum* Biourge	-	+	-	+	-
101	*Penicillium nigricans* Bainier ex Thom	+	-	-	+	+
102	*Penicillium rubrum* Stoll	-	-	+	+	+
103	*Periconia cookei* Manson & Ellis	-	+	+	-	-
104	*Phragmostilbe linderi* Subram.	+	-	+	-	-
105	*Rhynchosporium secalis* Davis	-	+	+	-	-
106	*Sclerococcum sphaerale* Fr.	-	+	+	-	-
107	*Sporidesmium vagum* C.G. Nees & T.F.L. Nees ex Link	+	+	-	-	-
108	*Stachybotrys chartarum* Hughes	-	+	+	-	+
109	*Stachybotrys pulchra* Speg.	-	+	+	+	-
110	*Trichoderma koningii* Oudem	+	+	+	-	-
111	*Trichoderma longibrachiatum* Rifai	-	-	+	-	+
112	*Trichoderma viride* Pers.	-	+	+	+	-
113	*Veronaea caprophola* M.B. Ellis comb. nov.	-	+	+	-	-
114	*Verticillium dahliae* Klebahn	+	-	+	+	-
115	*Verticillium luteo-album* Subram.	+	+	-	-	-
116	*Wardomyces anomala* Brooks & Hansf.	+	-	-	+	-
	COELOMYCETES					
117	*Phoma herbarum* Cooke	-	+	+	-	-
118	*Rhynchophoma raduloides* Karst.	-	+	-	+	-
	TOTAL	**60**	**76**	**84**	**48**	**35**

+ = Present, *- = Absent.,* Pep. - *Piper nigram* Linn. (Pepper), Cori. – *Coriandrum sativum* Linn. (Coriander) , Cumin - *Cuminum cyminum* Linn. (Cumin), Cardo. - *Elettaria cardomomum* Maton. (Cardamom), In. Cassia. - *Cinnamomum tamela* T. Nees & Eberm (Indian Cassia)

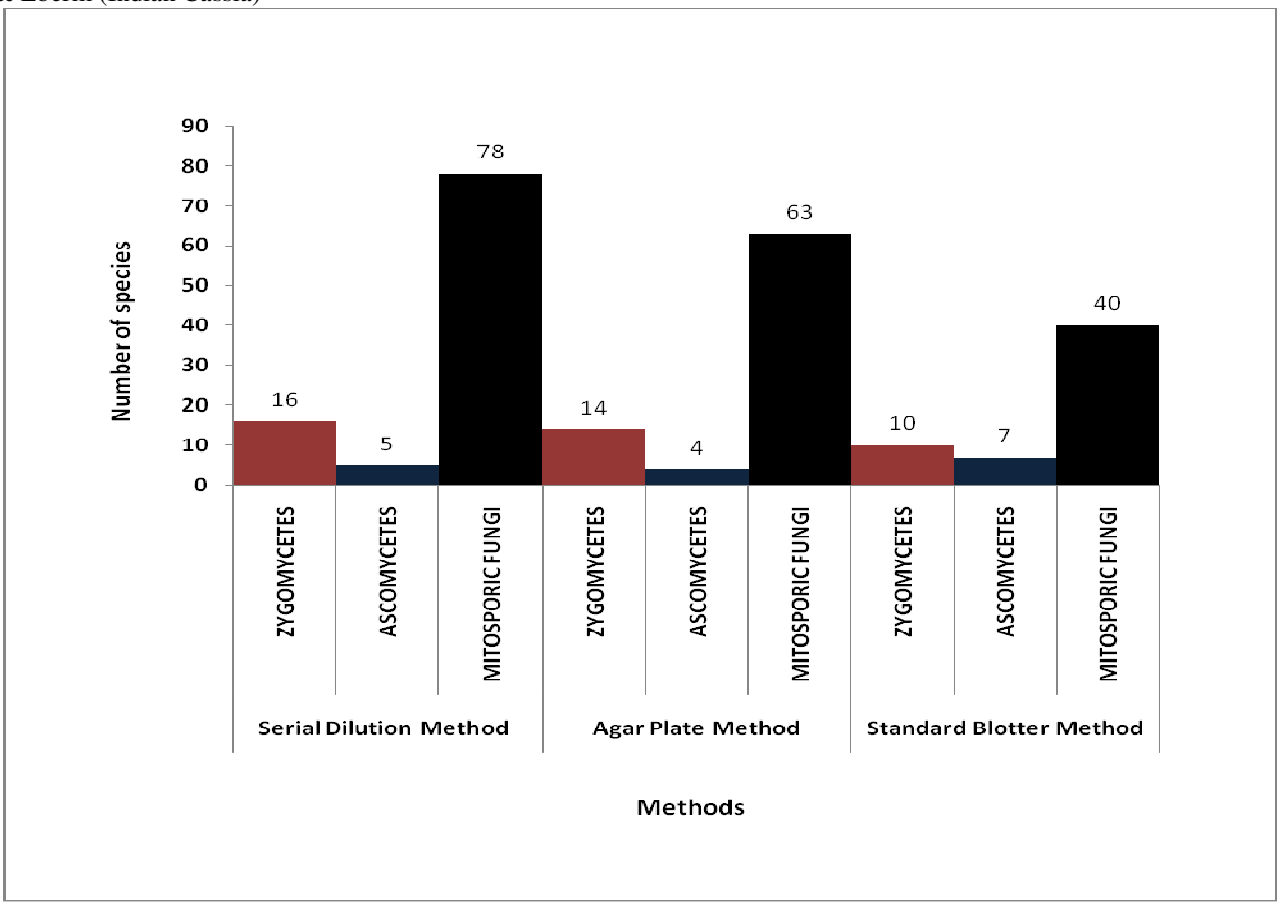

Figure-2
Isolation of different classes of fungi by using different methods

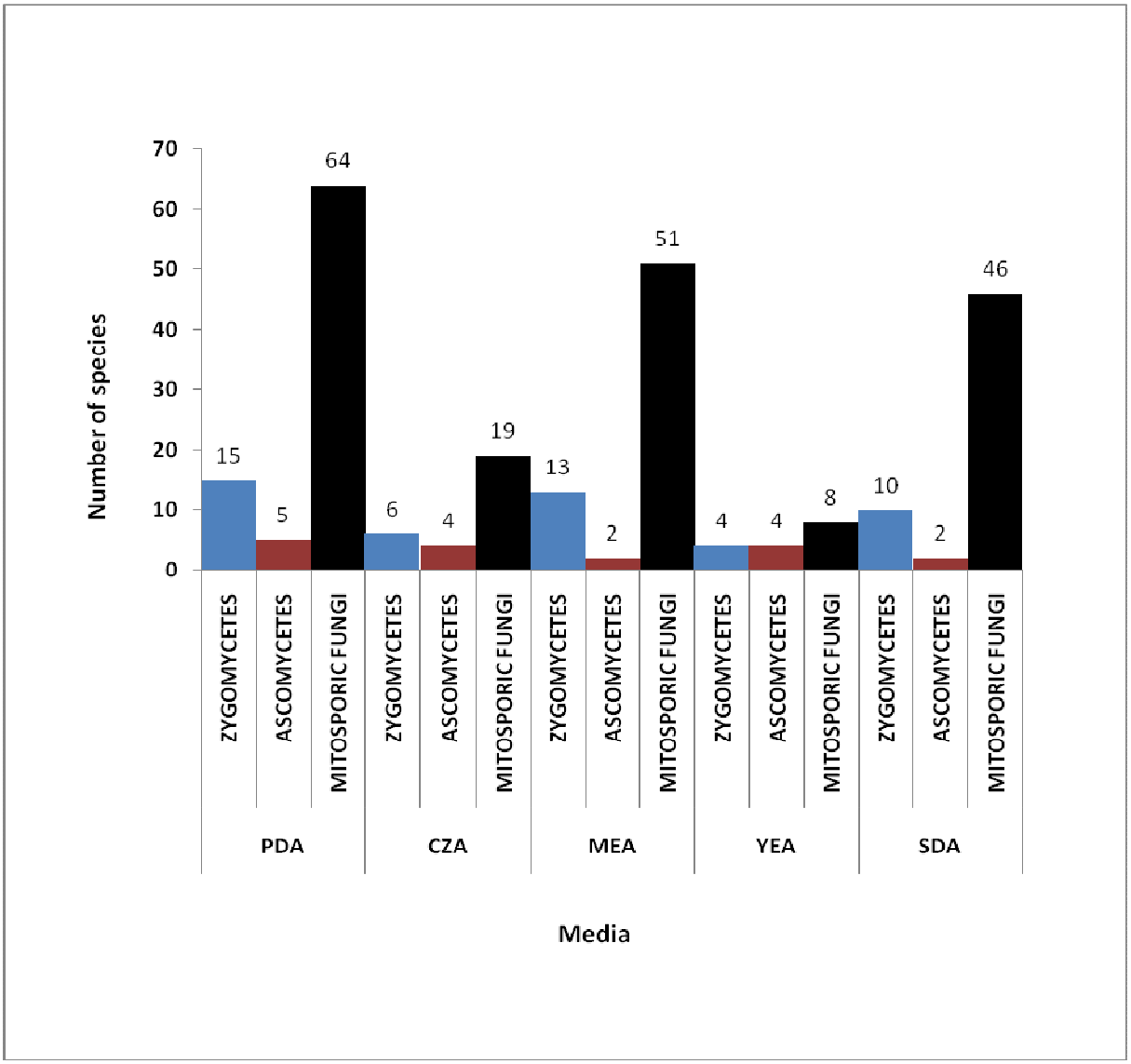

Figure-3
Distribution of different classes of fungi in different media

Conclusion

Spices are most important agricultural products because of their taste and aroma they are commonly used to flavor the food preparations. India is known as home of spices and one of the largest producer spices and they occupy a prominent place in traditional culinary practices, because of poor agriculture, storage and handling practices spices become contaminated at each level. When the contaminated mycoflora get conducive atmosphere for their growth; they grow and alter the quality and taste of the spices by secreting enzymes, toxins and other secondary metabolites during their growth and development.

The present investigation resulted in the isolation and identification of 118 species of fungi belongs to 44 genera out of which 18 species belonging to 08 genera of Zygomycetes, 07 species belonging to 02 genera of Ascomycets and 93 species belonging to 34 genera of Mitosporic fungi. In the present screening no basidiomycetes were encountered. Among the isolates majority of the fungi belongs to the Mitosporic fungi group, than Zygomycetes and Ascomycetes group respectively.

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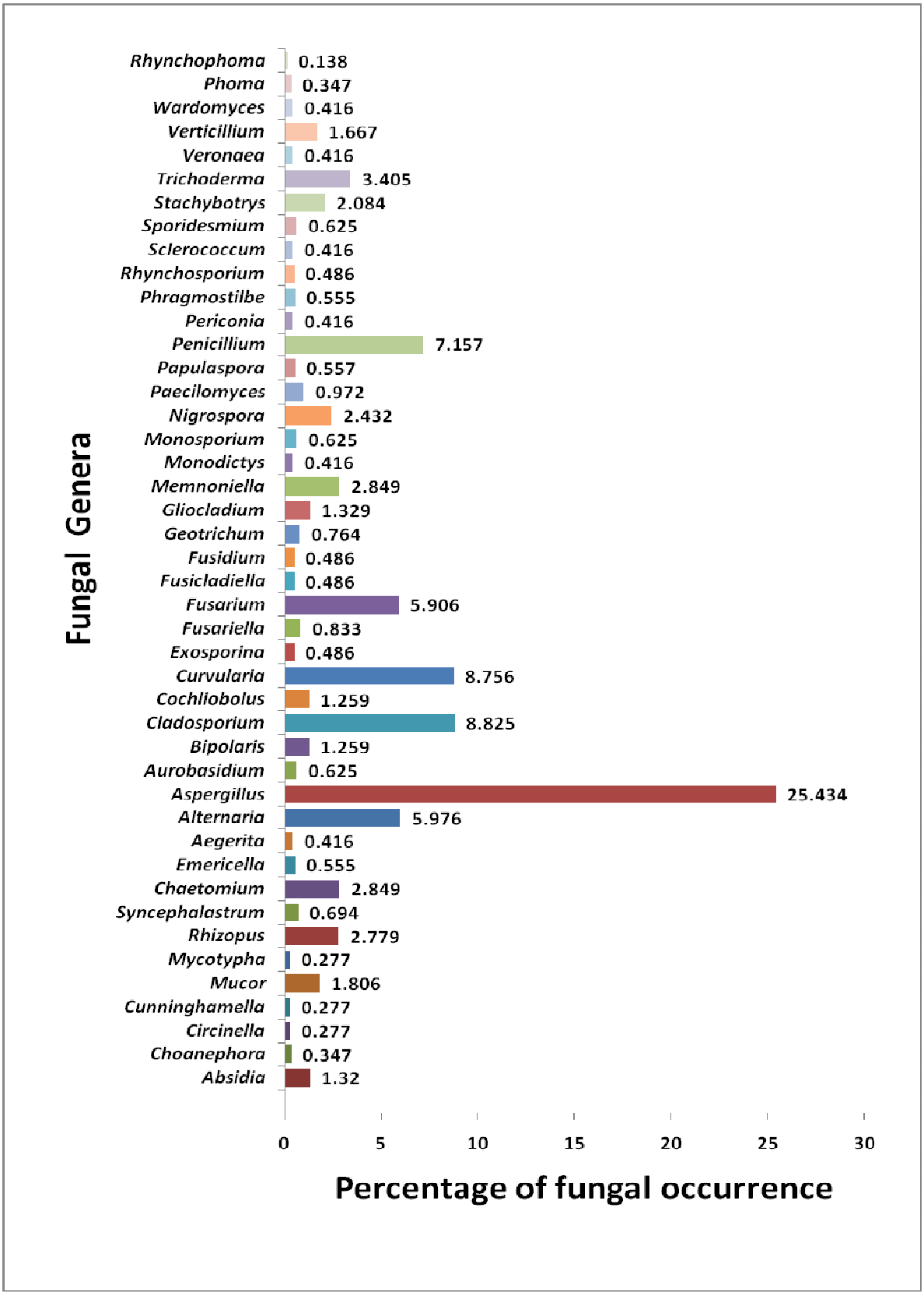

Figure-4
Percentage of fungal occurrence

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