

RESEARCH ARTICLE

Biodiversity of seed borne microfungal flora in storage on cauliflower (*Brassica oleracea* var. *botrytis*) from Nagpur region.

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Manuscript details:	ABSTRACT
<p>Date of publication 18.10.2014</p> <p>Available online on http://www.ijlsci.in</p> <p>ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Madavi Shivkumar and Bhajbhuj MN (2014) Biodiversity of seed borne microfungal flora in storage on cauliflower (<i>Brassica oleracea</i> var. <i>botrytis</i>) from Nagpur region., <i>Int. J. of Life Sciences</i>, Special issue A2: 43-47.</p> <p>Acknowledgement: The authors gratefully acknowledges the facilitation of this work by Prof. & Head, Dr Mrs. Alka Chaturvedi and Dr .R.P. Thakre, Ex- Professor, P.G. Department of Botany, RTM, Nagpur University, Nagpur.</p> <p>Copyright: © Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p>The mycological analysis of seed samples of Cauliflower (<i>Brassica oleracea</i> L. var. <i>botrytis</i>) from Nagpur region revealed prevalence of 38 fungal species belonging to 20 genera. Of these, 24 isolates encountered both as external and internal seed borne; 9 isolates were external and 5 isolates were internal seed borne. Deuteromycota dominated with nearly half of the total count of isolates followed by Ascomycota. <i>Aspergillus</i> dominated with highest count of species and contributed more than one quarter of the total incidence. <i>Fusarium</i> dominated with four; <i>Penicillium</i>, <i>Alternaria</i> and <i>Curvularia</i> each with three; <i>Chaetomium</i>, <i>Helminthosporium</i> with two species while remaining ones had single species. Ascomycota contributed greatest fungal incidence followed by Deuteromycota. <i>Rhizopus stolonifer</i>, <i>Fusarium</i>, <i>Alternaria</i> and <i>Penicillium</i> had significant level of infestation Moderate infestation has been recorded for <i>Cercospora</i>, <i>Chaetomium</i>, <i>Cladosporium</i>, <i>Mucor</i> <i>Curvularia</i>, <i>Helminthosporium</i>, <i>Nigrospora</i>, <i>Pyricularia</i>, <i>Trichothecium</i> and <i>Pythium</i> while others reported with little to mild infestation. Blotter paper method proved superior over agar plate.</p> <p>Key words: <i>Brassica oleracea</i> var. <i>botrytis</i>, seed borne, pathogens, susceptible, infestation, isolates.</p>
	<p>INTRODUCTION</p> <p>Cauliflower (<i>Brassica oleracea</i> L. var. <i>botrytis</i>) is a major winter vegetable cash crop extensively grown worldwide including India for its white inflorescence as it is low caloric, low fat, zero cholesterol content, vitamin and mineral rich, nutritious vegetable, propagated by seeds. It is a store house of health-benefiting antioxidants and several phytochemicals hence consumed as vegetable in curries, soups and pickles. The florets are used raw in mixed salad as it can be consumed by low carbohydrate dieters as a reasonable substitute for potatoes or rice. A fresh white cauliflower heads are roasted, boiled, fried, steamed and consumed. India ranks second among leading producer contributing around 32.9% of the global annual harvest after China (42.4%). Aside from being used as food, cauliflower has great demand in pharmaceuticals. Its extract has been reported to be effective in the inhibition of carcinogenesis; support the livers ability to neutralize toxins,</p>

maintains cardiovascular system, lowers cholesterol level; build a health immune system and also contribute to development of the fetus during pregnancy (Wikipedia, 2014).

Cauliflower is prone to attack by diverse group of fungal pathogens. Majority of them are reported to grow on stored seeds, causes physiological damage to seeds, resulting multi-fold loss to crop (Srivastava *et al.*, 2011). Prevalence of seed borne mycoflora concern to this crop has been highlighted by Srivastava *et al.*, (2011); Ismail *et al.*, (2012); Thakur *et al.*, (2013); Pscheidt and Ocamb (2014). A little is known from the Nagpur region concerning to biodiversity of seed mycoflora on *Brassica oleracea* L. var. *Botrytis*; it seems worthwhile to undertake systematic and comprehensive studies on biodiversity of seed mycoflora of this crop from Nagpur region.

MATERIALS AND METHODS

A composite seed sample of cauliflower from different retailers and stockiest of Nagpur region have been screened for isolation of fungal flora following standard blotter paper as well as agar plate technique (ISTA, 2014). The colonies developed on the untreated and pre-treated seeds were counted, isolated and identified after sub-culturing on tube slants containing Czapek's nutrient media. The species were identified on the basis of micro- & macro morphology; reverse and surface coloration of colonies grown on Czapek's medium and finally authenticated by authority. Fungal infestation level has been recorded as a percentage of infested seeds (Chukunda *et al.*, 2013).

RESULTS AND DISCUSSION

The seed borne organisms include a very large and heterogeneous group of organisms that exhibit an enormous diversity in life-history strategies. Healthy seeds may act as catalyst for realizing the potential of all other inputs. Health of seeds can be affected by direct infection of pathogen or through contaminated seeds by pathogenic propagules as contamination in, on or with the seeds or as concomitant contamination (Saskatchewan, 2013). The prevalence of propagules of pathogen in seed lot is vitally important because infected seed(s) may fail to germinate, cause infection to seedlings and reduce health of growing plants (Chukunda *et al.*, 2013).

The blotter and agar plate technique recommended for seed health testing and standardized time to time by ISTA (2013) for accuracy are applied for detection of seed borne fungal flora as these two tests are inevitable for getting a complete picture of the fungal infection/association with the seeds (Saskatchewan, 2013). Mycological analysis of a composite seed sample of cauliflower revealed the prevalence of population of total 38 fungal pathogens of diverse groups which fall under 20 genera in varying incidence (Table 1). A count of 24 isolates representing 13 genera has been isolated as both external and internal seed borne; 9 isolates of 7 genera confined only as external seed borne while 5 genera representing single species as internal seed-borne. Deuteromycota dominated with highest 47.4% fungal count followed by Ascomycota (39.5%), Zygomycota (7.9%). Oomycota had least count of isolates. Fungal spores from Basidiomycota did not persist on the seed surface. *Aspergillus* dominated with a higher count of 8 species; *Fusarium* with four species; three species each contributed by *Penicillium*, *Alternaria* and *Curvularia*; two species for *Chaetomium*, *Helminthosporium* while remainings had single species (Table 1).

The high level incidence was confined to cauliflower seeds. *Ascomycota* dominated with nearly half of the total fungal incidence (46.3%) followed by Deuteromycota (32.2%) and Zygomycota (19.1%) while Oomycota contributed least incidence. *Aspergilli* dominated with greater incidence; *Chaetomium* and *Penicillium* had moderate incidence while others representing Ascomycota had least level of incidence (Table 2). Amongst members of Deuteromycota, the *Fusarium moniliformae* had greater incidence while it was significant with *Alternaria alternata*, *A. brassicicola*, *Fusarium oxysporum*, *Curvularia ovoidea*, *C. lunata* and *Trichothecium roseum*. Moderate level was recorded for *Alternaria solani*, *Pyricularia* and *Rhizoctonia solani* while others had little incidence. In Oomycota, *Pythium* sp. had higher incidence over *Phytophthora infestans*. In Zygomycota, *Rhizopus stolonifer* had higher level of incidence. Out of the total, 62.8% incidence was recorded on blotter paper while it was 37.2% on agar plates. It is in confirmation with the findings of Saskatchewan (2013); Bhajbhujje (2013); Gayatri and Madhuri (2014) who reported higher fungal incidence from infested stored seeds of pulses, tomato and safflower respectively by blotter test.

Table 1: Percent incidence of fungal contaminants in storage on (*Brassica oleracea* L. var. *botrytis*) seeds.

Sr. No.	Name of fungal Species	Frequency (%) of fungal incidence		Total Frequency	% over total incidence ¹	
		Blotter	Agar		Species	Genus
A	Oomycota	6.5	2.5	9.0	2.37	2.37
1	<i>Phytophthora infestans</i> de Bary.	2.5	1.0	3.5	0.92	0.92
2	<i>Pythium</i> sp	4.0	1.5	5.5	1.45	1.45
B.	Zygomycota	50.0	22.5	72.5	19.13	19.13
3.	<i>Absidia corymbifera</i> (Cohn) Sacc. & Trotter	-	2.0	2.0	0.53	0.53
4	<i>Mucor pusillus</i> Lindt.	21.0	4.5	25.5	6.73	6.73
5	<i>Rhizopus stolonifer</i> (Ehrarb. Ex.Fr.) Lind.	29.0	16.0	45.0	11.87	11.87
C	Ascomycota	99	76.5	175.5	46.30	46.30
6	<i>Aspergillus flavus</i> Link	22.5	12.5	35.0	9.23	29.02
7	<i>Aspergillus fumigatus</i> Fres.	8.5	4.0	12.5	3.30	
8	<i>Aspergillus nidulans</i> (Eldam) Winter	3.5	-	3.5	0.92	
9	<i>Aspergillus niger</i> Van Tieghen	9.0	20.5	29.5	7.78	
10	<i>Aspergillus ochraceus</i> Wihelm	1.5	-	1.5	0.40	
11	<i>Aspergillus sulphureus</i> (Fres.) Thom & Church	2.5	-	2.5	0.66	
12	<i>Aspergillus terreus</i> Thom	12.0	10.5	22.5	5.94	
13	<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	-	3.0	3.0	0.79	
14	<i>Chaetomium glabosum</i> Kunne & Schm	10.0	5.5	15.5	4.09	6.86
15	<i>Chaetomium</i> sp.	5.0	5.5	10.5	2.77	
16	<i>Cladosporium fulvum</i> Cooke	6.0	2.5	8.5	2.24	7.39
17	<i>Penicillium oxalicum</i> Currie & Thom	13.5	8.5	22.0	5.81	
18	<i>Penicillium pallidum</i> (Cruick & Shank) Pitt.	2.0	1.0	3.0	0.79	
19	<i>Penicillium</i> sp	-	3.0	3.0	0.79	
20	<i>Phoma glomerata</i> (Corda) Wr. & Bochapfal	3.0	-	3.0	0.79	0.79
D.	Basidiomycota	-	-	-	-	-
E.	Deuteromycota	82.5	39.5	122	32.19	32.19
21	<i>Alternaria alternata</i> (Fr.) Keissler	6.0	4.5	10.5	2.78	7.27
22	<i>Alternaria solani</i> (E & M) Jones & Grout	4.0	3.5	7.5	1.98	
23	<i>Alternaria brassicicola</i> (Schweinitz, Wiltshire)	5.5	4.0	9.5	2.51	
24	<i>Botryodiplodia</i> sp	-	3.0	3.0	0.79	0.79
25	<i>Cercospora</i> sp	-	5.0	5.0	1.32	1.32
26	<i>Curvularia ovoidea</i> (H & W) Munt.	6.0	2.0	8.0	2.11	5.67
27	<i>Curvularia lunata</i> (Wakker) Boedijn	6.5	2.0	8.5	2.24	
28	<i>Curvularia</i> sp	5.0	-	5.0	1.32	
29	<i>Fusarium miniliformae</i> Sheldom	7.0	4.5	11.5	3.03	8.18
30	<i>Fusarium oxysporum</i> Schlecht	6.0	4.0	10.0	2.64	
31	<i>Fusarium semitectum</i> Berk & Rav.	4.0	-	4.0	1.06	
32	<i>Fusarium solani</i> (Mert.) APP. & Wollenw	3.5	2.0	5.5	1.45	
33	<i>Helminthosporium tetramera</i> Mc Kinney	4.0	2.0	6.0	1.58	2.24
34	<i>Helminthosporium</i> sp.	2.5	-	2.5	0.66	
35	<i>Nigrospora</i> sp.	2.5	-	2.5	0.66	0.66
36	<i>Pyricularia</i> sp.	6.0	1.5	7.5	1.98	1.98
37	<i>Rhizoctonia solani</i> Kuhn.	7.5	-	7.5	1.98	1.98
38	<i>Trichothecium roseum</i> Link	6.5	1.5	8.0	2.11	2.11
	Total fungal incidence	238	141	379	99.99	99.99

1. Values of incidence of fungal flora calculated in terms of percent incidence over total incidence recorded.

Ascomycota contributed greatest fungal incidence. The dominant microfungus genera of this group include *Aspergillus*, *Penicillium* and *Chaetomium*, of this *Aspergillus* contributed more than one quarter of the total incidence. Higher incidence of *Aspergillus* was reported on seeds of maize (Chukunda *et al.*, 2013); brinjal (Bhajibhujje, 2014). These results are in confirmation with earlier findings on oil seeds (Jain, 2008). *Alternaria*, *Curvularia*, *Fusarium*,

Helminthosporium, *Rhizoctonia* and *Pyricularia* of Deuteromycota were reported predominant. It is in agreement to finding of Kakde *et al.* (2012) who reported predominant occurrence of Deuteromycetous members on oil seeds.

The efficacy of both standard blotter and agar plate tests varied with nature of fungal flora. The members of Oomycota and Zygomycota developed more

Table 2: Distribution of seed borne fungal flora on seeds of *Brassica oleracia* var *botrytis*

Sr. no.	Fungal Division	Number of fungal pathogen recorded						Frequency (%) of incidence				
		Both external & internal seed borne		External seed borne only		Internal seed borne only		Total genera	Total species	Blotter test	Agar plate test	Total fungal incidence ³
		Species ¹	Genera ²	Species	Genera	Species	Genera					
1.	Oomycota	2 (8.3) ¹	2 (14.3) ²	-	-	-	-	2 (10.0)	2 (5.6)	6.5 (1.7)	2.5 (0.7)	9.0 (2.4) ³
2.	Zygomycota	2 (8.3)	2 (14.3)	-	-	1 (20.0)	1 (20.0)	3 (15.0)	3 (7.9)	50.0 (13.2)	22.5 (5.9)	19.13 (19.1)
3.	Ascomycota	9 (37.5)	4 (28.6)	4 (44.4)	2 (28.6)	2 (40.0)	2 (40.0)	5 (25.0)	15 (39.5)	99.0 (26.1)	76.5 (20.2)	175.5 (46.3)
4.	Deuteromycota	11 (45.8)	6 (42.9)	5 (55.6)	5 (71.4)	2 (40.0)	2 (40.0)	10 (50.0)	18 (47.4)	82.2 (21.8)	39.5 (10.4)	122.0 (32.2)
	Total	24	14	9	7	5	5	20	38	238.0 (62.8)	141.0 (37.2)	379.0 (99.99)

1 & 2. Values in parenthesis indicate percent fungal isolates over total isolates recorded.
3. Values in parenthesis calculated in terms of percent incidence over total incidence.

profusely on agar plate possibly because they require softer medium rich in moisture for their establishment and growth. Among the seed health test techniques, standard blotter method was proved comparatively superior over agar plate method to the fungal pathogens isolation. Chukunda *et al.*, (2013) pointed out the quick growing saprophytes adhering to the outer seed coat which may be troublesome to detect internal slow growing pathogen on agar plate. These variations may possibly attribute to the prolonged incubation that might lead to the development of deep seated infection (Hedawoo *et al.*, 2014). The physiochemical nature of the seed as well as agricultural practices and storage environment provided for the different crop seeds are also possibly responsible to variation in two methods (Gayatri and Madhuri, 2014).

Mycological analysis of disinfected and non-disinfected seeds gave only general information about inner seed infection by assuming that fungal propagules exist in non-disinfected seeds and absent in disinfected seeds and that fungal organism contaminated their surface and they do not penetrate the inner tissue. This information can be a starting point to determine proper strategies of seed treatment.

Aspergilli and *Penicilli* of Ascomycota as well as *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Rhizoctonia*, *Pyricularia* and *Trichothecium* of Deuteromycota contributed as major components on cauliflower seeds 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 (Gayatri and Madhuri, 2014). Deuteromycota had comparatively higher count of fungal isolates associated with stored seeds but Ascomycota had

greater level of incidence followed by Deuteromycota. It may possibly due to prevalence of greater count of fungal propagules associated with seed coat with their higher incidence. Moreover, members of this group are known facultative parasites on crop plants as well as involved as saprophyte in biodegradation of seeds, and debris of plant and animal origin (Jyoti and Malik, 2013). Under storage, in moist environment the seeds form the ideal organic substrate to the development of storage fungi (Bhajbhuj, 2014). Members of Deuteromycota complete their life cycle asexually producing abundant, resistant, thick walled conidia which may remain viable for longer duration in adverse climate (Gayatri and Madhuri, 2014). The conidia of *Helminthosporium*, *Cladosporium*, *Alternaria*, *Trichothecium*, and *Curvularia* remained in greatest abundance under storage even at low humidity during warmer climate (Kakde *et al.*, 2012). It was interesting to record that members of Basidiomycota did not persist on cauliflower seeds may be possibly attributed to mode of nutrition as majority of fungal organisms of these groups are obligate parasites of other crop plants.

The report revealed that Ascomycetous genera, *Aspergilli* and *Penicilli* which were the highly predominant on cauliflower seeds are among the most abundant and widely distributed organisms on the globe (Gayatri and Madhuri, 2014). *Aspergillus niger*, *A. flavus*, *A. fumigatus* etc. are known as obligate saprophyte and are commonly isolated from seeds, soil, plant litter, dried fruits and nuts (Kakde *et al.*, 2012; Jyoti and Malik, 2013). *Aspergillus niger* has potential to produce *ochratoxin-A*; *Aspergillus flavus* secretes aflatoxin which proved to be nephrotoxic in pigs and broilers (EFSA, 2011). *Penicillium* produce Penicillic acid causing systemic penicilliosis in AIDS patients in Southern Asia and proved to be nephrotoxic in

pigs and boilers (EFSA, 2011). Members of *Helminthosporium* have been reported to produce *Helminthosporin*; *Curvularia lunata* produces 2-methyl-(5-hydroxy methyl) furan-2 carboxylate; *Alternaria* secretes *Altersolarol-A* and *alternaric acid dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphytoxin III* (Brakhage and Schroeckh, 2011) that have been reported to cause a variety of toxic effects in both experimental animals and in human. *Fusarium solani* and *F. moniliformae* were reported to cause *keratitis* and also associated with wound and infections of the eyes and fingernails (EFSA, 2011). *Mucor pusillus* secretes Citrinin and Penetrem-A; *Cladosporium fulvum* produce glycosyl moiety; *Cercospora* secretes cercosporin. All these toxins are known to create physiological disorders to consumers (EFSA, 2011). Majority of fungal isolates involved in seed deterioration of cauliflower are xerophilic moulds such as *Aspergilli* and *Penicilli* of Ascomycota as well as *Alternaria, Curvularia, Fusarium, Helminthosporium* of Deuteromycota (Bhajibhuje, 2014). Planting of deteriorated seeds, increases chances of pathogen transmission to a new crop. The toxic metabolites secretion by these isolates may be one of the reasons to spoilage of stored seeds (Jyoti and Malik, 2013). Mutagenic and carcinogenic effect of mycotoxins has been highlighted by EFSA (2011). More than 300 fungal metabolites are reported to be toxic to man, animals and pose serious health hazard. Mycotoxins alter regular metabolism, induced physiological & biochemical changes in host cells resulting abnormal proliferation of plant cells (Jyoti and Malik, 2013).

CONCLUSION

Healthy seeds are important input for desired plant production and good economic harvest. The results revealed that cauliflower seeds harbor arrays of fungal contamination may be associated with the quality of seeds at the time of storage, environmental factors during pre- and post-harvest stages, moisture content, ambient relative humidity, temperature of storage environment and duration of seeds. The climate of winter season of Nagpur as well as improper storage condition contributes to make the storage environment extremely supportive for fungal attack on nutrient rich cauliflower seeds. In order to neutralize the potential of these fungal microbes surviving as agents of seed borne diseases, the steps must be initiated to develop a strategy to antagonize their

growth and survival in this seed commodity. Low temperature results in delayed seed deterioration, and, thereby leads to prolonged viability period. Thus seed storage under ambient temperature and relative humidity without deterioration in quality for a longer period is of immense importance for farmers. The farmers are advised to use improved scientific methods of storage to discourage proliferation of these organisms on seeds.

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