RESEARCH ARTICLE

Biodiversity of seed mycoflora in storage of *Brassica campestris* L.

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ABSTRACT

Seed is the basic and most critical input for substantial agriculture. A population of 41 fungal pathogens representing 20 genera has been recorded on stored seeds from a composite of 20 seed samples of mustard (*Brassica campestris* L.) obtained from each geographical areas of Nagpur district understudy comprising of five sub-divisions and 14 talukas. Mycological analysis revealed prevalence of total 41 fungal pathogens fall under 20 genera from survey area SD-I; 39 species representing 20 genera from SD-II; 39 species of 19 genera from SD-III; 35 isolates of 19 genera from SD-IV; and altogether 34 species belonging to 18 genera from SD-V in varying level of incidence. Members of Deuteromycota are most predominant followed by Ascomycota, Zygomycota and Oomycota. Basidiomycota did not persist on seeds. A population of 29 species of 15 genera have been reported from composite seed lots as both external and internal seed borne; 7 isolates of 5 genera as external while 5 species as internal seed borne isolates. Of the total, three-fourth incidence was recorded on blotter paper over agar plating. Ascomycota contributed nearly half of a total incidence followed by Deuteromycota, contributing one-third of total incidence. Zygomycota contributed moderate while Oomycota had least incidence. Among the predominant isolates, *Aspergillus* dominated with highest count of species followed by *Alternaria, Curvularia, Fusarium*, *Penicillium, Rhizopus* and *Helminthosporium* while remainings were reported with single species. *Aspergillus amstelodomi, A. sulphureus, A. versicolor, A. ochracious, Paecilomyces varioti* and *Cunninghamella elegans* were reported for first time on mustard seeds in India.

Key words: Seed mycoflora, Biodiversity, *Brassica campestris* L. incidence, susceptible.

INTRODUCTION

Mustard (*Brassica campestris* L.) of family Brassicaceae, is native to Europe but has become naturalized throughout the world for its oil-seeds as the seeds are used in prehistoric time quite intuitively for extraction of oil for cooking and burning purposes; curing many bodily disorders and thereby keep health in perfect state of fitness and lived a long life. The seed oil becomes a remedy for skin diseases hence crude oil is applied on skin for primary health care especially among those representing in remote areas, like tribal and other forest dwellers.

It is used in margarine soap, rubber lubrication and for oiling wool, as counter irritant and rebefacient in the form of poultice or plasters (Wikipedia, 2014). Nearly 90% human population of U.P., Punjab, Bihar, and Assam utilizes mustard seed oil for cooking as an alternative source to groundnut oil while in remaining parts of India, the mustard seed oil is utilized as preservative in response to strong antibacterial, antifungal and medicinal properties while oil cake is used as cattle feed & manure (Wikipedia, 2014). India holds a premier position in mustard economy and ranks third leading producer of mustard seeds in the world contributing around one-third of the global annual harvest after China and Canada. This crop accounts for nearly one-third of the oil produced in India, making it the country's key edible oilseed crop. Due to the gap between domestic availability and actual consumption of edible oils, India has resort to import of edible oils with a projected demand for edible oils at more than 20 mt in 2014-15 (Aradhey, 2011).

The literature survey reveals that seeds of *Brassica campestris* L. are known to carry several fungal pathogens which cause to alter physio-chemical properties of the seeds during storage, losses of the seed weight, germination potential, medicinal properties, and discolouration causing the losses to the extent of 24% (Ashraf and Choudhary, 2008). In India, various researchers have studied the incidence of seed borne-fungi of several species of *Brassica* under storage environment from various geographical locations (Gotarkar and Hedawoo 2010; Siddiqui, 2013; Ghugal and Thakre, 2014). It is very long that no investigations on bio-diversity of seed-borne fungal flora of mustard during storage is carried out pertaining to the area of Nagpur district comprising altogether 14 talukas fall under five sub-divisions. Keeping this in view, a survey on bio-diversity of fungal flora on stored seeds of mustard (*Brassica campestris* L.) of Nagpur District of Maharashtra State is undertaken.

MATERIALS AND METHODS

In present investigation, after collection of 20 seed samples of *Brassica campestris* L from cultivators and retailors of each tehsil of Nagpur district were screened preliminary for apparent deformities employing dry examination technique (CMI, 2010). A randomly selected four hundred seeds from a composite of seed sample and samples from each survey area were screened for prevalence of seed-borne fungal pathogens employing standard blotter and agar plate technique as recommended by International Seed Testing Association (ISTA, 2013). Two hundred seeds without pretreatment were screened for detection of external seed borne while same count of seeds pretreated with aqueous solution of 0.1% mercuric chloride were placed to sterile petri plate containing semi-solid agar nutrient sterile medium composed of peeled potato ($400gm^{-l}$), dextrose ($20gm^{-l}$**)** and agar ($20gm^{-l}$**)** in a liter of distilled water for isolation of internal seed borne fungal flora. After incubation for seven days in B.O.D incubator at 25±2°C under alternating cycles of 12 hours light and darkness, all untreated and pretreated seeds in petri plates were examined for fungal growth appeared on seeds surfaces. The fungal flora was identified with the help of colony colour and sporulation type. Fungal count and infestation level on untreated and pre-treated seeds have been recorded as a percentage of infested seeds in a sample following a technique reported earlier (CMI, 2010). The seed borne isolates were purified, sub-cultured and maintained on Czapek's Dox agar nutrient medium in sterile tube slants and species were identified on the basis of micro- & macro morphology; reverse and surface coloration of colonies grown in Czapek's medium (Neergaard, 1977) and finally authenticated by authority.

RESULTS AND DISCUSSION

Seed is both a symbol and foundation of life as it is a container of embryo(s) of a new generation and vehicle for the spread of new life hence pathogen free healthy seeds gives a clear picture of their glorious golden era (Saskatchewan (2013). Altogether 20 seed samples of mustard (*Brassica campestris* L.) has been collected from each geographical areas understudy comprising of 14 talukas belongs to five sub-divisions of Nagpur districts and screened for prevalence of seed borne mycoflora.

(a) Seed mycoflora of mixed seed samples:

Mycological analysis of composite seed samples of *Brassica campestris* L. revealed prevalence of a population of total 41 fungal pathogens fall under 20 genera in varying level of incidence (Table 1). Of

these, isolates belong to Deuteromycota are most predominant ones, represented by 8 genera and 18 species. Ascomycota are represented by 6 genera and 16 species. Zygomycota had 4 genera and 5 species. Oomycota are represented by 2 genera and 2 species. Member of Basidiomycota did not persist on the seeds. Individual genus, *Aspergillus* dominated with 9 species, followed by *Alternaria, Curvularia* and *Fusarium* with 4 species each. Three species of genus *Penicillium,*; two species of *Rhizopus, Helminthosporium* have been encountered as seed contaminants while genera recorded with single species included *Phytophthora, Pythium, Absidia, Mucor, Cunninghamela, Botrytis, Chaetomium, Cladosporium, Phoma, Nigrospora, Paecilomyces, Rhizoctonia* and *Trichothecium*. A population of seven isolates, *Aspergillus amstelodomi, A. sulphureus, A. versicolor, A. ochracious, Paecilomyces varioti* and *Cunninghamella elegans* has been reported as seed borne pathogens for a first time from *Brassica campestris* L seeds in India.

A fungal population of 29 species representing 15 genera have been isolated on both blotter paper and agar plate included *Alternaria alternata, A. solani,, A. brassicicola, A. brassicae, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia clavata, C. ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus, Paecilomyces variotii, Penicillium oxalicum, P. pallidum, Phytophthora infestans, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus stolonifer, R. nigricans* and *Trichothecium roseum*. Of the total count, *Aspergillus flavus, A. niger* and *Rhizopus stolonifer* were appeared to be most predominant exhibiting comparative higher incidence. The isolates recorded subdominant had incidence between 20-27% included *Aspergillus fumigatus, A. terreus, Penicillium oxalicum* and *Mucor pusillus* while others had 5.5 to 18.0% incidence. Little incidence was detected for *Penicillium pallidum, Curvularia clavata*, *Paecilomyces variotii* and *Trichothecium roseum* by both health testing techniques (Table 1).

A population of a total 7 fungal species belongs to 5 genera has been confined to blotter test only as external seed borne fungal pathogens, included *Aspergillus nidulans, A. ochracious, A. sulphureus Cunninghamella elegans*, *Curvularia intermedia, Nigrospora* and *Helminthosporium spiciferum.* Among these, *Aspergillus ochracious* and *Curvularia intermedia* were appeared to be most dominant with 6.5% incidence. The level of incidence, 4.5% was detected for *Cunninghamela elegans* and *Aspergillus sulphureus.* The isolates *Aspergillus nidulans* and *Helminthosporium spiciferum* had low frequency of incidence. Fungal isolates restricted only to agar plates included five genera, *Absidia corymbefera, Aspergillus versicolor,Botrytis cinera, Penicillium digitatum,* and *Phoma glomerata*. Member of Deuteromycota did not appear as internal seed borne. Excepting *Phoma glomerata*, others had incidence varies between 3.5 -5.5% (Table 1).Of the total, 66.1% incidence was recorded on blotter paper while 33.9% on agar plates. Ascomycota contributed nearly half of the total fungal incidence, represented by 47.0%. Deuteromycota contributed 34.6% of total incidence, followed by Zygomycota (15.3%) and Oomycota (3.1%) (Table 2).

(b) Seed mycoflora from SD-I

Mycological analysis of seed samples of *Brassica campestris* L from SD-I revealed prevalence of total 41 fungal pathogens fall under 20 genera in varying incidence. The isolates, *Aspergillus flavus, A. niger, A. terreus, Mucor pusillus* and *Penicillium oxalicum* were appeared to be most predominant with 20.5-37.5% incidence whereas low frequency, 2.0-4.5% was recorded for *Absidia corymbefera, Aspergillus ochracious, A. versicolor, Botrytis cinera, Curvularia clavata, Cunninghamella elegans, Helminthosporium specifectum, Nigrospora sp., Penicillium digitatum* and *Rhizopus nigricans.* The isolate *Aspergillus nidulans* had least incidence (Table 1).

The seed samples from SD-I were reported heavily infested with a fungal population comprising of 41 pathogens representing 20 genera (Table 1). Of them, 24 isolates of 14 genera has been detected in varying level of incidence as both external and internal seed borne by blotter paper and agar plate techniques, included *Alternaria alternata, A. solani,, A. brassicicola, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus, Paecilomyces variotii, Penicillium oxalicum, P. pallidum, Phytophthora infestans, Pythium aphanidermatum, Rhizopus stolonifer* and *Trichothecium roseum* (Table 1).

Table 1: Frequency (%) of incidence of fungal flora on seeds of mustard (*Brassica campestris* L.) received from various geographical locations of sub-divisions of Nagpur District.

Sr. No.	Fungal isolates	Frequency (%) fungal incidence on seeds of *Brassica campestris* L.																	
		Mixed seed sample			*Sub-div.- I			Sub-div.-II			Sub-div.- III			Sub-div.-IV			Sub-div.-V		
		Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence		
		B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T
A	**Oomycota**	**9.5**	**5.5**	**15.0**	**6.5**	**4.0**	**10.5**	**4.0**	**1.5**	**5.5**	**4.5**	**4.0**	**8.5**	**6.5**	**5.0**	**11.5**	**5.0**	**3.5**	**8.5**
1	*Phytophthora infestans* de Bary.	5.0 (1.0)	3.0 (0.6)	8.0 (1.6)	3.5 (0.9)	1.5 (0.4)	5.0 (1.3)	2.5 (0.8)	0.5 (0.1)	3.0 (0.9)	2.0 (0.6)	2.5 (0.7)	4.5 (1.3)	3.0 (0.9)	2.5 (0.7)	5.5 (1.6)	2.5 (0.9)	2.0 (0.7)	4.5 (1.7)
2	*Pythium aphanidermatum* (Edson) Fitzp.	4.5 (0.9)	2.5 (0.5)	7.0 (1.4)	3.0 (0.8)	2.5 (0.6)	5.5 (1.4)	1.5 (0.5)	1.0 (0.3)	2.5 (0.8)	2.5 (0.7)	1.5 (0.4)	4.0 (1.1)	3.5 (1.0)	2.5 (0.7)	6.0 (1.8)	2.5 (0.9)	1.5 (0.6)	4.0 (1.5)
B.	**Zygomycota**	57.0	18.0	75.0	51.0	15.5	66.5	38.0	21.0	59.0	50.0	24.5	74.5	49.0	20.0	69.0	30.5	16.0	46.5
3.	*Absidia corymbifera* (Cohn) Sacc. & Trotter	-	4.5 (0.9)	4.5 (0.9)	-	3.0 (0.8)	3.0 (0.8)	-	4.0 (1.2)	4.0 (1.2)	-	3.0 (0.8)	3.0 (0.8)	-	2.0 (0.6)	2.0 (0.6)	-	1.5 (0.6)	1.5 (0.6)
4	*Mucor pusillus* Lindt.	22.5 (4.6)	4.5 (0.9)	27.0 (5.5)	17.0 (4.3)	3.0 (0.9)	20.5 (5.2)	12.0 (3.7)	4.5 (1.4)	16.5 (5.1)	16.0 (4.5)	5.5 (1.6)	21.5 (6.1)	18.0 (5.3)	6.0 (1.8)	24.0 (7.1)	9.0 (3.3)	4.0 (1.5)	13.0 (4.8)
5	*Rhizopus stolonifer (Ehrarb.* Ex.Fr. Lind.	25.5 (5.2)	5.5 (1.1)	31.0 (6.3)	26.0 (6.6)	9.0 (2.3)	35.0 (8.9)	18.5 (5.7)	11.5 (3.5)	30.0 (9.2)	19.5 (5.5)	13.0 (3.7)	32.5 (9.2)	21.5 (6.4)	11.0 (3.2)	32.5 (9.6)	17.0 (6.3)	9.0 (3.3)	26.0 (9.6)
6	*Rhizopus nigricans* Demelius	4.5 (0.9)	3.5 (0.7)	8.0 (1.6)	4.5 (1.1)	-	4.5 (1.1)	5.5 (1.7)	1.0 (0.3)	6.5 (2.0)	6.5 (1.8)	3.0 (0.8)	9.5 (2.7)	3.5 (1.0)	1.0 (0.3)	4.5 (1.3)	4.5 (1.7)	1.5 (0.6)	6.0 (2.2)
7	*Cunninghamella elegans* Lender	4.5 (0.9)	-	4.5 (0.9)	3.5 (0.9)	-	3.5 (0.9)	2.0 (0.6)	-	2.0 (0.6)	8.0 (2.3)	-	8.0 (2.3)	6.0 (1.8)	-	6.0 (1.8)	-	-	-
C	**Ascomycota**	145.0	85.0	230.0	127.5	59.5	187.0	103.0	48.0	151.0	100.0	44.5	144.5	90.5	39.0	129.5	73.5	35.5	109.0
8	*Aspergillus amstelodomi* (Mang) Thom & Church	9.5 (1.9)	6.5 (1.3)	16.0 (3.3)	8.5 (2.2)	1.0 (0.3)	9.5 (2.4)	6.5 (2.0)	1.5 (0.5)	8.0 (2.5)	7.5 (2.1)	2.5 (0.7)	10.0 (2.8)	5.5 (1.6)	1.5 (0.4)	7.0 (2.1)	4.5 (1.7)	1.0 (0.4)	5.5 (2.0)
9	*Aspergillus flavus Link*	22.5 (4.6	11.5 (2.3)	34.0 (6.9)	18.5 (4.7)	10.5 (2.7)	29.0 (7.4)	16.0 (4.9)	6.5 (2.0)	22.5 (6.9)	18.0 (5.1)	7.5 (2.1)	25.5 (7.2)	14.0 (4.1)	6.5 (1.9)	20.5 (6.1)	12.0 (4.4)	7.5 (2.8)	19.5 (7.2)
10	*Aspergillus fumigatus Fres.*	18.0 (3.7)	5.5 (1.1)	23.5 (4.8)	12.5 (3.2)	4.5 (1.1)	17.0 (4.3)	14.0 (4.3)	5.5 (1.7)	19.5 (6.0)	18.0 (5.1)	2.5 (0.7)	20.5 (5.8)	16.0 (4.7)	3.5 (1.0)	19.5 (5.8)	6.0 (2.2)	3.0 (1.1)	9.0 (3.3)
11	*Aspergillus nidulans (Eldam) Winter*	2.5 (0.5)	-	2.5 (0.5)	3.5 (0.9)	-	3.5 (0.9)	-	-	-	2.5 (0.7)	-	2.5 (0.7)	-	-	-	-	-	-
12	*Aspergillus niger Van Tieghen*	24.5 (5.0)	9.5 (1.9)	34.0 (6.9)	29.0 (7.4)	8.5 (2.2)	37.5 (9.5)	22.0 (6.7)	9.5 (2.9)	31.5 (9.7)	18.0 (5.1)	8.5 (2.4)	26.5 (7.5)	17.0 (5.0)	6.5 (1.9)	23.5 (6.9)	14.0 (5.2)	4.5 (1.7)	18.5 (6.8)
13	*Aspergillus ochracious Wihelm*	6.5 (1.3)	-	6.5 (1.3)	2.5 (0.6)	-	2.5 (0.6)	-	-	-	1.0 (0.3)	-	1.0 (0.3)	-	-	-	1.5 (0.6)	-	1.5 (0.6)
14	*Aspergillus sulphureus* (Fres.) Thom & Church	4.5 (0.9)	-	4.5 (0.9)	1.5 (0.4)	-	1.5 (0.4)	2.5 (0.8)	-	2.5 (0.8)	-	-	-	1.0 (0.3)	-	1.0 (0.3)	-	-	-

Table 1: Continued…

Sr. No.	Fungal isolates	Frequency (%) fungal incidence on seeds of *Brassica campestris* L.																	
		Mixed seed sample			*Sub-div.- I			Sub-div.-II			Sub-div.- III			Sub-div.-IV			Sub-div.-V		
		Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence		
		B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T
15	*Aspergillus terreus* Thom	15.0 (3.1)	10.0 (2.0)	25.0 (5.1)	14.0 (3.6)	9.5 (2.4)	23.5 (5.9)	11.0 (3.4)	7.5 (2.3)	18.5 (5.7)	8.0 (2.3)	5.5 (1.6)	13.5 (3.8)	6.0 (1.8)	3.5 (1.0)	9.5 (2.8)	5.5 (2.0)	3.5 (1.3)	9.0 (3.3)
16	*Aspergillus versicolor* (Vuill.) Tiraboschi	-	4.5 (0.9)	4.5 (0.9)	-	2.5 (0.6)	2.5 (0.6)	-	3.5 (1.1)	3.5 (1.1)	-	-	-	-	-	-	-	2.5 (0.9)	2.5 (0.9)
17	*Botrytis cinera* Pets	-	3.5 (0.7)	3.5 (0.7)	-	2.5 (0.6)	2.5 (0.6)	-	1.5 (0.5)	1.5 (0.5)	-	3.5 (1.0)	3.5 (1.0)	-	2.0 (0.6)	2.0 (0.6)	-	1.0 (0.4)	1.0 (0.4)
18	*Chaetomium glabosum Kunne & Schm*	13.0 (2.7)	4.0 (0.8)	17.0 (3.5)	11.0	4.5 (1.1)	15.5	7.0 (2.1)	3.5 (1.1)	10.5 (3.2)	6.0 (1.7)	5.5 (1.6)	11.5 (3.3)	8.0 (2.4)	4.5 (1.3)	12.5 (3.7)	6.0 (2.2)	4.0 (1.5)	10.0 (3.7)
19	*Cladosporium fulvum Cooke*	12.0 (2.4)	6.0 (1.2)	18.0 (3.7)	6.0 (1.5)	2.5 (0.6)	8.5 (2.2)	4.0 (1.2)	1.5 (0.5)	5.5 (1.7)	8.0 (2.3)	3.5 (1.0)	11.5 (3.3)	9.0 (2.7)	4.5 (1.3)	13.5 (4.0)	11.0 (4.1)	5.0 (1.8)	16.0 (5.9)
20	*Penicillium oxalicum Currie & Thom*	14.0 (2.9)	8.0 (1.6)	22.0 (4.5)	13.5 (3.4)	8.5 (2.2)	22.0 (5.6)	9.5 (2.9)	4.5 (1.4)	14.0 (4.3)	7.5 (2.1)	3.5 (1.0)	11.0 (3.1)	9.5 (2.8)	5.5 (1.6)	15.0 (4.4)	8.0 (2.9)	3.5 (1.3)	11.5 (4.2)
21	*Penicillium pallidum* (Cruick & Shank) Pitt.	3.0 (0.6)	2.0 (0.4)	5.0 (1.0)	2.0 (0.5)	1.0 (0.3)	3.0 (0.8)	3.5 (1.1)	-	3.5 (1.1)	-	-	-	-	-	-	2.0 (0.7)	-	2.0 (0.7)
22	*Penicillium digitatum* (Pers. Ex. Fr.) Sacc.	-	5.5 (1.1)	5.5 (1.1)	-	4.0 (1.0)	4.0 (1.0)	-	3.0 (0.9)	3.0 (0.9)	-	2.0 (0.6)	2.0 (0.6)	-	1.0 (0.3)	1.0 (0.3)	-	-	-
23	*Phoma glomerata* (Corda) Wr. & Bochapfal	-	8.5 (1.7)	8.5 (1.7)	5.0 (1.3)	-	5.0 (1.3)	7.0 (2.1)	-	7.0 (2.1)	5.5 (1.6)	-	5.5 (1.6)	4.5 (1.3)	-	4.5 (1.3)	3.0 (1.1)	-	3.0 (1.1)
D.	**Deuteromycota**	111.5	57.5	169.0	91.0	38.0	129.0	77.0	33.5	110.5	84.5	40.0	124.5	87.0	41.0	128.0	76.0	31.0	107.0
24	*Alternaria alternata* (Fr.) Keissler	9.5 (1.9)	4.5 (0.9)	14.0 (2.9)	8.0 (2.2)	3.5 (0.9)	11.5 (2.9)	6.5 (2.0)	2.0 (0.6)	8.5 (2.6)	8.0 (2.3)	2.5 (0.7)	10.5 (3.0)	9.5 (2.8)	3.0 (0.9)	12.5 (3.7)	7.5 (2.8)	2.5 (0.9)	9.5 (3.5)
25	*Alternaria solani (E & M) Jones & Grout*	6.5 (1.3)	4.5 (0.9)	11.0 (2.2)	6.0 (1.5)	2.5 (0.6)	8.5 (2.2)	5.0 (1.5)	1.5 (0.5)	6.5 (2.0)	6.0 (1.7)	3.5 (1.0)	9.5 (2.7)	7.5 (2.2)	3.5 (1.0)	11.0 (3.2)	7.5 (2.8)	1.5 (0.6)	9.0 (3.3)
26	*Alternaria brassicicola* (Schweinitz, Wiltshire)	10.0 (2.0)	5.5 (1.1)	15.5 (3.2)	8.5 (2.2)	5.0 (1.3)	13.5 (3.4)	10.5 (3.2)	4.0 (1.2)	14.5 (4.4)	9.5 (2.7)	6.0 (1.7)	15.5 (4.4)	10.0 (2.9)	7.0 (2.1)	17.0 (5.0)	8.0 (2.9)	5.0 (1.8)	13.0 (4.8)
27	*Alternaria brassicae*	5.5 (1.1)	4.5 (0.9)	10.0 (2.0)	-	4.5 (1.1)	4.5 (1.1)	-	5.5 (1.7)	5.5 (1.7)	-	6.5 (1.8)	6.5 (1.8)	-	2.5 (0.7)	2.5 (0.7)	-	1.5 (0.6)	1.5 (0.6)
27	*Alternaria brassicae*	5.5 (1.1)	4.5 (0.9)	10.0 (2.0)	-	4.5 (1.1)	4.5 (1.1)	-	5.5 (1.7)	5.5 (1.7)	-	6.5 (1.8)	6.5 (1.8)	-	2.5 (0.7)	2.5 (0.7)	-	1.5 (0.6)	1.5 (0.6)
28	*Curvularia clavata* Jain	4.5 (0.9)	2.5 (0.5)	7.0 (1.4)	-	3.5 (0.9)	3.5 (0.9)	3.5 (1.1)	1.5 (0.5)	5.0 (1.5)	4.5 (1.3)	-	4.5 (1.3)	3.0 (0.9)	-	3.0 (0.9)	-	-	-
29	*Curvularia ovoidea* (H & W) Munt.	5.5 (1.1)	3.5 (0.7)	9.0 (1.8)	5.0 (1.3)	2.5 (0.6)	7.5 (1.9)	5.0 (1.5)	2.5 (0.8)	7.5 (2.3)	4.5 (1.3)	3.5 (1.0)	8.0 (2.3)	6.5 (1.9)	2.5 (0.7)	9.0 (2.7)	4.5 (1.7)	1.5 (0.6)	6.0 (2.2)
30	*Curvularia lunata* (Wakker) Boedijn	7.5 (1.5)	4.5 (0.9)	12.0 (2.4)	8.0 (2.0)	3.0 (0.8)	11.0 (2.8)	6.0 (1.8)	3.0 (0.9)	9.0 (2.8)	8.0 (2.3)	2.5 (0.7)	10.5 (3.0)	9.0 (2.7)	4.5 (1.3)	13.5 (4.0)	7.0 (2.6)	4.0 (1.5)	11.0 (4.1)

Table 1: Continued...

Sr. No.	Fungal isolates	Frequency (%) fungal incidence on seeds of *Brassica campestris* L.																	
		Mixed seed sample			*Sub-div.- I			Sub-div.-II			Sub-div.- III			Sub-div.-IV			Sub-div.-V		
		Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence			Per cent incidence		
		B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T
31	*Curvularia intermedia* (Tracy & Barle) Boedjim	6.5 (1.3)	-	6.5 (1.3)	6.0 (1.5)	-	6.0 (1.5)	4.5 (1.4)	-	4.5 (1.4)	7.5 (2.1)	-	7.5 (2.1)	9.5 (2.8	-	9.5 (2.8)	6.5 (2.4)	-	6.5 (2.4)
32	*Fusarium miniliformae* Sheldom	10.0 (2.0)	3.5 (0.7)	13.5 (2.8)	9.0 (2.3)	3.5 (0.9)	12.5 (3.2)	7.0 (2.1)	2.5 (0.8)	9.5 (2.9)	5.0 (1.4)	2.5 (0.7)	7.5 (2.1)	4.0 (1.2)	2.5 (0.7)	6.5 (1.9)	5.5 (2.0)	1.5 (0.6)	7.0 (2.9)
33	*Fusarium oxysporum Schlecht*	4.5 (0.9)	3.5 (0.7)	8.0 (1.6)	3.0 (0.8)	2.0 (0.5)	5.0 (1.3)	3.0 (0.9)	2.0 (0.6)	5.0 (1.5)	2.0 (0.6)	1.0 (0.3)	3.0 (0.8)	3.0 (0.9)	1.5 (0.4)	4.5 (1.3)	3.5 (1.3)	0.5 (0.2)	4.0 (1.5)
34	*Fusarium semitectum* Berk & Rav.	5.5 (1.1)	5.5 (1.1)	11.0 (2.2)	5.0 (1.3)	-	5.0 (1.3)	3.5 (1.1)	-	3.5 (1.1)	4.5 (1.3)	-	4.5 (1.3)	2.5 (0.7)	-	2.5 (0.7)	3.5 (1.3)	-	3.5 (1.3)
35	*Fusarium solani* (Mert.) APP. & Wollenw	6.0 (1.2)	3.5 (0.7)	9.5 (1.9)	5.5 (1.4)	2.5 (0.6)	8.0 (2.0)	3.5 (1.1)	2.5 (0.8)	6.0 (1.8)	4.5 (1.3)	3.5 (1.0)	8.0 (2.3)	5.5 (1.6)	3.5 (1.0)	9.0 (2.7)	6.5 (2.4)	4.0 (1.5)	10.5 (3.9)
36	*Helminthosporium tetramera* Mc Kinney	6.5 (1.3)	4.0 (0.8)	10.5 (2.1)	6.0 (1.5)	2.5 (0.6)	8.5 (2.2)	4.5 (1.4)	2.0 (0.6)	6.5 (2.0)	5.0 (1.4)	3.5 (1.0)	8.5 (2.4)	4.5 (1.3)	3.5 (1.0)	8.0 (2.4)	4.0 (1.5)	3.0 (1.1)	7.0 (2.6)
37	*Helminthosporium specifectum* (Bain) Nicol	3.5 (0.7)	-	3.5 (0.7)	3.5 (0.9)	-	3.5 (0.9)	3.0 (0.9)	-	3.0 (0.9)	4.0 (1.1)	-	4.0 (1.1)	-	-	-	-	-	-
38	*Nigrospora sp.*	4.0 (0.8)	-	4.0 (0.8)	3.5 (0.9)	-	3.5 (0.9)	2.5 (0.8)	-	2.5 (0.8)	-	-	-	-	-	-	-	-	-
39	*Paecilomyces variotii* Bainier	4.0 (0.8)	3.5 (0.7)	7.5 (1.5)	4.0 (1.0)	1.5 (0.4)	5.5 (1.4)	3.0 (0.9)	1.0 (0.3)	4.0 (1.2)	2.0 (0.6)	1.0 (0.3)	3.0 (0.8)	3.0 (0.9)	1.5 (0.4)	4.5 (1.3)	3.5 (1.3)	2.5 (0.9)	6.0 (2.2)
40	*Rhizoctonia solani* Kuhn.	7.0 (1.4)	2.0 (0.4)	9.0 (1.8)	6.5 (1.6)	-	6.5 (1.6)	4.5 (1.4)	1.0 (0.3)	5.5 (1.7)	5.5 (1.6)	2.5 (0.7)	8.0 (2.3)	5.0 (1.5)	2.0 (0.6)	7.0 (2.1)	4.5 (1.7)	1.5 (0.6)	6.0 (2.2)
41	*Trichothecium roseum* Link	5.0 (1.0)	2.5 (0.5)	7.5 (1.5)	3.5 (0.9)	1.5 (0.4)	5.0 (1.3)	1.5 (0.5)	2.5 (0.8)	4.0 (1.2)	4.0 (1.1)	1.5 (0.5)	5.5 (1.6)	4.5 (1.3)	3.5 (1.0)	8.0 (2.4)	4.5 (1.7)	2.5 (0.9)	7.0 (2.6)
	Total fungal incidence	323	166	489	276	117	393	222	104	326	239	113	352	233	105	338	185	86	271
	Per cent of total incidence	66.1	33.9	100	70.2	29.8	100	68.1	31.9	100	67.9	32.1	100.0	68.9	31.1	100.0	68.3	31.7	100
	* Sub. Div.-I (Saoner -tah. Kalmeshwar & Saoner); II (Katol - tah. Narkhed & Katol); III- (Umred- tah. Kuhi, Umred & Bhiwapur); IV- (Ramtek- tah. Parshioni, Ramtek & Mouda) and Sub.Div.-V (Nagpur - tah. Kamptee, Nagpur (City), Hingna & Nagpur (Rural). B= Blotter paper test; A= Agar plating test; T= total per cent incidence																		

*** values in parenthesis indicates per cent incidence over sum total*

Table 2: Distribution of external and internal seed borne fungal pathogen on seeds of mustard (*Brassica campestris* L) received from various geographical locations of Nagpur District.

Sr. No.	Fungal Division	Parameters		Seed borne fungal isolates and their frequency(%) of incidence											
				Mixed seed sample		Seed sample SD-I		Seed sample SD-II		Seed sample SD-III		Seed sample SD-IV		Seed sample SD-V	
				Isolate/ Incidence	% of total	Isolate/ Incidence	% of total	Isolate/ Incidence	% of total	Isolate/ Incidence	% of total	Isolate/ Incidence	% of total	Isolate/ Incidence	% of total
A	Oomycota	Genera		2	10.00	2	10.00	2	10.53	2	10.53	2	10.53	2	11.11
		Species		2	4.88	2	4.88	2	5.41	2	5.71	2	5.71	2	5.88
		Percent incidence	B	9.5	1.94	6.5	1.65	4.0	1.23	4.5	1.28	6.5	1.35	5.0	1.84
			A	5.5	1.12	4.0	1.02	1.5	0.46	4.0	1.13	5.0	1.65	3.5	1.29
			T	15.0	3.1	10.5	2.67	5.5	1.69	8.5	2.41	11.5	3.40	8.5	3.14
B	Zygomycota	Genera		4	20.00	4	20.00	4	21.05	4	21.05	4	21.05	3	16.67
		Species		5	2.2	5	12.2	5	13.51	5	14.28	5	14.28	4	11.76
		Percent incidence	B	57.0	11.6	51.0	12.9	38.0	11.6	50.0	14.2	49.0	14.5	30.5	11.3
			A	18.0	03.7	15.5	03.9	21.0	06.4	24.5	07.1	20.0	05.9	16.0	06.1
			T	75.0	15.3	66.5	16.9	59.0	18.1	74.5	21.3	69.0	20.4	46.5	17.2
C	Ascomycota	Genera		6	30.00	6	30.00	6	31.58	6	31.58	6	31.58	6	33.33
		Species		16	39.02	16	39.02	14	37.84	13	37.14	12	34.29	12	35.29
		Percent incidence	B	145.0	29.6	127.5	32.4	103.0	31.6	100.0	28.4	90.5	26.8	73.5	27.1
			A	85.0	17.4	59.5	14.6	48.0	14.7	44.5	12.6	39.0	11.5	35.5	13.1
			T	230.0	47.0	187.0	47.6	151.0	46.3	144.5	41.0	129.5	38.3	109.0	40.2
D	Deuteromycota	Genera		8	40.00	8	40.00	8	42.11	7	36.84	7	36.84	7	46.67
		Species		18	43.9	18	43.9	18	48.65	17	48.57	16	45.71	15	44.12
		Percent incidence	B	111.5	22.8	91.0	23.1	77.0	23.6	84.5	24.0	87.0	25.7	76.0	28.1
			A	57.5	11.7	38.0	09.7	33.5	10.3	40.0	11.4	41.0	12.1	31.0	11.4
			T	169	34.5	129.0	32.8	110.5	33.9	124.5	35.4	128.0	37.8	107.0	39.5
	Total genera			20	-	20	-	19	-	19	-	19	-	18	18
	Total species			41	-	41	-	37	-	35	-	35	-	34	34
	Cumulative frequency (Blotter)			323	66.1	276	70.2	222	68.1	239	67.9	233	68.9	185	68.3
	Cumulative frequency (Agar)			166	33.9	117	29.8	104	31.9	113	32.1	105	31.1	86	31.7
	Cumulative frequency (Total)			489	-	393	-	326	-	352	-	338	-	271	-

B = Blotter paper test; A = Agar plating test; T = Total per cent incidence

Only six isolates were restricted to agar plate as internal seed borne with an incidence level varies from 2.5-4.5%, included *Absidia corymbefera, Alternaria brassicae*, *Aspergillus versicolor, Botrytis cinera, Curvularia clavata* and *Penicillium digitatum*. A fungal population of 11 isolates representing 9 genera has been confined to blotter paper only with 1.5-6.5% incidence included *Aspergillus nidulans, A. ochracious, A. sulphureus, Curvularia intermedia,Cunninghamella elegans, Fusarium semitectum, Helminthosporium specifectum, Nigrospora sp. Phoma glomerata, Rhizoctonia solani* and *Rhizopus nigricans* (Table 1). All seed borne fungal pathogens have been detected with sum total of 393 per cent incidence by both seed health tests. Of the total, 70.2% incidence was recorded on blotter paper while 29.8% was confined on agar plates. Ascomycota contributed 47.6% incidence of the total followed by Deuteromycota (32.8%), Zygomycota (16.9%) and Oomycota (2.7%) (Table 2).

(c) Seed mycoflora from SD-II

Mycological analysis of seed samples obtained from localities of SD-II revealed the prevalence of fungal population of altogether 39 species fall under 20 genera in varying incidence (Table 1). The isolates of Deuteromycota are most predominant ones, represented by 8 genera and 18 species. Ascomycota contributed 6 genera and 14 species. *Zygomycota* had 4 genera and 5 species. Oomycota are represented by 2 genera and 2 species. Member of the Basidiomycota did not appear on the seeds (Table 2).

The isolate, *Aspergillus* dominated with seven species, followed by *Alternaria, Curvularia* and *Fusarium* with four species each. Three species of genus *Penicillium*,; two species of *Rhizopus, Helminthosporium* have been confined as seed contaminants while remaining genera had single species. The isolates, *Aspergillus flavus and A. niger* were appeared to be most predominant with 31.5% and 22.5% incidence respectively whereas *Mucor pusillus, Aspergillus fumigatus, A. terreus, Chaetomium glabosum, Penicillium oxalicum* and *Alternaria brassicicola* have been detected sub-dominant with frequency of incidence ranged between 10.5 to 19.5%. The low level of incidence, 1.5-2.5% has been encountered for *Pythium aphanidermatum, Cunninghamella elegans, Aspergillus sulphureus, Botrytis cinera* and *Nigrospora sp*. while remaining isolates had 3-10% incidence (Table 1).

Altogether, 26 fungal pathogens representing 15 genera have been detected on both blotter paper and agar plates included *Alternaria alternata, A. solani,, A. brassicicola, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia clavata, C. ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus, Paecilomyces variotii, Penicillium oxalicum, Phytophthora infestans, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus stolonifer, R. nigricans* and *Trichothecium roseum*. Of these, two isolates, *Aspergillus flavus* and *Aspergillus niger* were appeared to be most predominant on the seeds exhibiting higher incidence. The isolates, *Aspergillus fumigatus* and *Mucor pusillus* has been reported to be subdominant on seeds of *Brassica campestris* L. by both seed health techniques (Table 1).

A population of total 8 fungal species, each representing single genera has been confined to blotter test only as external seed borne pathogens. These isolates included *Aspergillus nidulans, Curvularia intermedia, Cunninghamella elegans, Fusarium semitectum, Helminthosporium specifectum, Penicillium pallidum, Phoma glomerata* and *Nigrospora* sp. Among these, *Phoma glomerata* and *Curvularia intermedia* were appeared to be most predominant with 7.0% and 4.5% incidence. *Fusarium semitectum* and *Penicillium pallidum* has been detected with 3.5% incidence while remainings had low level of incidence ranged between 2-3%. Least infestation has been recorded for *Cunninghamella elegans*. Fungal isolates restricted only to agar plates included five genera, *Absidia corymbefera, Aspergillus versicolor, Alternaria brassicae, Botrytis cinera* and *Penicillium digitatum*. Excepting *Botrytis cinera*, others had incidence varies between 3.5 -5.5% (Table 1). A population of fungal pathogen adhering to seed surfaces has been encountered with sum total of 326 per cent incidence by both seed health tests. Of the total, 68.1% incidence was confined on blotter paper while 31.9% on agar plates. Ascomycota contributed 46.3% incidence followed by Deuteromycota with 33.9% of total incidence. Moderate incidence was recorded for Zygomycota (16.9%) while Oomycota had least incidence (Table 2).

(d) Seed mycoflora from SD-III

Mycological analysis of seed samples from localities of SD-III revealed prevalence of total 39 fungal pathogens belonging to 19 genera in varying incidence (Table 1). The isolates of Deuteromycota are most predominant ones, represented by 7 genera and 17 species. Ascomycota contributed 6 genera and 13 species. Zygomycota are represented by 4 genera and 5 species. Oomycota had 2 genera and 2 species. No isolates of Basidiomycota encountered to seeds of mustard (Table 2).

The isolate, *Aspergillus* dominated with 7 species followed by *Alternaria, Curvularia* and *Fusarium* with 4 species each. Two species of *Helminthosporium Penicillium* and *Rhizopus* has been confined to seeds as fungal contaminants while remaining genera had single species. The isolates, *Rhizopus stolonifer* was appeared to be most predominant with 32.5% incidence, exhibiting higher frequency level against others followed by *Aspergillus flavus, A. niger* and *A. fumigatus, Mucor pusillus* with frequency of incidence varied between 21.5-25.5%. The moderate incidence varied between 10.0-15.5% has been detected for *Alternaria alternata, Aspergillus amstelodomi, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia lunata, and Penicillium oxalicum*. The low frequency, 2.0-4.0% was recorded for *Absidia corymbefera, Botrytis cinera, Helminthosporium semitectum, Fusarium oxysporum,* and *Paecilomyces* variotii. *Aspergillus ochracious* had least while remainings had 4-10% incidence (Table 2).

A population of total 25 fungal pathogens belonging to 15 genera have been detected by both seed health tests included *Alternaria alternata, A. solani,, A. brassicicola, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus, Paecilomyces variotii, Penicillium oxalicum, Phytophthora infestans, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus stolonifer, R. nigricans* and *Trichothecium roseum*. Of these, four isolates, *Aspergillus flavus, A. fumigatus, A. niger Rhizopus stolonifer* were appeared to be most predominant exhibiting higher incidence. *Alternaria alternata, Aspergillus amstelodomi, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia lunata, and Penicillium oxalicum* has been reported to be subdominant by both seed health tests (Table 1).

Altogether 8 fungal species which fall under 6 genera has been confined to blotter test only included *Aspergillus nidulans, A. ochracious, Curvularia clavata, C. intermedia, Cunninghamella elegans, Fusarium semitectum, Helminthosporium specifectum* and *Phoma glomerata*. Among these, *Cunninghamella elegans was* appeared to be most dominant with 8.0% incidence while remaining isolates had low frequency of incidence ranged between 2.5-5.5%. *Aspergillus ochracious* had least incidence (Table 1). Fungal isolates restricted only to agar plates included four genera, *Absidia corymbifera, Alternaria brassicae, Botrytis cinera* and *Penicillium digitatum*. The isolate *Alternaria brassicae* has been recorded with 6.5% incidence, exhibiting highest incidence over other internal borne pathogens which had incidence level varies between 2.0 -3.5% (Table 1). Seed mycoflora from localities of SD-III has been detected with sum total of 352 per cent incidence by both seed health tests. Fungal incidence, 67.9% was detected by blotter paper while 32.1% by agar plate tests. Ascomycota contributed higher, 41.0% incidence. Deuteromycota had 35.4% while Zygomycota contributed 21.2% of total incidence. Least incidence contributed by Oomycota (Table 2).

(e) Seed mycoflora from SD-IV

Seed samples received from localities of SD-IV revealed the prevalence of fungal population total 35 isolates representing 19 genera in varying incidence. The isolates of Deuteromycota contributed 7 genera and 16 species, exhibited highest count of isolates over others (Table 3). Ascomycota are represented by 6 genera and 12 species. Zygomycota had 4 genera and 5 species while Oomycota are represented by 2 genera and 2 species. Member of Basidiomycota did not confine to seeds of mustard (Table 2).

Individual genus, *Aspergillus* dominated with 6 species, followed by *Alternaria, Curvularia* and *Fusarium* with 4 species each. *Penicillium* and *Rhizopus* had two while remainings represented by single species. *Rhizopus stolonifer* was encountered with higher, 32.5% incidence. Significant level of incidence varied between 20.5 to 24.0% has been detected for *Aspergillus flavus, A. niger* and *Mucor pusillus* while *Alternaria alternata, A. solani, A. brassicicola,*

Aspergillus fumigatus, Chaetomium glabosum, Cladosporium fulvum, Curvularia lunata and *Penicillium oxalicum* has been encountered with fungal incidence varied between 11.0-19.5%. The low frequency, 1.0-4.5% was recorded for *Absidia corymbifera, Alternaria brassicae, A. sulphureus, Botrytis cinera, Curvularia clavata, Fusarium oxysporum, F. semitectum, Paecilomyces variotii, Penicillium digitatum, Phoma glomerata* and *Rhizopus nigricans* while remainings had 5-10% incidence (Table 2).

Altogether 25 fungal pathogens representing 15 genera have been detected on both blotter paper and agar plates included *Alternaria alternata, A. solani,, A. brassicicola, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus, Paecilomyces variotii, Penicillium oxalicum, Phytophthora infestans, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus stolonifer, R. nigricans* and *Trichothecium roseum*. Of these, *Aspergillus flavus, A. niger Mucor pusillus* and *Rhizopus stolonifer* were appeared to be most predominant on the seeds exhibiting higher incidence. The isolates, *Alternaria alternata, A. solani, A. brassicicola, Aspergillus fumigatus, Chaetomium glabosum, Cladosporium fulvum, Curvularia lunata, and Penicillium oxalicum* has been reported to be subdominant on seeds. The least per cent incidence has been reported for *Fusarium oxysporum, Paecilomyces variotii* and *Rhizopus nigricans* (Table 2).

Fungal population of total 6 species belonging to 5 genera viz., *Aspergillus sulphureus, Curvularia clavata, C. intermedia, Cunninghamella elegans, Fusarium semitectum* and *Phoma glomerata* has been encountered on blotter only. Among these, *a Curvularia intermedia was* confined to be most dominant with 9.5% incidence while remaining isolates had 2.5-6.0% incidence. Least per cent incidence has been recorded for *Aspergillus sulphureus* (Table 2). Altogether four fungal isolates confined only to agar plates, each representing single genus included *Absidia corymbifera, Alternaria brassicae, Botrytis cinera* and *Penicillium digitatum*. Excluding *Penicillium digitatum*, other isolates has been recorded with frequency of incidence varies between 2.0-2.5% (Table 2). The sum total of fungal incidence from seed of *Brassica campestris* L from localities of sub-div. Ramtek of Nagpur District has been estimated to be 338 per cent by both seed health tests. Of the total, 68.9% incidence has been confined on blotter paper while 31.1% incidence on agar plates. Ascomycota contributed highest, 38.3% incidence, followed by Deuteromycota with 37.9% of total incidence. Zygomycota contributed 20.4% incidence while Oomycota had least incidence (Table 2).

(f) Seed mycoflora from SD-V

Mycological analysis of seed samples obtained from localities of SD-V revealed the prevalence of a population of altogether 34 fungal pathogens belonging to 18 genera in varying incidence. *Deuteromycota* had 7 genera and 15 species, exhibited comparatively highest count of isolates, followed by Ascomycota with 6 genera and 12 species. Zygomycota represented by 3 genera and 4 species while Oomycota had 2 genera and 2 species. Member of Basidiomycota did not persist to seeds of *Brassica campestris* L (Table 1).

Ascomycetous isolate, *Aspergillus* dominated with 7 species, followed by *Alternaria,* and *Fusarium* with 4 species; *Penicillium* and *Curvularia with* 3 species each*. Rhizopus* had two while remaining's had single species. The isolates, *Rhizopus stolonifer* was detected with 26.0% incidence, exhibiting higher infestation compared to others. Significant fungal incidence varied between 16.0 to 19.5% has been detected for *Aspergillus flavus, A. niger* and *Cladosporium fulvum* while *Curvularia lunata, Fusarium solani, Mucor pusillus* and *Penicillium oxalicum* has been encountered with fungal incidence varied between 10.5-13.0%. *Absidia corymbifera, Alternaria brassicae, A. ochracious, A. versicolor, Botrytis cinera, Fusarium oxysporum, F. semitectum, Penicillium pallidum, Phoma glomerata* Phytophthora infestans and Pythium aphanidermatum have been recorded with low frequency of incidence to the extent of 1.5-4.5% while remainings had 5.0-9.5% incidence (Table 1).

A population of total 25 fungal species belonging to 15 genera, including *Alternaria alternata, A. solani, A. brassicicola, Aspergillus amstelodomi, A. flavus , A. fumigatus, A. niger, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia ovoides, C. lunata, Fusarium moniliformae,, F. oxysporum, F. solani, Helminthosporium tetramera, Mucor pusillus,*

Paecilomyces variotii, Penicillium oxalicum, Phytophthora infestans, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus stolonifer, R. nigricans and *Trichothecium roseum* have been detected by both seed health tests. Among these *Rhizopus stolonifer* was appeared to be most dominant exhibiting greater incidence over others. *Mucor pusillus, Aspergillus flavus, A. niger, Cladosporium fulvum, Penicillium oxalicum, Chaetomium glabosum, Curvularia lunata* and *Fusarium solani* have been reported to be subdominant on seeds. *Fusarium oxysporum, Paecilomyces variotii* and *Rhizopus nigricans* had least incidence (Table 1). Altogether five isolates, each representing single genera and species have been confined to blotter paper only as external seed borne pathogen, included *Aspergillus ochracious, Curvularia intermedia, Fusarium semitectum, Penicillium pallidum* and *Phoma glomerata*. Among these, *Curvularia intermedia* have been reported with higher incidence while remainings had 2.0-3.5% incidence. Least incidence has been recorded for *Aspergillus ochracious* (Table 1).Only four fungal isolates confined only to agar plates, each representing single genus included *Absidia corymbifera, Alternaria brassicae, Aspergillus versicolor* and *Botrytis cinera*. Excluding *Botrytis cinera*, other isolates has been recorded with frequency of incidence varies between 1.5-2.5% (Table 1).

A population of fungal pathogen adhering to seed surfaces has been detected with sum total of 271 per cent incidence by both seed health tests (Table 1). Of the total, 68.3% fungal incidence was recorded by blotter paper test while 31.7% incidence was detected with agar plate technique. Ascomycota and Deuteromycota contributed nearly equal estimate of incidence, representing 40.2% and 39.5% respectively. Moderate incidence was recorded for Zygomycota (17.2%) and while Oomycota contributed least, 3.1% incidence (Table 2).

The screening of seed samples of *Brassica campestris* L. by dry examination technique revealed prevalence of diverse group micro-propagules of fungal origin such as spores, conidia, debries, acervuli, pycnidia etc. tend to be restricted in variable count on seed coats, cells of embryo and seed endosperm exhibiting an enormous heterogeneity in life-history strategies that occupy position of great economic importance in agriculture in developing countries. The routine seed health tests recommended by International Seed Testing Association comprising blotter and agar plating 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 (ISTA, 2013). A population of altogether 41 fungal micro-organisms falls under 20 genera has been confined to in the seeds surface of mixed samples understudy both as external and internal seed borne pathogens. Deuteromycota contributed highest, 44% fungal count over the total isolates followed by Ascomycota ranking second highest contribution. Zygomycota contributed moderate while Oomycota had least count (Fig. 1). The count of both external as well as internal seed borne isolates was appeared to be greater over count of either only external or internal seed borne. Similarly count of external seed borne isolates was recorded greater in count against internal seed borne. Moreover, nearly equal count of fungal isolates was recorded from seeds of all study area excepting composite seed samples (Fig.2).

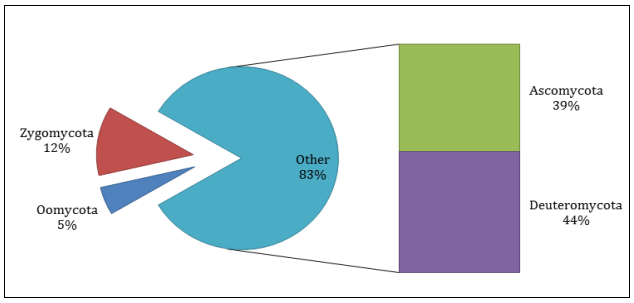

Fig. 1: Division wise distribution of fungal isolates on composite seed samples of *Brassica compestris* L.

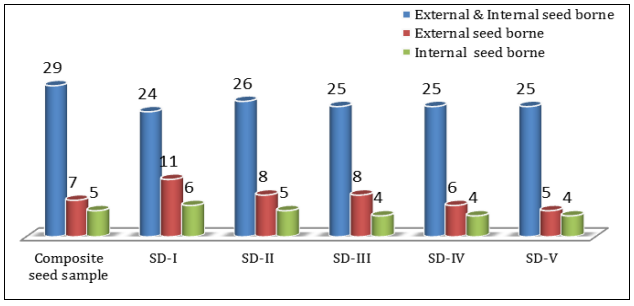

Fig. 2: Distribution of fungal isolates on seed samples of *Brassica compestris* L. form various geographical location understudy.

The prevalence of greater count of species confined to genus, *Aspergillus* followed by *Alternaria, Curvularia* and *Fusarium* which were recorded subdominant (Table 1). These results are in confirmation with earlier findings of Madavi and Bhajbhuje (2014) who have reported comparable higher count of *Aspergillus niger, A. terreus, A. fumigatus, A. flavus, A. nidulens, A ochracious, A. sulphureus* and *A. versicolor* on seeds

of *Brassica oleracia* var. botrytis. *Alternaria solani, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramera* and *Trichoderma viride* were confined in variable count in infested seeds on maize seeds (Chukunda et al., 2013). Bhajbhuje (2013) reported prevalence of *Aspergillus, Alternaria, Penicillium, Cladosporium, Fusarium* and *Stachybotrys atra* in infested seeds of *Solanum melongena L* .

Among total, seed borne nature of seven isolates, *Aspergillus amstelodomi, A. sulphureus, A. versicolor, Aspergillus ochracious, Paecilomyces varioti* and *Cunninghamella elegans* were reported for a first time in India from *Brassica campestris* L seeds (Table 1). Prevalence of these isolates on seeds of other crop confirmed their seed borne nature. *Aspergillus amstelodomi, Cunninghamela elegans* and *Paecilomyces varioti* were reported with *Solanum melongena* L seeds (Bhajbhuje, 2013) These reports are in conformity of seeds borne nature for first time isolated fungal pathogen from *Brassica campestris* L. seeds.

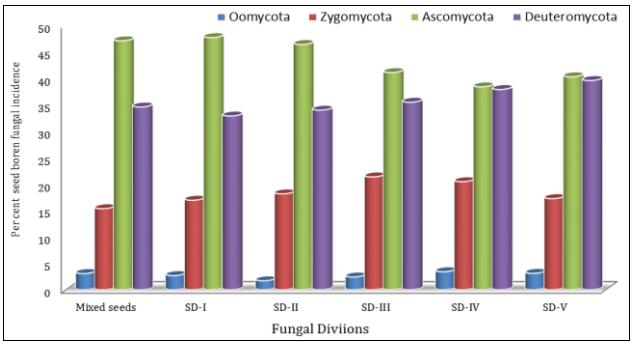

Fig. 3: Division wise percent contribution on seeds *Brassica compestris* L. in storage from area understudy.

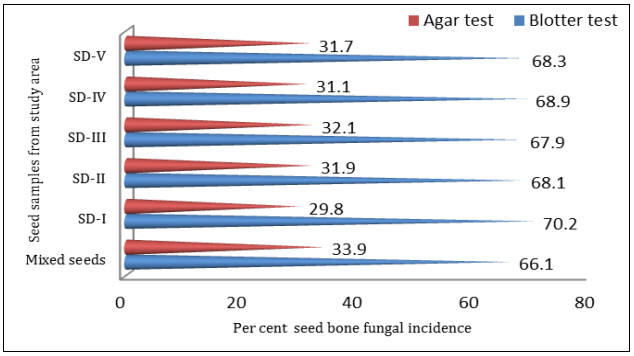

Fig. 4: Percent incidence of fungal isolates on *Brassica Compestris* L. Seeds confined to standard health tests.

The count of colonies appeared on blotter paper and agar plates gave estimates of fungal incidence on the seeds. Ascomycota contributed nearly half per cent incidence followed by Deuteromycota contributing one-third over total incidence; Zygomycota contributed moderate while Oomycota had least fungal incidence over total incidence from composite seed samples and SD-I, & SD-II (Table 2). The remaining samples from other area understudy had similar trend of infection excepting SD-IV where Ascomycota & Deuteromycota contributed identical level of incidence (Fig. 3). These results confirmed with earlier findings on crops involving *Solanum melongena* (Bhajbhuje (2013). It may be attributed to uniform climatic condition in storage in most of the area and environment fluctuation in few area understudies. Jyoti and Malik (2013) pointed out that climate of the storage environment including temperature and moisture content of seeds determines the rate of their biodeterioration in response to growth and proliferation of fungal organisms. Of the total 71.1% fungal incidence was confined to blotter paper while 28.9% incidence was detected on agar plates from seeds of mixed samples of all cultivars (Fig. 3).

The efficacy of blotter paper and agar plating test varied considerably. Fungal flora isolated from composite seed samples revealed that Ascomycota dominated with 29.6% and 17.4% incidence followed by Deuteromycota with 22.8% and 11.4%; while Oomycota had 3.3% and 1.9% fungal incidence by blotter and agar plate test respectively (Table 2). It is noted that nearly three-fourth of the total fungal incidence of isolates was recorded by blotter paper technique from seeds of all the lots understudy including composite seed sample over agar plate test (Fig. 4). These results are in conformity with earlier findings from other region of the country. Recently Madavi and Bhajbhuje (2014) Saskatchewan (2013) recorded higher frequency of fungal pathogens from stored seeds of *Brassica oleracea* var. botrytis on blotter paper over agar plate. Several investigators reported similar findings by blotter test from infested stored seeds involving oil seeds (Jain. 2008), solanaceous vegetables (Ismael, 2010); pulses (Saskatchewan, 2013), *Solanum melongena* L.(Bhajbhuje, 2013).

The standard blotter paper technique was proved comparative superior over agar plating to the fungal pathogens isolation. The greater count of seed borne fungal isolates with higher level of incidence in was encountered to blotter paper over agar plate test (Fig. 4). A population of members belongs to *Zygomycota* developed rapidly on blotter paper while *Ascomycota*

and *Deuteromycota* proliferate more profusely on agar plating possibly because they require softer medium rich in moisture for their establishment and growth. Several researchers have pointed out that a quick growing saprophytes adhering to the outer seed coat may be troublesome to detect internal slow growing pathogens as these saprophytes comparatively grow rapidly and restricting growth of pathogenic forms (Al-Askar et al., 2013; Madavi and Bhajbhuje, 2014). Other possibility for such divergence might be possibly attributed to the prolonged incubation that might lead to the development of deep seated infection (Lew-Smith, 2013). Siddiqui (2014) have reported that physiochemical nature of seed as well as agricultural practices and storage environment provided for seeds are also possibly responsible to variation in two methods. Mycological studies on disinfected and non-disinfected seeds gave only general information about inner seed infection, with assuming that fungal propagules exist in non-disinfected seeds while absent in disinfected seeds and that fungi were contaminated their surface, did not penetrate the inner tissues (Lew-Smith, 2013; Madavi and Bhajbhuje, 2014). This information, although not very precise, can be a starting point to determine proper strategies of seed treatment.

Mycological survey of seed borne fungal flora revealed that fungal isolates belong to genera, *Aspergilli* and *Penicilli* of Ascomycotina as well as *Alternaria, Curvularia, Fusarium and Helminthosporium* of Deuteromycotina contributed as major components on the seeds (Fig. 5); 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 (Saskatchewan, 2013). Deuteromycota contributed greater count of isolates (Fig. 5) while Ascomycota had greater fungal incidence over remainings (Fig. 3) may be possibly attributed to prevalence of greater propagules of fungal micro-organisms associated with seed coat with significant incidence. Moreover, majority members of these groups are known facultative parasites on crop plants as well as involved as saprophyte in biodegradation of substrates including seeds and debris of plant and animal origin (Bhajbhuje, 2013). Under storage, in humid environment the seeds form an ideal organic substrate to the development of storage fungi (Jain, 2008). Deuteromycota members have short life cycle, and proliferate asexually producing numerous resistant, thick walled conidia which may remain viable for longer period in adverse climatic environment (Jyoti and Malik, 2013). The conidia *Cladosporium, Alternaria, Helminthosporium, Trichothecium* and *Curvularia* tend to persist in greatest abundance under storage even at low humidity, mostly during warmer climate (Jain, 2008). Basidiomycotina members did not persist on seeds lots may be possibly attributed to mode of nutrition as majority of fungal organisms of these groups are obligate parasites of other crop plants.

Majority of fungal isolates from Deuteromycota including *Alternaria, Curvularia, Helminthosporium, Fusarium, Paecilomyces, Rhizoctonia, Trichothecium* and Ascomycota including *Aspergillus, Cladosporium* and *Penicillium* confined to be highly predominant on seeds samples of *Brassica campestris* L. from all the geographical area of Nagpur district understudy.

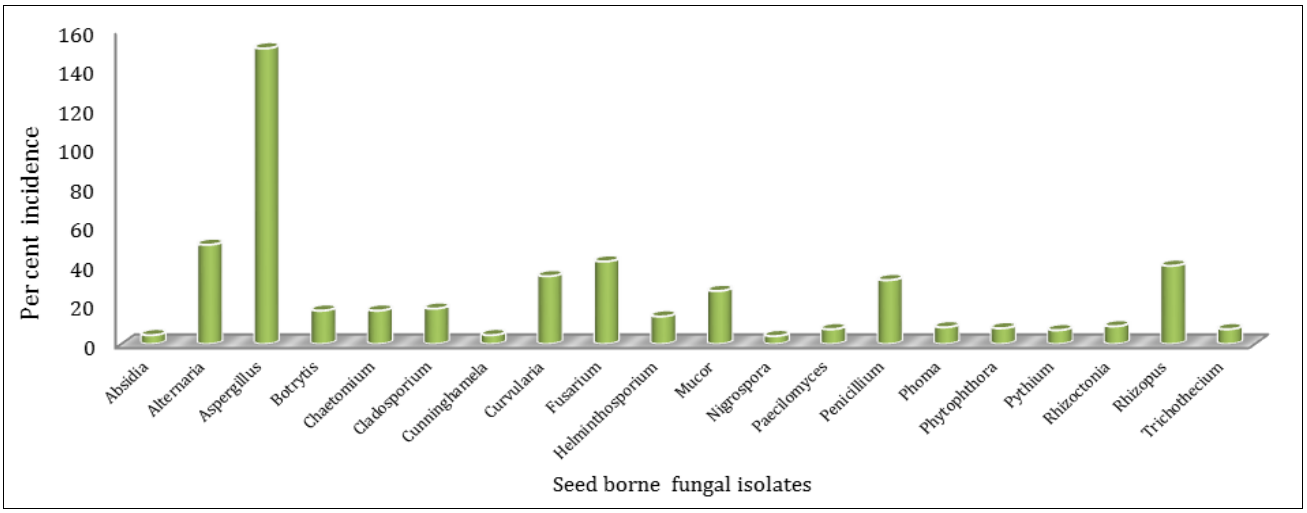

Fig. 5: Percent incidence of microfungal genera on stored comosite seed samples of Brassica compestris L.

These isolates are among the most abundant and widely distributed organisms on the globe (Lew-Smith, 2013). *Aspergilli* exist as obligate saprophytes on nutrient rich stored food material and survive in the environment without causing disease (Bhajbhuje, 2013; Jyoti and Malik, 2013). *Aspergillus amstelodomi, A. flavus, A. fumigatus and A. niger* had greater frequency of incidence. *Alternaria, Curvularia Fusarium* contributed second higher count of species over other genera. Of these, *Fusarium* exists under very wet storage environment as saprophytes on seeds and plant debris or parasites of many crops causes wilting. These ubiquitous species are mostly restricted to testa of stored seeds and other substrates, plant litter, dried fruits and nuts (Jain, 2008).

Mutagenic and carcinogenic effect of mycotoxins has been highlighted by Brakhage and Schroeckh (2011) and EFSA (2011). Mycotoxins are known to cause chromosomal breakage, create disturbances in normal mitotic cell division, alter regular metabolism & cell membrane permeability and also induce physiological as well as biochemical changes in metabolically active meristematic host cells (Bhajbhuje, 2013). *Fusarium* secretes a diverse range of mycotoxins includes *trichothecenes* (*T-2 toxin, HT-2 toxin, deoxy-nivalenol & nivalenol)*, *zearalenone* and *fumonisins* that have been reported to cause a variety of toxic effects in both experimental animals and livestock and also suspected of causing toxicity in human. *Fusarium. solani* and *F. moniliformae* were reported to cause *keratitis* and also associated with wound; and infections of eyes & fingernails (Shephard, 2012). *Aspergillus niger* has potential to produce *ochratoxin-A*; *A. flavus* secretes aflatoxins as well as other toxic compounds including *strigmatocystin, cyclopiazonic acid, kojic acid, β-nitropropionic acid, aspertoxin, aflatrem, gliotoxin* and *aspergillic acid*. *Penicillium* 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 (EFSA, 2011). *Helminthosporium* have been reported to produce *Helminthisporin*, four different HC toxins; *Paecilomyces varioti* secretes epoxysuccinic acid; *Curvularia lunata* produces 2-methyl-(5-hydroxy methyl) furan-2 carboxylate. Majority species of *Alternaria* are reported to secrete *Altersolarol-A* and *alternaric acid dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III* and induce mutagenic and cytotoxic effects (Brakhage and Schroeckh, 2011).

The data on fungal diversity and their incidence may be of a great importance in the region for predicting the extent of pre-and post-infections. Results indicated that *Brassica campestris* L. seeds harbor arrays of fungal contamination by diverse group of fungal flora as in response to improper storage management (Clemson, 2013). Majority of isolates reported in this survey, had been encountered to various kinds of stored seeds (Jain. 2008; Ismael, 2010; Saskatchewan, 2013; Bhajbhuje, 2013; Madavi and Bhajbhuje, 2014). The practices associated with quality of seeds at the time of storage; environmental factors during pre- & post-harvest stages, ambient relative humidity, temperature of storage environment, duration of storage and biotic agents, processing and handling of seeds may be responsible for its contamination (Jyoti & Malik, 2013). Moreover, proliferation of fungal flora on stored seeds in ideal climatic environment results to changes associated with various cellular, metabolic and chemical alterations, including DNA damage, impairment of RNA and protein synthesis, enzymes degradation & inactivation, loss of membrane integrity, declining of ATP, lowering in sugar and protein content, inability of ribosomes to dissociate, starvation of meristematic cells, increase in seed leaches & fatty acid content, reduced respiration and accumulation of toxic substances lead to spoilage of seeds (Jyoti and Malik, 2013). On the other hand, the prevalence of active fungal spores in seeds suggests an imminent public health danger since their metabolites (mycotoxins) produced in seeds may lead serious and devastating clinical conditions in the consumers (EFSA, 2011).

Majority of fungal isolates involved in seed deterioration of *Brassica campestris* L are xerophilic moulds such as *Aspergilli, Cladosporium* and *Penicilli* of *Ascomycotina* as well as *Alternaria, Curvularia, Fusarium, Helminthosporium, Paecilomyces and Trichothecium* of Deuteromycotina (Bhajbhuje, 2013). Sowing of deteriorated seeds increases chances of pathogen transmission to a new crop; the seedling emergence may be poor exhibiting stunted growth. The toxic metabolites secretion by these isolates may one of reason to spoilage of stored seeds. It is henceforth important to develop a strategy to antagonize their growth and survival in this seed commodity in order to neutralize the potential of these organisms surviving as agents of seed borne diseases. Low temperature and humidity results in delayed seed deterioration process and thereby leads to prolonged viability period (Jyoti and Malik, 2013).

CONCLUSION

Seed constitute basic agricultural productivity. Seed borne pathogens may help to spread diseases generation to generation and also involve in seed deterioration in storage, hence availability of pathogen free, healthy seed is the need of hours to overcome the food demand of growing mouth on the globe**.** The results of present survey revealed that all the seed lots of *Brassica campestris* L from various geographical regions of Nagpur districts, are more prone to fungal attack and carried greater count of fungal propagules on seed surface, leads to seed spoilage. The deeply seated fungal pathogen in the embryonic or endospermic tissues of seed may transmit to next generation, proliferate their population causing multifold losses in productivity. Only pathogen free and non-deteriorated seeds, respond better to all inputs thus seeds can be stored under ambient temperature and relative humidity at very low cost, without quality deterioration for periods of subsequent season is of immense importance for farmers. The farmers are advised to adopt improved scientific technologies of storage to discourage proliferation of seed borne fungal flora.

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