Final Report

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Title of Project:

"A survey on bio-diversity of fungal flora of stored oil seeds of Nagpur District & their impact on oil quality"

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Title of Project: A survey on bio-diversity of fungi on stored oil seeds of Nagpur District & their impact on oil quality.

Summary

Seed is the basic and most critical input for substantial agriculture. It 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. The pathogen free, and healthy seed and the way for cultivated the crops and gives had given a clear picture of their glorious golden era. Altogether 20 seed samples of mustard (*Brassica campestris* L.) has been collected from the each geographical areas comprising of the five sub-divisions of 14 talukas of Nagpur districts and screened for seed viability test and oil-extraction. The entire test samples resulted above 75% seed viability; it was maximum, 96% in most of the seed samples. Mycological analysis revealed the prevalence of total 41 fungal pathogens fall under 20 genera from sub-div. Saoner; 39 species representing 20 genera from Katol; 39 species of 19 genera from Umred; 35 isolates representing 19 genera from Ramtek; and altogether 34 species belonging to 18 genera from Nagpur in varying incidence.

Altogether 41 fungal pathogens representing 20 genera have been reported on seeds from composite of entire seed samples. Deuteromycota are most predominant ones with 8 genera and 18 species, followed by Ascomycota (6 genera and 16 species), Zygomycota (4 genera and 5 species) and Oomycota (2 genera and 2 species). Member of the Basidiomycota did not persist on the seeds. A population of 29 species of 15 genera have been reported as both external as well as internal seed borne; 7 isolates of 5 genera as external while 5 species as internal seed borne isolates. Of the total, 66.1% fungal 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% followed by Deuteromycota (34.6%), Zygomycota (15.3%) and Oomycota (3.1%). Among the predominant isolates, *Aspergillus* dominated with highest count of species followed by *Alternaria, Curvularia, Fusarium* , *Penicillium, Rhizopus, Helminthosporium* while remainings reported with single species. *Aspergillus amstelodomi, A. sulphureus, A. versicolor,A. ochracious, Paecilomyces varioti* and *Cunninghamella elegans* were reported for the first time on mustard seeds in India.

The analysis of data obtained during the monthly isolation of fungal seed mycoflora revealed prevalence maximum count in winter against minimum in summer. The storage period of October and November had moderate counts and it was declined during warmer summer. Of the total, 17 species of 11 genera existed throughout a year of storage; 4 species representing 4 genera confined to warmer summer (April to September); 13 species of 10 genera occurred during winter season (October to March) while 7 species representing single genera each did not exhibit any consistence of their recurrence in relation to changing and fluctuating climate.

Moreover, heavy infestation was confined to a month of January followed by February, December and March and it declined in summer which reported to low in May. It was again enhanced to an initiation period (October) of the winter. Seed viability for initial storage period of five months did exhibit little or negligible change and thereafter, it began to decline from September and finally reduced to 28 per cent in March. Freshly collected mixed seeds yielded 38.9 per cent pale yellow edible oil with saponification value 170. Phytochemical analysis of stored seed samples reveals reduction in oil content, saponification value, iodine value acid value and activity of peroxide was enhanced during storage of *Brassica campestris* L. seeds.

(A) Introduction:

Seeds are important input for crop production and hence pathogen free healthy seeds are considered as one of the vital factor for desired plant population and good economic harvest. India ranks third largest producer of mustard in the world after China and Canada with 12 per cent of world's total production. India holds a premier position in mustard economy of the world with 2nd and 3rd rank in area and production respectively. 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 to resort to import of edible oils with a projected demand for edible oils at more than 20 mt in 2014-15 (Kumar et al., 2009).

The oil-seeds of this crop is used by people in the prehistoric times quite intuitively for extraction of oil for cooking & burning purposes and curing many bodily disorders and thereby kept their health in perfect state of fitness and lived a long life. The crude oil applied on skin for the primary health care especially among those representing in the remote areas, like tribal and other forest dwellers. It becomes a remedy for skin diseases. It is also used in margarine soap, rubber lubrication and for oiling wool, as counter irritant and rebefacient in the form of poultice or plasters (Kokate et al., 1990).

As per the ICAR bulletin, it is estimated that 90% population of U.P., Punjab, Bihar, and Assam depends upon mustard seed oil for cooking as an alternative source to the groundnut oil. In other parts of India, the mustard seed oil is used as preservative due to its pungent smell, strong antibacterial, antifungal & medicinal properties. The oil cake is used as cattle feed & manure.

The literature survey reveals that seeds of *Brassica campestris* L. are known to carry several fungal pathogens which cause to alter the physio-chemical properties of the seeds during storage, losses of the seed weight, germination potential, medicinal properties, and discolouration. During storage, nearly 24% loss is caused by seed-borne pathogens. Some fungal species may bring about certain biochemical changes and produce mycotoxins which pose serious health hazard. Certain of these diseases are quite divesting but occur now at low rates in most of the countries, as conspicuous progress has been made to control seed-borne diseases.

In India, various researchers have studied the incidence of seed borne-fungi of *Brassica campestris* L under storage environment from various geographical locations. It it is very long that no investigations on bio-diversity and seasonal variations of seed-borne fungal flora of mustard during storage is carried out pertaining to the area of **Nagpur district** where temperature varies greatly from 10-15^{0}C in winter and 42-46^{0}C in summer. 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 & its impacts on oil quality are undertaken.

(B) Material and Methods:

(I) Selection of plant material:

Mustard (*Brassica campestris* L.) has been selected as an experimental material as it is rich source of oil content with high erucic acid which is used for cooking and has potent medicinal properties.

(II) Collection of seed samples:

Nagpur district comprises altogether 14 talukas which are grouped into 5 sub-divisions including Saoner (tah. Kalmeshwar and Saoner); Katol (tah. Narkhed and Katol); Umred (tah. Kuhi, Umred and Bhiwapur); Ramtek (tah. Parshioni, Ramtek and Mouda) and Nagpur (tah. Kamptee, Nagpur (City), Hingna and Nagpur (Rural).

A survey for collection of seed samples of post-harvest crop has been conducted from the area of all tehsils of Nagpur. Altogether 20 seed samples of *Brassica campestris* L were collected in polythene bags from different cultivators from each tehsil of Nagpur district. These seed samples were brought to laboratory and transferred aseptically to the cloth bags and stored in laboratory condition.

(III) Seed viability test:

The viability of the collected seeds for all the samples was performed employing roll-towel technique as suggested earlier by Musket (1948). The germination papers were washed with 1% hydrochloric acid to remove the impurities and any contamination. These papers were thoroughly washed with distilled sterile water for ten consecutive times and removed the traces of the acid. Four hundred seeds from each sample were placed with the help of sterilized forcep, on a sheet of damp paper toweling, covered by another layer, the lower 5 cm turned over and whole sheet rolled up and secured by elastic bands. The rolls having seeds were incubated at 25 ± 1^0C in B.O.D. incubator. The moisture content of rolled paper containing seeds has been maintained by addition of sterile distilled water when required. The seedlings were counted at sixth day's interval. At the end of incubation, a count of un-germinated seeds (including rotten seeds) was recorded. The ungerminated seeds were categorized into hard and dead seeds. The seedlings raised from germinating seeds were graded as normal (seedlings with well-developed root and shoot and free of disease symptoms) and abnormal (seedlings with underdeveloped root, shoot or both exhibiting disease symptoms) defined by Ismail et al., (2012).

(IV) Screening of seed samples:

Preliminary screening of seeds was done by dry examination (ISTA, 2013). A thin layer of seeds was spread in sterile Petri plates and seeds were examined under stereo-binocular microscope for their apparent deformities or discoloration.

Preliminary screening of seeds revealed prevalence of many types of deformities, deposition of acervuli, cleistothecia, clot of mycelia etc. on the seed surfaces. Some of the seeds appeared to be discolored.

(V) Seed Health Testing:

The entire collected samples of each variety and all the varieties of crop were mixed together and screened for prevalence of fungal flora associated with seeds. Seed surface fungal contaminants were isolated employing blotter paper as well as agar plate method as recommended by ISTA (2013). Four hundred seeds were used to perform seed health test. Out of these 50% seeds were screened for blotter test and remaining for agar plate test.

(i) Blotter test**:** Two hundred seeds from each variety and mixed seed sample were screened for isolation of external seed borne fungal pathogens. Twenty seeds without pretreatment were placed in ten sterile Petri plates containing three layered sterile moistened absorbent blotter paper to provide enough moisture for the duration of test. The experiment was carried out under laminar airflow to avoid chances of external contamination. Only external seed-borne fungi were recorded.

(ii) Agar test**:** Infested seeds of cauliflower were screened for isolation of internal seed borne mycoflora employing standard agar plating method (ISTA, 2013). Potato-dextrose medium composed of peeled potato ($400gm^{-l}$); dextrose ($20gm^{-l}$**)** and agar ($20gm^{-l}$**)** in a liter of distilled water was prepared and pH was adjusted to 6.5. After sterilization in autoclave at 15 p.s.i. for 20 minutes, 25mg/lit streptomycin sulphate was added in medium to prevent the growth of bacteria. The sterilized PDA mediums were poured aseptically under Laminar Flow in sterilized Petri plates. These Petri plates containing liquid medium were allowed to cool for two hours at room temperature to jellify medium. To the isolation of internal seed borne pathogens, two hundred seeds were pretreated with aqueous 0.1% mercuric chloride solution for a minute and rinsed with sterile distilled water for five consecutive times. The pretreated twenty seeds were transferred aseptically under Laminar Flow to each sterile Petri plate containing semi-solid medium and placed them equidistantly with the help of sterilized forcep.

All Petri plates containing seeds were incubated for seven days under the alternating cycle of 12 hours NUV (Near ultraviolet) exposure and 12 hours darkness at 25 ± 1^0C in B.O.D. incubator. The seeds were observed under stereo-binocular on eighth day for appearance of fungal growth. 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.

(VI) Monthly check up of seed health under storage**:**

The seed samples were stored in small cotton bags under normal room conditions for a period of one year (April 2013 to March. 2014). After every month 400 seeds were taken out randomly and per cent incidence of fungal pathogens as well as per cent seed germination was recorded by usual blotter method (ISTA, 2013).

(VII) Identification of seed borne fungal pathogens:

The fungal pathogens appeared on untreated as well as pre-treated seed surfaces were examined on eighth day under stereo-binocular microscope and colonial features were studied using compound microscope. An isolate of pathogen was sub-cultured on Czapek's Dox agar medium (Ainsworth, 1983). A pure culture of the isolate was obtained by serial transfer of the culture appeared on seed surface on regular basis. 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 determination of morphological structure of fungal organisms was carried out after being mounted on glass slide in lecto-phenol and cotton blue covered with cover slip. The fungal types were analyzed for each day. The developed colonies of some fungal organisms were identified up to genus level. The species were identified on the basis of micro- & macro morphology; reverse and surface coloration of colonies grown in Czapek's medium. The morphological characteristics of isolates were compared with the available literature, standard mycological books (Neergaard, 1977; Gilmen, 2001; Mokadam et al., 2006) and manuals and finally authenticated by authority.

The Czapek's Dox agar nutrient medium was prepared by dissolving the ingredients such as Sodium nitrate (NaNO3), 2.0g; Potassium dihydrogen phosphate (KH2PO4), 1.0g; Magnesium sulphate, (MgSO4.7H2O, 0.5g; Potassium chloride (KCl), 0.5g; Ferrous sulphate (FeSO4.7H2O), 0.01g; Sucrose, 30 g; agar powder in one liter distilled water as prescribed by Ainsworth (1983). The pH of the medium was adjusted to 6.8 and it was sterilized in autoclave at 15 p.s.i for 20 minutes. The slightly cooled medium was used for tube slant preparation. Purified fungal isolates were propagated and maintained on Czapek's Dox agar nutrient medium in sterile slants. The medium is also used for sub-culturing of fungal isolates.

(VIII) Ecological studies:

For ecological studies, at the end of incubation period, fungal count and infestation level have been recorded as a percentage of infested seeds in a sample following a technique reported earlier (Chukunda et al., 2013).

$$\text{Per cent incidence} = \frac{\text{No. of seeds containing fungi}}{\text{Total no. of seeds assessed}} \text{ X } 100$$

(IX) Phytochemical analysis of oil of freshly collected stored seed samples:

Mustard (*Brassica campestris* L) seeds are considered to be one of the rich source of vegetable oil for cooking. Initially, entire collected seed samples from various sub-divisions of Nagpur Districts were mixed together and stored at laboratory condition. After a period of one year, the stored seed samples were screened to oil extraction.

Physicochemical analysis of the oils was conducted using the standard methods reported earlier (Warra et al., 2011). The parameters analyzed were the iodine value, saponification value, acid value and peroxide value:

(a) **Crude oil content**:

The oil of the seeds of mustard was extracted following Soxhlet extraction method reported earlier (Kalia et al., 2002; Straccia et al., 2012). Prior to processing, the stored seeds were ground in domestic grinder to convert into fine flour (powder) of particle size. The dried flour of seeds was packed in plastic tube and kept at low temperature until analyses.

(i) Operation of Soxhlet extractor:

The powdered seed sample (10g each) was placed into porous filter paper thimble and was inserted in the centre of the extractor. A round bottom flask containing 300 ml petroleum ether (with boiling point of 40- 60^{o}C) was placed in the heating mantle. The Soxhlet was heated at 40-60^{0}C. When the solvent was boiling the vapour rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contained the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for 6 hrs. It was then removed from thimble, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil extracted. The extraction of oil from residue was carried at an interval of 6 hrs. up to constant value. The experiment was repeated thrice. The weight of oil extracted was determined for each 6 hrs. interval. The oil was obtained after the removal of solvent with rotary evaporator and refluxing at 70^{o}C. The oil was then stored in freezer at low temperature for subsequent physicochemical analyses.

(ii) Oil Yield Determination (%):

The oil thus obtained after the extraction was transferred into a measuring cylinder which was placed over water bath for 30 min at 70^{o}C to ensure complete evaporation of solvent. The volume of the oil was recorded and expressed in percentage as oil content.

$$\text{Oil content (\%)} = \frac{\text{Weight of the oil}}{\text{Weight of the sample}} \times 100$$

(b) **Saponification Value:**

About 2g of the oil sample was added to a flask with 30ml of ethanolic KOH and was then attached to a condenser for 30 minutes to ensure that the sample is fully dissolved. After cooling the content, 1ml of phenolphthalein was added and titrated with 0.5 M HCl until appearance of pink colour, indicated the end point.

The expression for saponification value (S.V.) is given by:

$$\text{Saponification Value} = 56.1\ N\ (V0 - V1) / M$$

where V0 = the volume of the solution used for blank test;
VI = the volume of the solution used for determination;
N = actual normality of the HCl used;
M = Mass of the sample.

(c) **Iodine Value**:

The powdered seed sample (0.4g) was taken into a conical flask and 20ml of carbon tetrachloride was added to dissolve the oil. Then 25ml of Dam's reagent was added to the flask using a safety pipette. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours 30 minutes. At the end, 20ml of 10% aqueous potassium iodide and 125ml of water were added to the flask containing sample. The content was titrated with 0.1M sodium thio-sulphate solution until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thio-sulphate drop wise until blue coloration disappeared after vigorous shaking. The same was performed for blank test and other samples.

The iodine value (I.V.) is given by the expression:

$$\text{Iodine Value} = 12.69\ C\ (V1 - V2) / M$$

Where C = Concentration of sodium thio-sulphate used;
V1 = Volume of sodium thio-sulphate used for blank;
V2 = Volume of sodium thio-sulphate used for determination,
M = Mass of the sample.

(d) Acid Value:

A beaker containing 100 ml of neutral ethyl alcohol was heated with 10g of oil or fat sample until the mixture began to boil. The heating was stopped and the solution was titrated with N/10 KOH solution, using two drops of phenolphthalein as indicator with consistent shaking until development of pink colour, indicating end point.
The Acid value was calculated using the expression

$$\text{Acid Value (A.V.)} = 0.56 \times \text{No. of ml. N/10 KOH used.}$$

(e) **Peroxide Value:**

Exactly 1.0g of KI and 20ml of solvent mixture (glacial acetic acid: chloroform, 2:1 v/v) were added to 1.0g of the oil sample and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20ml of 5% KIO_3 solution. Few drops of starch solution were added to the mixture and the latter was titrated with 0.025M $Na_2S_2O_3$ solution.

(C) Experimental Results:

On receiving the seed samples from various geographical locations of sub-divisions of Nagpur District, the experiments on various aspects were conducted to study biodiversity of fungal flora associated with seeds of *Brassica campestris* L in the laboratory and results are presented in the tables 1-5.

(I) Seed viability test:

The seed viability test has been performed for twenty seed samples of *Brassica campestris* L employing rolled-towel technique (Musket, 1948). A count of un-germinated seeds (hard and dead seeds including rotten seeds) as well as seedlings raised from germinating seeds (normal and abnormal seedlings) are recorded in table I.

The entire test samples of *Brassica campestris* resulted above 75% seed viability. The samples exhibited highest 96% seed viability while it was appeared minimum, 76.5%.

Out of the 20 seed samples obtained from **Sub.-div. Saoner**, sample viz., BC-11 had 96% seed germination, exhibiting highest seed viability compared to others. The seed samples, BC-8, 9, 16 & 17 had above 90% seed germination. The least seed viability, 78.5% has been recorded for BC-8. Altogether, 4.0-21.5 seeds did not germinate, of them, 0.5-8.5% seeds had hard seed coat and remaining seeds were found dead. The seed sample BC-11 had least count of hard and dead seeds (Table 1).

Majority of seeds from **Sub.-div. Katol** were appeared to be viable. The sample BC-19 had highest, 96% seed viability. Seed germination, above 90% has been recorded for samples, BC-3, 4 and 20 while it was only 78% for BC-7, exhibiting least seed viability against others. Altogether, 4 – 22% seeds unable to germinate, of them, 1.0-5.5% seeds had hard seed coat and remaining seeds were found dead (Table 1).

The seed sample, BC-9 from **Sub.-div. Umred** had greater, 96% seed viability and least count of hard & dead seeds against others. Single seed sample, BC-17 had above 90% seed germination while least seed viability, 76.5% has been recorded for BC-19. Out of the 4.0- 23.5% ungerminated seeds, 1.5-4.5% seeds had hard seed coat and remaining seeds were found dead (Table 1).

The seed sample, BC-18 from **Sub.-div. Ramtek**, had 96% seed germination exhibiting highest seed viability against others. Seed germination, above 90% has been recorded for three samples, BC-10, 11 and 20. Sample, BC-5 had least seed viability. Altogether, 4 – 22% seeds did not germinate, of them, 1.0-5.5% seeds had hard seed coat and remaining seeds were found dead (Table 1).

The seed sample, BC-7 from **Sub.-div. Nagpur** had greater, 96% seed viability as well as least count of hard & dead seeds against others. Seed germination, above 90% has been recorded for samples, BC-4, 6 and 8. Sample, BC-12 had 76.5% seed germination, exhibiting least seed viability. The ungerminated seeds were reported to the extent of 4.0-23.5%, of them, 1-4% seeds had hard seed coat and remaining were found dead (Table 1).

Table 1 : Per cent seed viability of the seed samples of mustard (*Brassica campestris* L.) obtained from various geographical locations of sub-divisions of Nagpur District.

Sample No.	Per cent germination of seeds of various seed samples of *Brassica campestris* L.																								
	Sub-div.: Saoner					Sub-div.: Katol					Sub-div.: Umred					Sub-div.: Ramtek					Sub-div.: Nagpur				
	Seedlings		Ungerminated seeds		Total seed viability	Seedlings		Ungerminated seeds		Total seed viability	Seedlings		Ungerminated seeds		Total seed viability	Seedlings		Ungerminated seeds		Total seed viability.	Seedlings		Ungerminated seeds		Total seed viability
	N	A	H	D	T	N	A	H	D	T	N	A	H	D	T	N	A	H	D	T	N	A	H	D	
BC-01	81.5	1.0	5.5	12.0	82.5	80.0	3.0	5.5	11.5	83.0	86.5	1.0	2.5	10.0	87.5	81.5	1.0	5.5	12.0	82.5	81.5	1.0	5.5	12.0	82.5
BC-02	80.0	0.5	8.5	11.0	80.5	91.5	1.0	1.5	6.0	92.5	76.0	3.0	3.5	17.5	79.0	81.0	2.5	2.5	14.0	83.5	87.5	2.0	3.0	7.5	89.5
BC-03	76.5	2.0	2.0	19.5	78.5	94.0	1.5	0.5	4.0	95.5	80.0	2.0	4.0	12.0	84.0	72.0	6.5	3.5	18.0	78.5	88.5	1.0	2.5	8.0	89.5
BC-04	78.5	1.0	6.5	14.0	79.5	88.5	1.0	2.5	8.0	89.5	87.5	2.0	3.0	7.5	89.5	86.5	1.0	2.5	10.0	87.5	91.0	1.5	1.5	6.0	92.5
BC-05	87.0	1.5	3.0	8.5	88.5	78.5	2.0	1.5	18.0	80.5	80.0	3.0	1.5	15.5	83.0	90.5	1.5	1.5	6.5	92.0	85.5	1.0	2.5	11.0	86.5
BC-06	85.5	1.5	3.5	9.5	87.0	76.0	3.0	3.5	17.5	79.0	78.0	4.0	2.0	16.0	82.0	72.0	6.0	3.5	18.5	78.0	91.5	1.0	1.5	6.0	92.5
BC-07	88.5	1.0	2.5	8.0	89.5	76.0	2.0	2.5	19.5	78.0	85.5	1.0	2.5	11.0	86.5	88.5	1.0	2.5	8.0	89.5	94.0	2.0	1.0	3.0	96.0
BC-08	91.0	1.5	1.5	6.0	92.5	87.5	2.0	3.0	7.5	89.5	75.0	3.5	2.5	19.0	78.5	87.0	1.5	2.5	9.0	88.5	91.5	1.5	1.5	5.5	93.0
BC-09	90.0	2.0	2.5	5.5	92.0	85.5	1.0	2.5	11.0	86.5	94.0	2.0	1.0	3.0	96.0	77.0	3.0	2.0	18.0	80.0	76.0	3.0	3.5	17.5	79.0
BC-10	86.5	2.0	1.5	10.0	88.5	81.0	2.5	2.5	14.0	83.5	75.0	3.5	2.5	19.0	78.5	91.0	1.5	1.5	6.0	92.5	88.5	1.0	2.5	8.0	89.5
BC-11	94.5	1.5	0.5	3.5	96.0	71.0	6.5	3.5	19.0	77.5	88.5	1.0	2.5	8.0	89.5	91.5	1.0	1.5	6.0	92.5	78.5	2.0	1.5	18.0	80.5
BC-12	80.5	1.5	5.0	14.0	82.0	80.0	2.0	4.0	12.0	84.0	72.0	6.0	3.5	18.5	78.0	80.0	2.0	4.0	12.0	84.0	73.0	3.5	4.0	19.5	76.5
BC-13	81.5	2.5	5.5	12.0	84.0	73.0	3.5	4.0	19.5	76.5	87.0	1.5	2.5	9.0	88.5	88.5	1.0	2.5	8.0	89.5	86.5	1.0	2.5	10.0	87.5
BC-14	86.0	3.5	2.5	9.0	89.5	78.0	3.5	2.5	16.0	81.5	76.5	2.0	2.0	19.5	78.5	85.5	1.0	2.5	11.0	86.5	87.0	1.5	2.5	9.0	88.5
BC-15	85.5	1.0	2.5	11.0	86.5	86.5	1.0	2.5	10.0	87.5	72.0	6.5	3.5	18.0	78.5	78.5	2.0	1.5	18.0	80.5	71.0	6.5	3.5	19.0	77.5
BC-16	91.5	1.5	1.5	5.5	93.0	72.0	6.0	3.5	18.5	78.0	78.0	4.5	2.5	15.0	82.5	88.5	1.0	2.5	8.0	89.5	80.0	2.0	4.0	12.0	84.0
BC-17	89.5	1.0	2.5	7.0	90.5	79.0	4.0	1.5	15.5	83.0	89.5	2.0	2.5	7.0	91.5	76.0	3.0	3.5	17.5	79.0	81.0	2.5	2.5	14.0	83.5
BC-18	80.0	3.5	5.5	12.0	83.5	87.0	1.5	2.5	9.0	88.5	86.5	1.0	2.5	10.0	87.5	94.0	2.0	1.0	3.0	96.0	85.5	1.0	2.5	11.0	86.5
BC-19	86.5	1.0	2.5	10.0	87.5	94.0	2.0	1.0	3.0	96.0	74.0	2.5	4.5	19.0	76.5	87.5	2.0	3.0	7.5	89.5	88.5	1.0	2.5	8.0	89.5
BC-20	80.0	2.0	4.0	12.0	84.0	90.5	1.5	1.5	6.5	92.0	77.0	3.0	2.0	18.0	80.0	92.0	1.5	1.5	5.0	93.5	76.5	2.0	2.0	19.5	78.5

* All the values are calculated in terms of percentage.
BC – *Brassica campestris*
N = Normal seedlings; A = Abnormal seedlings; H = Hard seeds; D = Dead seeds

(II) Screening of seed samples:

Preliminary screening of seeds obtained from various locations of sub-divisions of the Nagpur District revealed prevalence of many types of deformities, deposition of acervuli, cleistothecia, clot of mycelia etc. on the seed surfaces. Some of the seeds appeared to be discolored. It is concluded that majority of the seeds from the seed samples may lost their germinability in due course of storage due to activities of the fungal flora adhered on seed coats in set of favourable environment.

(III) Seed Health Testing

The entire collected seed samples from each sub-divisions and from all the sub-divisions of Nagpur District were mixed together and screened for prevalence of fungal flora associated with seeds employing blotter paper as well as agar plate method as recommended by ISTA (2013). At the end of incubation period, fungal count and infestation level on the untreated and pre-treated seeds have been recorded as a per cent infested seeds in a sample following a technique reported earlier (Chukunda et al., 2013). The results are presented in Tables 2-4.

(a) Seed mycoflora of mixed seed samples:

Mycological analysis of mixed seed samples of *Brassica campestris* L. revealed the prevalence of total 41 fungal pathogens fall under 20 genera in varying incidence (Table 3). 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 the Basidiomycota did not persist on the seeds (Table 2).

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 confined as seed contaminants while the genera such as *Phytophthora, Pythium, Absidia, Mucor, Cunninghamela, Botrytis, Chaetomium, Cladosporium, Phoma, Nigrospora, Paecilomyces, Rhizoctonia* and *Trichothecium* had single species. A total of seven isolates, *Aspergillus amstelodomi, A. sulphureus, A. versicolor, Aspergillus ochracious, Paecilomyces varioti* and *Cunninghamella elegans* were reported as seed borne pathogens on seeds for the first time from *Brassica campestris* L seeds in India (Table 2).

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 isolates, *Aspergillus flavus, Aspergillus*

niger and *Rhizopus stolonifer* were appeared to be most predominant on the mustard seeds exhibiting higher incidence. The isolates recorded subdominant had incidence between 20-27% included *Aspergillus fumigatus, A. terreus, Penicillium oxalicum* and *Mucor pusillus* while others were detected with 5.5 to 18.0% incidence. Low level of fungal incidence was detected for *Penicillium pallidum, Curvularia clavata* and *Paecilomyces variotii* and *Trichothecium roseum* by both health testing techniques (Table 2).

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 frequency of Incidence, 4.5% was detected for *Cunninghamela elegans* and *Aspergillus sulphureus.* The isolates *Aspergillus nidulans* and *Helminthosporium spiciferum* had low frequency of incidence (Table 2).

The fungal isolates from mixed seed samples 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 fungal pathogen. Excepting *Phoma glomerata*, others were detected with incidence frequency varies between 3.5 -5.5% (Table 2).

The mixed seed samples seeds were highly infested by fungal pathogens. Of the total, 66.1% fungal incidence was recorded on blotter paper while 33.9% incidence was detected 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 4).

(b) Seed mycoflora from Sub.-div. Saoner

Mycological analysis of mixed seed samples of *Brassica campestris* L. revealed the 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% has been recorded for *Absidia corymbefera, Aspergillus ochracious, A. versicolor, Botrytis cinera, Curvularia clavata Cunninghamella elegans, Helminthosporium specifectum, Nigrospora sp., Penicillium digitatum, , Rhizopus nigricans,* The isolate *Aspergillus nidulans* had least incidence (Table 2).

The seed samples from **sub-div Saoner** were reported heavily infested with a fungal population comprising of 41 pathogens representing 20 genera (Table 3). Of them, 24 fungal isolates of 14 genera has been detected in varying frequencies of incidence as both external and internal seed borne pathogens by standard blotter paper and agar plate techniques. These fungal flora 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 2).

Only six isolates were restricted to agar plate as internal seed borne pathogens in the incidence level varies from 2.5-4.5%, included *Absidia corymbefera, Alternaria brassicae, Aspergillus versicolor, Botrytis cinera, Curvularia clavata and Penicillium digitatum* (Table 2).

A fungal population of 11 isolates representing 9 genera has been confined to blotter paper only in frequency of incidence varying from 1.5-6.5% included *Aspergillus nidulans, A. ochracious, A. sulphureus, Curvularia intermedia, Cunninghamella elegans, Fusarium semitectum, Helminthosporium specifectum, Nigrospora sp. Phoma glomerata, Rhizoctonia solani Rhizopus nigricans* (Table 2).

All the fungal pathogens have been detected with sum total of 393 per cent incidence by both seed health tests. Of the total, 70.2% fungal incidence was recorded on blotter paper while 29.8% incidence was confined on agar plates. Ascomycota contributed 47.6% incidence which was nearly half of the total incidence recorded. Deuteromycota contributed 32.8% of total incidence, followed by Zygomycota (16.9%) and Oomycota (2.5%) (Table 4).

(c) Seed mycoflora from Sub-div. Katol:

Mycological analysis of seed samples obtained from localities of **sub.-div. Katol** revealed the prevalence of fungal population of altogether 39 species fall under 20 genera in varying incidence (Table 3). 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 frequency of incidence varies between 3-10% (Table 2).

Table 2 : Frequency (%) of incidence of fungal flora on seeds of mustard (*Brassica campestris* L.) obtained from various geographical locations of sub-divisions of Nagpur District.

S.No	Fungal isolates	Frequency (%) fungal incidence on seeds of *Brassica campestris* L.																	
		Mixed seed sample			Sub-div.: Saoner			Sub-div.: Katol			Sub-div.: Umred			Sub-div.: Ramtek			Sub-div.: Nagpur		
		Per cent fungal incidence			Per cent fungal incidence			Per cent fungal incidence			Per cent fungal incidence			Per cent fungal incidence			Per cent fungal incidence		
		Blotter	Agar	Total	Blotter	Agar	Total	Blotter	Agar	Total	Blotter	Agar	Total	Blotter	Agar	Total	Blotter	Agar	Total
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)	-	-	-
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)
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)
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	100.0	70.2	29.8	100.0	68.1	31.9	100.0	67.9	32.1	100.0	68.9	31.1	100.0	68.3	31.7	100.0

Table 3: Distribution of external and internal seed borne fungal pathogen on seeds of mustard (*Brassica campestris* L obtained from various geographical locations of sub-divisions of Nagpur District.

S. N	Cultivars	A count of seed borne fungal pathogens 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
		Species	Genera	Species	Genera	Species	Genera					
1	Mixed seed samples	29	15	07	05	05	05	20	41	323 (66.1)	166 (33.9)	489
2	Seeds from Sub. Div. Saoner	24	14	11	09	06	06	20	41	276 (70.2)	117 (29.8)	393
3	Seeds from Sub. Div. Katol	26	15	08	08	05	05	20	39	222 (68.1)	104 (31.9)	326
4	Seeds from Sub. Div. Umred	25	15	08	06	04	04	19	37	239 (67.9)	113 (32.1)	352
5	Seeds from Sub. Div. Ramtek	25	15	06	05	04	04	19	35	233 (68.9)	105 (31.1)	338
6.	Seeds from Sub. Div. Nagpur	25	15	05	05	04	04	18	34	185 (68.3)	86 (31.7)	271
1. Values in parenthesis is calculated in terms of cumulative per cent fungal incidence												

Table 3: Distribution of external and internal seed borne fungal pathogen on seeds of mustard (*Brassica campestris* L.) obtained from various geographical locations of sub-divisions of Nagpur District.

S. N	Cultivars	A count of seed borne fungal pathogens 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
		Species	Genera	Species	Genera	Species	Genera					
1	Mixed seed samples	29	15	07	05	05	05	20	41	323 (66.1)	166 (33.9)	489
2	Seeds from Sub. Div. Saoner	24	14	11	09	06	06	20	41	276 (70.2)	117 (29.8)	393
3	Seeds from Sub. Div. Katol	26	15	08	08	05	05	20	39	222 (68.1)	104 (31.9)	326
4	Seeds from Sub. Div. Umred	25	15	08	06	04	04	19	37	239 (67.9)	113 (32.1)	352
5	Seeds from Sub. Div. Ramtek	25	15	06	05	04	04	19	35	233 (68.9)	105 (31.1)	338
6.	Seeds from Sub. Div. Nagpur	25	15	05	05	04	04	18	34	185 (68.3)	86 (31.7)	271

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 2).

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 dominant with 7.0% and 4.5% incidence incidence. *Fusarium semitectum* and *Penicillium pallidum* has been detected with 3.5% incidence while remaining isolates had low frequency of incidence ranged between 2-3%. Least infestation has been recorded for *Cunninghamella elegans* (Table 2).

The 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 were detected with incidence frequency varies between 3.5 -5.5% (Table 2).

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% fungal incidence confined to on blotter paper while 31.9% incidence has been detected with agar plates. Ascomycota contributed 46.3% incidence followed by Deuteromycota with 33.9% of total incidence. Moderate incidence was recorded for Zygomycota (16.9%) and while Oomycota contributed least, 1.7% incidence (Table 4).

(d) Seed mycoflora from Sub-div. Umred:

Mycological analysis of seed samples obtained from localities of **sub.-div Umred** revealed the prevalence of total 39 fungal pathogens belonging to 19 genera in varying incidence (Table 3). 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 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, four isolates, *Aspergillus flavus, A. fumigatus, A. niger Rhizopus stolonifer* were appeared to be most predominant on the seeds exhibiting higher incidence. The isolates, *Alternaria alternata, Aspergillus amstelodomi, A. terreus, Chaetomium glabosum, Cladosporium fulvum, Curvularia lunata, and Penicillium oxalicum* has been reported to be subdominant on seeds L. by both health testing techniques.

Altogether 8 fungal species which fall under 6 genera has been confined to blotter test only. These isolates 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 2)

The 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%.

Seed mycoflora of *Brassica campestris* L from localities of sub-div. Umred of Nagpur District has been detected with incidence sum total of 352 per cent by both seed health tests . The fungal incidence, 57.9% was detected by blotter paper test while 32.1% incidence by agar plate technique. Ascomycota contributed 41.0% incidence, exhibiting highest contribution against others. Deuteromycota had 35.4% while Zygomycota contributed 21.2% total incidence. Least incidence contributed by Oomycota (Table 4).

(e) Seed mycoflora from Sub-div. Ramtek:

Seed samples received from localities of **sub.-div Ramtek** 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 confined 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 species while remainings had single species. The isolates, *Rhizopus stolonifer* was encountered with higher, 32.5% incidence. Significant fungal 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 4).

(f) Seed mycoflora from Sub-div. Nagpur:

Mycological analysis of seed samples obtained from localities of **sub.-div Nagpur** revealed the prevalence of a population of altogether 34 fungal pathogens belonging to 18 genera in varying incidence (Table 3). The isolates of *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 2).

Individual genus, *Aspergillus* dominated with 7 species, followed by *Alternaria,* and *Fusarium* with 4 species; *Penicillium* and *Curvularia* with 3 species each. *Rhizopus* had two species 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 pathogens 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 on both blotter paper and agar plates techniques. Among these Rhizopus stolonifer, was appeared to be most dominant, detected with higher 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

fungal contaminants on seeds. *Fusarium oxysporum, Paecilomyces variotii* and *Rhizopus nigricans* had least per cent incidence (Table 2).

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 remaining isolates had 2.0-3.5% incidence. Least per cent incidence has been recorded for *Aspergillus ochracious* (Table 2).

Altogether 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 2).

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 3). Of the total, 68.3% fungal incidence was recorded by blotter paper test while 31.7% incidence was detected with agar plate technique (Table 3). *Ascomycota* and Deuteromycota contributed nearly equal estimate of incidence, representing 40.2% and 39.5% incidence respectively. Moderate incidence was recorded for Zygomycota (17.2%) and while Oomycota contributed least, 3.1% incidence (Table 4).

(VI) Monthly check up of seed health under storage:

An incidence of seed mycoflora of randomly selected stored seeds of mixed samples of *Brassica campestris* L. have been detected from diverse geographical locations of Nagpur Districts at an interval of a month for a period of one year (April 2013 to March. 2014) following standard blotter test . The fungal incidence in terms of percentage as well as per cent seed germination has been presented in Table 5 & 6.

The standard blotter test proved superior over others was used for periodic detection of seed surface contaminants to record seasonal diversity in storage climate. Considering diverse fungal population count and variable infection level in monthly period of season, the fungal isolates may be categories into *four* categories:

Category- (a) fungal flora prevailing throughout a year;
Category- (b) fungal flora prevailing in summer season only;
Category- (c) Fungal flora prevailing in winter season only and
Category- (d) Fungal flora of rare occurrence without showing any specificity to a time of recurrence.

(i) Category- (a) fungal flora prevailing throughout a year:

A fungal population of forty one diverse isolates was seemed to be prevailing on seed surface in storage. Of these, a population of 17 species of 11 genera was encountered

throughout a year of storage representing category-(a). The fungal pathogens prevailing throughout the year on seeds included *Absidia corymbefera, Alternaria alternata, A. brassicicola, A. brassicae A. solani, Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Fusarium miniliformae, F. solani Mucor pusillus Nigrospora sp., Penicillium oxalicum, Phytophthora infestans, Rhizoctonia solani* and *Rhizopus stolonifer*.

Among the population of fungal pathogens, *Rhizopus stolonifer* was appeared to be predominant, exhibiting 214.5 per cent cumulative incidence followed by *Aspergillus fumigatus* with 198.0 per cent total incidence; Mucor pusillus was detected with 174.5 per cent cumulative incidence followed by *Aspergillus flavus* (150.5%); *Absidia corymbifera* (123.0%); *Aspergillus niger* (89.5%); *Nigrospora sp* (81.5%). Moderate level of cumulative incidence, ranged between 51-77% has been recorded for *Alternaria alternata, A. solani, A. brassicicola, A. brassicae*; *Curvularia lunata, Phytophthora infestans* and *Penicillium oxalicum*. The two isolates, *Fusarium moniliformae* and *Rhizoctonia solani* had little level of cumulative incidence (Table 5).

(ii) Category- (b) fungal flora prevailing in summer season only:

Fungal population of seed surface contaminants survived in storage throughout warmer summer season (April to September) representing category-(c). Of the total isolates, a population of 4 species representing 4 genera namely *Aspergillus ochracious, A. terreus, Chaetomium glabosum,* and *Paecilomyces varioti* have been encountered to seeds throughout summer season in varying degree of infestation. The isolate, *Chaetomium glabosum* was appeared to be predominant with cumulative 70.50 per cent incidence followed by *Paecilomyces variotii* (35.0%) and *Aspergillus terreus*(31.0%); Least cumulative incidence has been recorded for *Aspergillus ochracious* (Table 5).

(iii) Category- (c) fungal flora prevailing in winter season only:

The seed surface contaminants confined to seeds of *Brassica campestris* L in the winter season (October to March) representing category-(b), contributed a population comprising total of 13 species belonging to 10 genera. These isolates included *Aspergillus amstelodomi, Botrytis cinera, Cladosporium fulvum, Cunninghamela elegans, Curvularia clavatus, C. ovoidea, C. intermedia, Fusarium oxysporum, Helminthosporium specifectum, H. tetramera, Phoma glomerata, Pythium aphanidermatum* and *Rhizopus nigricans* in varying level of infestation (Table 5).

An isolate of *Zygomycota*, *Rhizopus nigricans* was seemed to be major components in the winter season, contributing cumulative 42.5 per cent incidence followed by *Helminthosporium tetramera* (41.0%) and *Cladosporium fulvum* (35.0%)., Moderate level of the cumulative incidence varied between 20.0 to 29.5 per cent incidence has been detected for *Cunninghamela elegans, Phoma glomerata, Curvularia clavatus, C. ovoides, C. intermedia, F. oxysporum and Helminthosporium tetramera* while it was recorded at low level for *Aspergillus amstelodomi* and *Botrytis cinera*. An isolate of Oomycota, *Pythium aphanidermatum* had least incidence (Table).

Similar trend for infestation level to seeds was confined to a month of *January* as recorded for category-(a). It is premised on the hypothesis that climate of winter season between *January* and *February* favors propagation of majority of propagules. Results revealed that a population of a total of 14 species belong to 12 genera, *Aspergillus amstelodomi, A. sydowi, Aureobasidium pullulans, Botryodiplodia theobromae, Curvularia ovoidea, Fusarium semitectum, Helminthosporium anomalous, H. spiciferum, Penicillium pallidum, Phoma glomerata, Pythium sp., Stemphylium botryosum, Trichoderma lignorum* and *Trichothecium roseum* were appeared in the months of *January, February* and rarely in *March* of the winter season. Both the species of *Helminthosporium* and *Pythium* sp. were seemed to be predominant with greater, 8.0 -11.5 per cent incidence. Little to mild incidence level varying between 1.0-4.75 per cent has been detected for *Aureobasidium pullulans, Botryodiplodia theobromae* and *Trichoderma lignorum* while others had moderate level of infestation (Table 5).

(iv) Category- (d) Fungal flora of rare occurrence without showing any specificity to a time of recurrence.

A few fungal isolates detected as seed surface contaminants, but did not exhibit any consistence of their recurrence in relation to changing and fluctuating climate, representing category-(d). It included only a population of 7 isolates belongs to 7 genera *Aspergillus nidulans, A. sulphureus, A. versicolor, Fusarium semitectum, Penicillium pallidum, P. digitatum* and *Trichothecium roseum*. An isolate of Deuteromycota, *Fusarium semitectum* was seemed to be most predominant detected with cumulative 12.0 per cent incidence followed by *Aspergillus sulphureus* (9.0%); *Trichothecium roseum* (9.0%), *Penicillium pallidum* and *Aspergillus pallidum* (5.0%). Least incidence has been detected for *Penicillium digitatum* (Table 5).

A population of all fungal seed borne isolates encountered to seeds for a year of storage in a set of environment has been categorized under various divisions and count of isolates as well as their per cent incidence for individual division is presented in table 2. Oomycota contributed maximum 2 genera and 2 species to a month of January to March. The greater level of cumulative incidence, 15 % has been detected in the month of January while other each month period had single isolate. Incidence level varied between, 3.5 – 15.0 per cent during the winter while it was detected 0.5-3.5 per cent in summer (Table 5).

Zygomycota contributed 4 species belonging to 3 genera during winter while only 3 species, each representing single genus confined to seeds in summer. Significant level of fungal incidence has been recorded during winter. Higher incidence, 83.0% has been detected in February, 83.0 while it was 71.5% and 66.5% for the month of March and January respectively. During winter, fungal incidence varied between 49.5 – 83.0 per cent while it was recorded 27-35 per cent in summer, exhibited fungal incidence has been recorded double in winter than in summer (Table 5).

Ascomycota had higher count of 14 species and 6 genera, with cumulative 94.5 per cent incidence in a month of March followed by January and February contributing 91.0

and 79.5 per cent incidence respectively. An isolate count of 6-8 species and 3-4 genera has been confined to the seeds in summer against 6-14 species and 5-6 genera in winter. Winter dominates with heavy infestation ranged between 40.5 – 94.5 per cent while it was recorded 48.0%-81.8 per cent during summer (Table 5).

Similar trend was observed for Deuteromycota, contributing higher count of 16 species and 7 genera to a month of January with significant level of infestation, varied between 49.0 to 133.0 per cent in winter against 10-12 isolates representing 6 genera with cumulative 20.5 to 31.5 per cent incidence in summer. Greater cumulative 133.0 per cent incidence has been confined to seeds in January followed by December (107.8%), February (89.5%) and March (76.5%). Basidiomycota did not contribute any isolate throughout the incubation period both in winter as well as in the summer (Table 5).

Fungal spore concentration on seed surface varies with seasonal climate. Prevalence of higher count of isolates, contributing greater incidence during winter elicited response to climate of this season. Greater count of isolates comprising of 35 species and 17 genera was detected to a month of January followed by March, contributing 33 species and 18 genera while December and February had 28 and 26 species fall under 16 genera respectively (Table 6).

Storage period of October and November had moderate counts (Table 6). A population of an isolates was observed decline during summer. Similar trend has been reported for fungal infestation. Heavy infestation was confined in middle period of winter season, estimated maximum, 305.5 per cent to a month of January followed by February (261.0%), December (252. 5%) and March (249.5%) while moderate, total 143.5 and 182.5 per cent in October and November respectively. It was observed decline, in summer to the months of June (121.0%); April (115.5); July (117.5) & August (117.0) ; March (152.75%) and detected low in May (107.0%). It was again enhanced to 143.5 per cent in an initiation period (October) of the winter season (Table 6). Seed viability for initial storage period of five months did exhibit little or negligible change, representing 82-85 per cent seed germination. Thereafter, it began to decline from September and finally reduced to 28 per cent in March (Table 6).

(IX) Phytochemical analysis of oil of freshly collected mixed seed samples:

Mustard (*Brassica campestris* L) seeds yield edible oil which is used for cooking and medicinal purposes. The oil of the seeds of freshly collected seed samples from various sub-divisions of Nagpur District was extracted by Soxhlet method (Straccia et al., 2012) and the modified cold percolation method (Kalia et al., 2002).

The seeds of mustard are reddish brown, about 1 mm in diameter and spherical in shape. The seed yield 38.91 per cent edible oil. The colour of oil is pale yellow with saponification value 160-170. The oil contains major fatty acid such as oleic acid; linoleic acid; eicosanoid acid; erucic acid. It also contains glycerides.

Table 5: Month wise seasonal distribution of seed mycoflora of *Brassica campestris* L obtained from various geographical locations of divisions of Nagpur District

S. N	Name of fungal isolates	Frequency (%) of fungal incidence												Total frequency
		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
A	**Oomycota**	**2.0 (0.09)**	**2.5 (0.12)**	**1.0 (0.05)**	**1.0 (0.05)**	**0.5 (0.02)**	**3.5 (0.17)**	**3.5 (0.17)**	**6.5 (3.11)**	**9.5 (0.45)**	**15.0 (0.72)**	**9.0 (0.43)**	**7.0 (0.34)**	**61.0 (2.92)**
1.	*Phytophthora infestans* de Bary	2.0 (0.09)[1]	2.5 (0.12)	1.0 (0.05)	1.0 (0.05)	0.5 (0.02)	3.5 (0.17)	3.5 (0.17)	6.5 (3.11)	9.5 (0.45)	12.0 (0.57)	5.5 (0.26)	4.5 (0.21)	52.0 (2.49)
2.	*Pythium aphanidermatum* (Edson) Fitzp..	-	-	-	-	-	-	-	-	-	3.0 (0.14)	3.5 (0.17)	2.5 (0.12)	9.0 (0.43)
B	**Zygomycota**	**35.0 (1.68)**	**35.5 (1.70)**	**30.0 (1.44)**	**27.0 (1.29)**	**32.5 (1.56)**	**34.5 (1.65)**	**49.5 (2.37)**	**52.5 (2.51)**	**58.5 (2.80)**	**66.5 (3.18)**	**83.0 (3.98)**	**71.5 (3.42)**	**576.0 (27.6)**
3.	*Absidia corymbifera* (Cohn) Sacc. & Trotter	16.0 (0.77)	18.5 (0.89)	17.0 (0.81)	14.0 (0.67)	13.0 (0.62	13.5 (0.65)	2.5 (0.12)	2.5 (0.12)	2.0 (0.09)	2.0 (0.09)	9.0 (0.43)	13.0 (0.62)	123.0 (5.89)
4	*Mucor pusillus* Lindt.	10.0 (0.48)	4.0 (0.19)	6.5 (0.31)	8.0 (0.38)	10.5 (0.50)	10.5 (0.50)	14.0 (0.67)	17.0 (0.81)	21.0 (1.01)	24.0 (1.15)	28.0 (1.34)	21.0 (1.01)	174.5 (8.36)
5	*Cunninghamella elegans* Lendner	-	-	-	-	-	-	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	6.0 (0.29)	2.5 (0.12)	3.5 (0.17)	21.50 (10.3)
6.	*Rhizopus nigricans Demelius*	-	-	-	-	-	-	9.5 (0.45)	7.5 (0.36)	6.0 (0.29)	5.5 (0.26)	9.5 (0.45)	4.5 (0.21)	42.5 (2.04)
7	*Rhizopus stolonifer* Eh. Ex.Rr.)Lind.	9.0 (0.43)	13.0 (0.62)	6.5 (0.31)	5.0 (0.24)	9.0 (0.43)	10.5 (0.50)	20.0 (0.96)	23.0 (1.10)	26.0 (1.25)	29.0 (1.39)	34.0 (1.63)	29.5 (1.41)	214.5 (10.3)
C	**Ascomycota**	**48.5 (2.32)**	**48.0 (2.30)**	**58.5 (2.80)**	**66.0 (3.16)**	**54.0 (2.59)**	**48.5 (2.32)**	**40.5 (1.94)**	**59.0 (2.83)**	**77.0 (3.69)**	**91.0 (4.36)**	**79.5 (3.81)**	**94.5 (4.53)**	**765.0 (36.6)**
8	*Aspergillus amstelodomi (Mang) Thom & Church*	-	-	-	-	-	-	-	-	-	3.0 (0.14)	4.5 (0.22)	6.5 (0.31)	14.0 (0.67)
9	*A. flavus* Link.	5.0 (0.24)	4.5 (0.21)	6.0 (0.29)	9.5 (0.45)	12.0 (0.57)	14.0 (0.67)	14.0 (0.67)	17.0 (0.81)	21.0 (1.01)	22.5 (1.08)	17.0 (0.81)	8.0 (0.38)	150.5 (7.21)
10	*A. fumigatus* Fres.	18.5 (0.89)	17.0 (0.81)	20.0 (0.96)	21.5 (1.03)	16.0 (0.77)	15.0 (0.72)	11.0 (0.53)	14.0 (0.67)	13.5 (0.65)	12.0 (0.57)	18.5 (0.89)	21.0 (1.01)	198.0 (9.48)
11	*A. nidulans* (Eidam) Winter	-	-	-	2.5 (0.12)	-	-	-	-	-	1.5 (0.07)	-	3.5 (0.17)	7.5 (0.36)
12	*A. niger* Van Tieghen	5.5 (0.26)	3.0 (0.14)	3.5 (0.17)	6.0 (0.29)	5.0 (0.24)	2.5 (0.12)	3.5 (0.17)	6.5 (0.31)	12.0 (0.57)	18.0 (0.86)	16.0 (0.77)	8.0 (0.38)	89.5 (4.29)
13	*A. ochracious* Wihelm	6.0 (0.29)	4.5 (0.21)	5.0 (0.24)	3.5 (0.17)	1.5 (0.07)	-	-	-	-	-	1.5 (0.07)	-	22.0 (1.05)
14	*A. sulphureus* (Fres.)Thom & Church	-	-	-	-	2.5 (0.12)	-	-	-	-	2.0 (0.09)	-	4.5 (0.21)	9.0 (0.43)
15	*A. terreus* Thom.	3.5 (0.17)	8.0 (0.38)	7.5 (0.36)	5.5 (0.26)	3.5 (0.17)	-	-	-	-	-	-	3.5 (0.17)	31.5 (1.51)
16	*Aspergillus versicolor* (Vuill.) Tiraboschi	-	-	-	-	-	-	-	-		2.5 (0.12)	-	2.5 (0.12)	5.0 (0.24)
17	*Botrytis cinera* Pets.	-	-	-	-	-	-	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	4.0 (0.19)	-	2.5 (0.12)	16.0 (0.77)
18	*Chaetomium glabosum Kunze & Schm*	7.0 (0.34)	9.0 (0.43)	12.0 (0.57)	14.0 (0.67)	9.5 (0.45)	10.5 (0.50)	-	-	-	-	-	8.5 (0.41)	70.5 (3.38)
19	*Cladosporium fulvum* Cooke.	-	-	-	-	-	2.5 (0.12)	3.0 (0.14)	7.0 (0.34)	6.5 (0.31)	3.0 (0.14)	4.5 (0.21)	8.5 (0.41)	35.0 (1.68)
20	*Penicillium oxalicum* Currie & Thom.	3.0 (0.14)	2.0 (0.09)	4.5 (0.21)	3.5 (0.17)	4.0 (0.19)	4.0 (0.19)	5.5 (0.26)	7.5 (0.36)	10.5 ((0.50)	9.0 (0.43)	13.0 (0.62)	10.0 (0.48)	76.5 (3.66)
21	*Penicillium pallidum* (Cruick & Shank) Pitt.	-	-	-	-	-	-	-	-	3.5 (0.17)	4.5 (0.21)	-	-	8.0 (0.38)
22	*Penicillium digitatum* (Pers. Ex. Fr.) Sacc.	-	-	-	-	-	-	-	-	-	1.5 (0.07)	-	2.5 (0.12)	4.0 (0.19)
23	*Phoma glomerata* (Corda) Wr. & Hocha	-	-	-	-	-	-	-	4.5 (0.21)	6.5 (0.31)	7.5 (0.36)	4.5 (0.21)	5.0 (0.24)	28.0 (1.34)
D	**Basidiomycota**	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**Deuteromycota**	**30.0 (1.44)**	**30.0 (1.44)**	**31.5 (1.51)**	**23.5 (1.13)**	**30.0 (1.44)**	**20.5 (0.98)**	**49.5 (2.37)**	**64.5 (3.09)**	**107.5 (5.15)**	**133.0 (6.37)**	**89.5 (4.29)**	**76.5 (3.66)**	**686.0 (32.9)**
24	*Alternaria alternata* (Fr.) Keissler	4.0 (0.19)	3.5 (0.17)	3.5 (0.17)	2.5 (0.12)	2.5 (0.12)	1.5 (0.07)	2.5 (0.12)	6.5 (0.31)	10.5 (0.50)	12.0 (0.57)	6.5 (0.31)	4.5 (0.21)	60.0 (2.87)
25	*Alternaria solani (E & M) Jones & Grout*	3.5 (0.17)	2.5 (0.12)	2.0 (0.09)	3.0 (0.14)	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	5.5 (0.26)	8.5 (0.41	14.0 (0.67)	7.5 (0.36)	5.5 (0.26)	61.5 (2.95)
26	*Alternaria brassicicola* (Schweinitz, Wiltshire)	3.5 (0.17)	4.0 (0.19)	3.5 (0.17)	3.5 (0.17)	3.0 (0.14)	1.0 (0.05)	3.5 (0.17)	4.0 (0.19)	6.5 (0.31)	12.5 (0.60)	4.5 (0.21)	4.0 (0.19)	53.5 (2.56)
27	*Alternaria brassicae*	5.5 (0.26)	0.5 (0.02)	4.5 (0.21)	3.0 (0.14)	3.5 (0.17)	1.5 (0.07)	3.0 (0.14)	4.5 (0.21)	7.5 (0.36)	14.5 (0.69)	5.5 (0.26)	4.5 (0.21)	62.5 (2.99)
28	*Curvularia clavata* Jain	-	-	-	-	-	-	8.5 (0.41	-	7.5 (0.36)	6.5 (0.31)	4.0 (0.19)	3.0 (0.14)	29.5 (1.41)

29	*Curvularia ovoidea* (H & W) Munt.	-	-	-	-	-	-	-	5.5 (0.26)	7.0 (0.34)	-	5.0 (0.24)	3.5 (0.17)	21.0 (1.01)
30	*Curvularia lunata* (Wakker) Boedijn	1.5 (0.07)	3.0 (0.14)	3.0 (0.14)	2.5 (0.12)	7.0 (0.34)	5.0 (0.24)	7.5 (0.36)	8.0 (0.38)	10.0 (0.48)	9.0 (0.43)	7.5 (0.36)	7.0 (0.34)	7.0 (0.34)
31	*Curvularia intermedia* (Tracy & Barle) Boedjin	-	-	-	-	-	-	8.5 (0.41)	-	7.5 (0.36)	6.5 (0.31)	-	-	22.5 (1.08)
32	*Fusarium miniliformae* Sheldom	3.0 (0.14)	0.5 (0.02)	1.5 (0.07)	1.5 (1.07)	2.5 (0.12)	2.0 (0.09)	4.0 (0.19)	4.5 (0.21)	5.5 (0.26)	9.5 (0.45)	7.0 (0.34)	6.0 (0.29)	47.5 (2.27)
33	*Fusarium oxysporum* Schlecht	-	-	0.5 (0.02)	-	-	2.0 (0.09)	-	4.5 (0.21)	2.5 (0.12)	7.5 (0.36)	6.5 (0.31)	2.5 (0.12)	26.0 (1.25)
34	*Fusarium semitectum* Berk & Rav.	-	-	-	-	-	-	-	-	-	-	5.5 (0.26)	6.5 (0.31)	12.0 (0.57)
35	*Fusarium solani* (Mert.) APP. & Wollenw	3.5 (0.17)	1.5 (0.07)	3.5 (0.17)	5.0 (0.24)	2.0 (0.09)	2.5 (0.12)	3.5 (0.17)	5.0 (0.24)	7.5 (0.36)	9.5 (0.45)	7.5 (0.36)	4.5 (0.21)	51.0 (2.44)
36	*Helminthosporium* spiciferum (Bain.) Nicol	-	-	-	-	-	-	-	-	4.5 (0.21)	7.0 (0.34)	4.0 (0.19)	4.5 (0.21)	20.0 (0.96)
37	*Helminthosporium tetramera* G & A	-	-	-	-	-	-	-	8.5 (0.41	7.5 (0.36)	13.5 (0.65)	5.0 (0.24)	6.5 (0.31)	41.0 (1.96)
38	*Nigrospora sp.*	1.5 (0.07)	1.5 (0.07)	1.5 (0.07)	2.0 (0.09)	2.5 (0.12)	2.0 (0.09)	2.5 (0.12)	3.0 (0.14)	6.5 (0.31)	4.0 (0.19)	3.5 (0.17)	3.0 (0.14)	81.5 (3.90)
39	*Paecilomyces variotii* Bainier	2.5 (0.12)	7.5 (0.36)	6.5 (0.31)	3.5 (0.17)	2.5 (0.12)	-	-	-	-	-	-	2.5 (0.12)	35.0 (1.68)
40	*Rhizoctonia solani* Kuhn.	1.5 (0.07)	1.0 (0.05)	1.5 (0.07)	1.5 (0.07)	1.0 (0.05)	2.5 (0.12)	2.5 (0.12)	5.0 (0.24)	8.5 (0.41)	7.0 (0.34)	6.0 (0.29)	3.5 (0.17)	41.5 (1.99)
41	*Trichothecium roseum* Link	-	-	-	-	-	-	-	-	-	-	4.0 (0.19)	5.0 (0.24)	9.0 (0.43)
	Total frequency	**115.5 (5.53)**	**116.0 (5.55)**	**121.0 (5.80)**	**117.5 (5.63)**	**117.0 (5.60)**	**107.0 (5.12)**	**143.0 (6.85)**	**182.5 (8.74)**	**252.5 (12.1)**	**305.5 (14.6)**	**261.0 (12.5)**	**249.5 (11.9)**	**2088**
	1. Values in parenthesis indicates per cent fungal incidence over total frequency of incidence.													

Table 6: Month wise count of seed borne fungal pathogens on seeds of *Brassica campestris* L obtained from various geographical locations of sub-divisions of Nagpur District

S N	Division	Parameter	Seed borne fungal isolates and their frequency(%) of incidence												Total
			Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
A	**Oomycota**	Genera	1	1	1	1	1	1	1	1	1	2	2	2	
		Species	1	1	1	1	1	1	1	1	1	2	2	2	
		Percent incidence	2.0 (0.09)[1]	2.5 (0.12)	1.0 (0.05)	1.0 (0.05)	0.5 (0.02)	3.5 (0.17)	3.5 (0.17)	6.5 (3.11)	9.5 (0.45)	15.0 (0.72)	9.0 (0.43)	7.0 (0.34)	61.0 (2.92)
B	**Zygomycota**	Genera	3	3	3	3	3	3	3	3	3	3	3	3	
		Species	3	3	3	3	3	3	4	4	4	4	4	4	
		Percent incidence	35.0 (1.68)	35.5 (1.70)	30.0 (1.44)	27.0 (1.29)	32.5 (1.56)	34.5 (1.65)	49.5 (2.37)	52.5 (2.51)	58.5 (2.80)	66.5 (3.18)	83.0 (3.98)	71.5 (3.42)	576.0 (27.6)
C	**Ascomycota**	Genera	3	3	3	3	3	4	4	5	5	5	4	6	
		Species	7	7	7	8	8	6	6	7	8	13	8	14	
		Percent incidence	48.5 (2.32)	48.0 (2.30)	58.5 (2.80)	66.0 (3.16)	54.0 (2.59)	48.5 (2.32)	40.5 (1.94)	59.0 (2.83)	77.0 (3.69)	91.0 (4.36)	79.5 (3.81)	94.5 (4.53)	765.0 (36.6)
D	**Basidiomycota**	Genera	-	-	-	-	-	-	-	-	-	-	-	-	-
		Species	-	-	-	-	-	-	-	-	-	-	-	-	-
		Percent incidence	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**Deuteromycota**	Genera	6	6	6	6	6	6	6	7	7	7	7	7	
		Species	10	10	11	10	10	11	12	15	13	16	14	13	
		Percent incidence	30.0 (1.44)	30.0 (1.44)	31.5 (1.51)	23.5 (1.13)	30.0 (1.44)	20.5 (0.98)	49.5 (2.37)	64.5 (3.09)	107.5 (5.15)	133.0 (6.37)	89.5 (4.29)	76.5 (3.66)	686.0 (32.9)
	Total genera		13	13	13	13	13	14	14	16	16	17	16	18	
	Total species		21	21	22	22	22	21	23	27	26	35	28	33	
	Cumulative frequency		115.5 (5.53)	116.0 (5.55)	121.0 (5.80)	117.5 (5.63)	117.0 (5.60)	107.0 (5.12)	143.0 (6.85)	182.5 (8.74)	252.5 (12.1)	305.5 (14.6)	261.0 (12.5)	249.5 (11.9)	2088
	Per cent seed viability		85.0	85.0	84.0	82.0	82.0	73.0	61.0	52.0	46.0	37.0	33.0	28.0	
	1. Values in parenthesis indicates percent fungal incidence over total frequency of incidence.														

Table 7: Bio-deteriorative changes in percent seed oil content and values of saponification, iodine, acid and peroxidase

S. No.	Parameters	Initial value	Values after one year of storage	Per change over initial value
1.	Seed oil content (% wt/wt)	38.9	27.6	-29.1%
2.	Saponification value (mgKOH/g)	170	145	-14.7%
3.	Iodine value (g I_2/100g)	97	54	-44.3%
4.	Acid value (mgKOH/g)	1.89	1.76	-6.9%
5.	Peroxide value (meg/Kg)	3.12	3.34	+7.1%

(a) Changes in oil content :

The data presented in Table 7 revealed that the freshly collected seed samples contain 38.9 per cent seed oil. The oil content of the one year stored Brassica *campestris* L. seeds was declined to 27.6% indicating 29.1% reduction due to biodeterioration of seed oil by fungal flora associated with seeds during storage.

(b) Changes in saponification value:

The saponification value of freshly collected seed samples was reported to be 170 mgKOH/g and it was reduced to 145 mgKOH/g in stored seed samples of Brassica *campestris* L. Seed borne fungal flora declined saponification value by 14.7% during storage of seeds (Table 7).

(c) Iodine value:

The freshly collected seed samples had 97 g I_2/100g iodine value and it was declined by 44.3% during biodeterioration of seed mycoflora in storage (Table 7).

(d) Acid value:

The acid value of freshly collected seed samples Brassica *campestris* L was reported to be 1.89 mgKOH/g and it was reduced to 1.76 mgKOH/g in stored seed samples exhibiting declining in acid value by 6.9% during storage of seeds (Table 7).

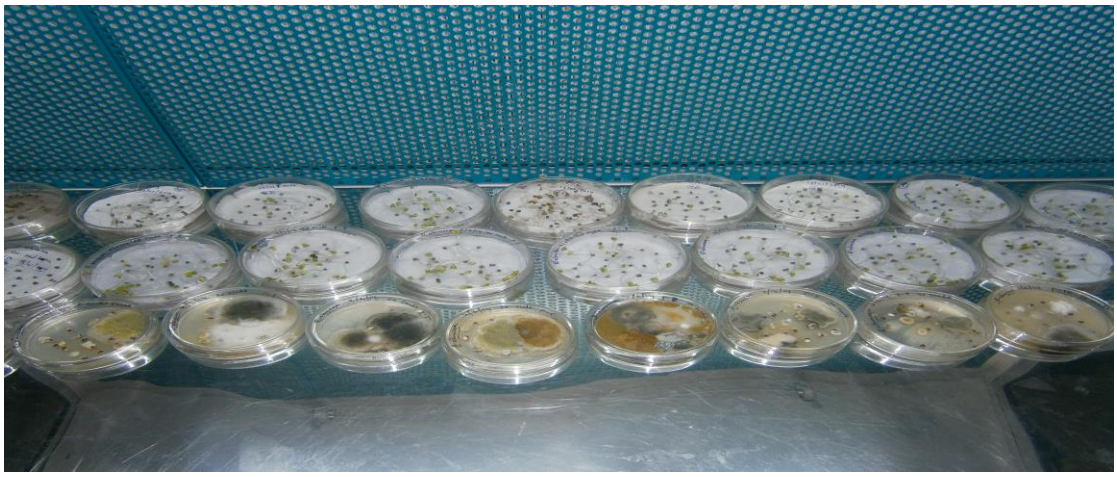
(e) Peroxide value:

The freshly collected seed samples had 3.12 peroxide value. In stored seed this value was increased to 3.34 meg/Kg indicating gaining by 7.1% during storage (Table 7).

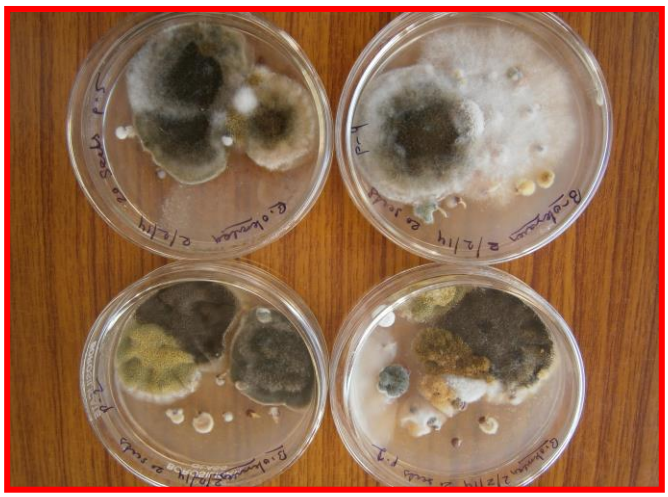
PLATE - I

Healthy seeds of *Brassica campestris* L.

Infested seeds of *Brassica campestris* L.



Isolation of seed mycoflora of Brassica campestris L.
by standard blotter paper and agar plate method



Seed borne mycoflora on agar plates

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).

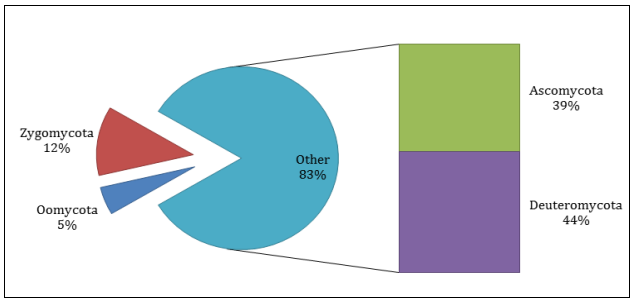

Fig. 1: Division wise distribution of fungal isolates on composite seed samples of *Brassica compestris* L.

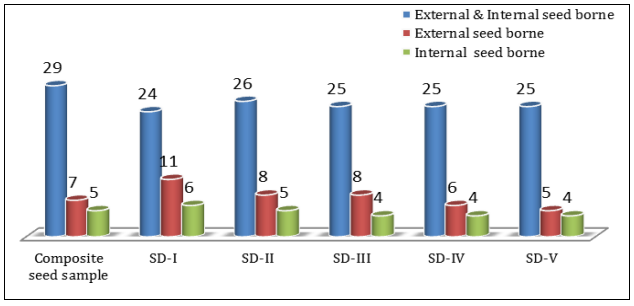

Fig. 2: Distribution of fungal isolates on seed samples of *Brassica compestris* L. form various geographical location understudy.

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.

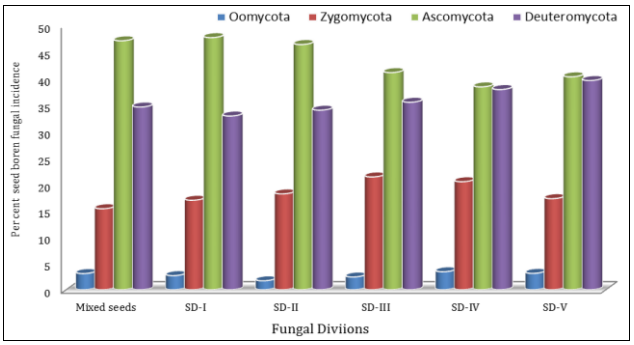

Fig. 3: Division wise percent contribution on seeds *Brassica compestris* L. in storage from area understudy.

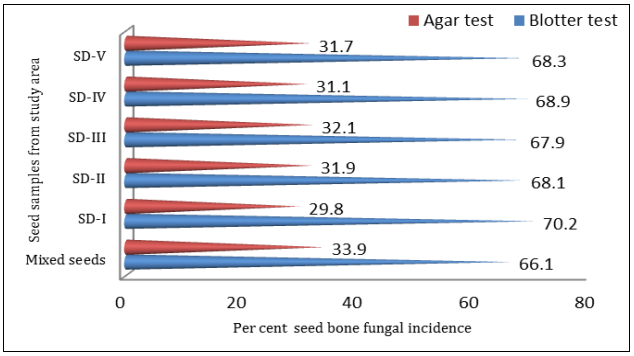

Fig. 4: Percent incidence of fungal isolates on *Brassica Compestris* L. Seeds confined to standard health tests.

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.

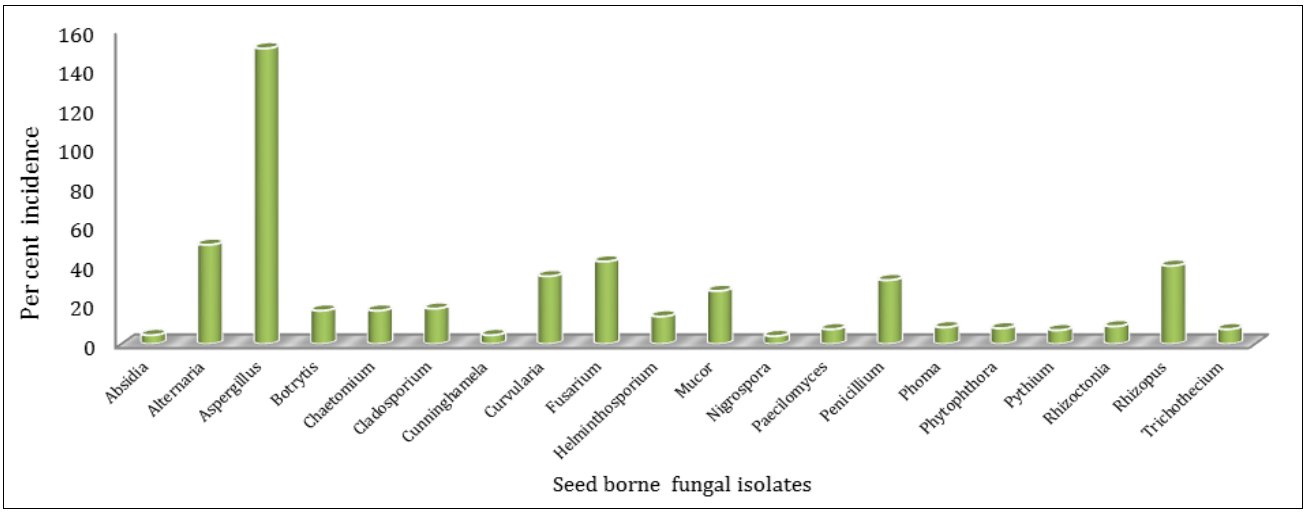

Fig. 5: Percent incidence of microfungal genera on stored comosite seed samples of Brassica compestris L.

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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RESEARCH ARTICLE

Diversity and seasonal variation of seed borne fungal flora of *Brassica campestris* L. in storage

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ABSTRACT

Seasonal variation pattern, vegetation diversity, pre- and post-harvest climate determine periodic distribution and concentration of microfungal propagules on seed coats in any geographic area.. Prevalence of enormous, high concentrations of diverse microfungal propagules on stored seeds of *Brassica campestris* L., widely considered as some pathogenic, phytotoxic and few carcinogenic, have been reported in a set of storage climate for a year employing standard blotter paper technique, screening four hundred seeds monthly from composite seed sample of diverse geographic location of Nagpur district comprising of five sub-divisions and 14 talukas. It has been proposed for an establishment of seasonal variation patterns as well as its possible correlation with climatological factors. Mycological analysis revealed that out of a total fungal population of forty one isolates categorize under twenty genera; nine species of *Aspergillus* are among the most predominant components of mesophilic seed mycoflora. Deuteromycota contributed greater count of isolates followed by *Ascomycota* while Oomycota had least count. Winter is dominated by nearly two-third of a total fungal incidence over one-third in summer. Heavy infestation was confined to a month of January followed by February, December and March and it gradually declined to lowest in May of summer season. It was again enhanced to an initiation period (October) of the winter indicated storage period of winter season was seemed to be supportive for fungal infestation, rapid proliferation and sporulation. Level of infestation for mesophilic isolates declined while it was enhanced for thermo-tolerant in summer. A count of seed borne fungal flora and their infestation level varied considerably throughout a year of storage. Report on seasonal variation provides a basis for estimating functional role of seed mycoflora in an ecosystem.

Keywords: Seed borne mycoflora, infestation, incidence, frequency, *Brassica campestris* L.

INTRODUCTION

Environmental climate of ambient humidity, moderate temperature, cloudy weather, seed nutritive content and high level of seed moisture content of post-harvest crop are proved supportive measures for seed contamination with diverse group of fungal micro-propagules (Ramesh *et al*, 2013). Majority of parasitic mycoflora attack matured pre-harvested seeds, penetrate deep contaminating internal seed coats, tissues of embryo endosperm while others contaminate external seed surface in

storage in response to ambient storage environment (Bhajbhuje, 2014). Planting infected seeds leads to a widespread distribution of diseases within crop, and an increased count of initial infection sites from which disease can spread. High rate of seed-to-seedling transmission of pathogens create alarming situation, even a small percentage of infected seed can result in significant seedling infection in a field (Saskatchewan, 2013). Moreover, the infested seeds are considered highly effective means for transporting diverse pathogenic fungal micro-propagules over long distance (Archana and Prakash, 2013). Literature review suggest for international spread of diseases as a result of importation of seeds that were infested with viable propagules of plant pathogens and their economic impact has increased in recent years with concern to many kinds of crop worldwide (Lew-Smith, 2013). The fungal flora associated with seed coats as surface contaminants elicits response causing seed abortion, seed rot, seed necrosis, loss of seed viability as well as seedling damage resulting disease development at later stages of plant growth by systemic or local infection (Gupta *et al*, 2012) thereby adversely reducing yield potential to the extent of 20-30% (Lew-Smith, 2013)

Mustard (*Brassica campestris* L.) of family Brassicaceae, is native to Europe but has become cultivated extensively on every part of the globe for its oil rich seeds as the oil from seeds are used quite intuitively for cooking, burning and curing many bodily disorders including skin diseases and thereby keep health in perfect state of fitness and lived a long life. From the prehistoric time, in response to medicinal property, a 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). Indian human population particularly of Punjab, U.P., Bihar, and Assam, utilizes mustard seed-oil for cooking as an alternative source to groundnut oil while in remaining areas, it is added to variety of achar as preservative due to its strong antimicrobial property 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 on the globe contributing around one-third of the World's annual harvest after China and Canada. It accounts for nearly one-third of the oil produced in India, making it the country's key edible oilseed crop. Due to a 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).

Variable distribution of diverse seed borne fungal flora differs from region to region attributed to diversity in vegetation, climatic fluctuation and storage environment (Bhajbhuje, 2014). The literature survey revealed that several researchers have made attempt on incidence of seed borne-fungi of several species of *Brassica* under storage environment from various geographical locations on the globe with concern to seed spoilage in a set of environment (Siddiqui, 2013; Ghugal and Thakre, 2014; Madavi and Bhajbhuje, 2014). A report on survey of seasonal variation of seed borne pathogens in fluctuating climate of Nagpur district might be of some significance in establishing correlation between fungal sensitization and bio-deteriorative prevention of post-harvest crops. Since biodeterioration of post-harvest crop in storage attributed to seasonal diversity in fungal flora is a common problem in Nagpur region, has not so far been investigated, it seemed to be worthwhile to undertake a more comprehensive & systematic study to report seasonal variation of seed borne fungal flora of *Brassica campestris* L. originating from diverse geographic locations of Nagpur district in set of storage environment.

MATERIAL AND METHODS:

(a) Selection of plant material:

Mustard (*Brassica campestris* L.) has been selected as an experimental material as its seed is rich source of oil content with high erucic acid which is used for cooking and has potent medicinal properties.

(b) Collection of seed samples:

During survey, a total of twenty seed samples of post-harvest crop has been collected in polygene bags from diverse group of cultivators and retailors of 14 taluka belongs to five sub-divisions of Nagpur district, brought to laboratory and immediately after mixing, they were transferred to small cloth bags and maintained under normal laboratory environment for a period of one year (April 2013 to March. 2014).

(c) Monthly check up of seed health under storage:

At the end of periodic interval for a month, isolation of seed borne fungal flora was made from randomly selected 400 seeds of the stored seed samples employing standard blotter paper test (ISTA, 2013). Twenty five seeds without pretreatment were laid down aseptically under Laminar flow on three layered sterilized moistened blotter papers in sterilized petri-dishes and incubated for seven days in B.O.D incubator at 25±1°C under alternating cycles of 12 hours light and darkness. The moisture content of blotter paper

containing seeds had been maintained by addition of sterile distilled water when required. After incubation, these plates containing seeds were examined directly for appearance of fungal growth under stereoscopic microscope.

(d) Identification of seed borne fungal pathogens: Identification up to species level was done on the basis of micro- & macro morphology, reverse as well as surface colony colour, sporulation type, spores diversity, fruiting bodies and finally authenticated by authority. A count of fungal isolates and their infection levels have been recorded as a percentage of infected seeds in a sample following a technique reported earlier (CMI, 2010). Purified fungal isolates were propagated and maintained on Czapek's Dox agar nutrient medium in sterile slants.

RESULTS AND DISCUSSION

The seeds play crucial role in crop productivity on the globe and are vulnerable to attack by diverse group of fungal pathogens and spread of diseases, in turns adversely affect the global agricultural economy (Saskatchewan, 2013; Bhajbhuje, 2013). It has been proposed for an establishment of seasonal variation patterns as well as its possible correlation with climatological factors (Chanu and Chhetry, 2012). The viable microfungal propagules survive as a single unit, spores rarely as hyphal fragments, conidiophores, associated with seed surface and their distribution differs with respect to count, type, time, weather, and geography may attributed to variation in surrounding climate, seasonal fluctuation, changing vegetation diversity and storage condition (Stephan. 2013; Bhajbhuje, 2014). Seasonal fluctuating climate including temperature and moisture content determines a periodic distribution of fungal flora on various components of seeds and their propagules elicited varying response to fluctuating temperature and humidity implicated in deterioration of nutrients and seed spoilage and their high conc. of mycotoxins may cause health hazards (Jyoti and Malik, 2013). The chemical constituents of seeds are known for sporistatic, fungistatic and fungicidal activities possibly helps in variable reduction of level of fungal spore population in a set of storage environment. Raising of seedlings from infected seeds favours pathogen transmission of "*seed-to-seedling*" leads to a widespread distribution of diseases; emergence of diseased unhealthy fruit bearing shoots, premature defoliation, limits ability to anabolism and reduced yield potential of crop (Saskatchewan, 2013).

Mycological analysis of monthly isolated seed mycoflora of *Brassica campestris* L by standard blotter test for a period of April 2013 to March 2014 from area understudy of Nagpur district revealed an existence of a fungal population of 41 species belongs to 20 genera in varying degree of incidence. Deuteromycota are predominant with 18 species and 8 genera, exhibiting greater count of isolates followed by *Ascomycota* with 16 species and 6 genera. *Zygomycota* contributed 5 species and 4 genera while *Oomycota* had 2 species and 2 genera. *Basidiomycota* member did not appear on mustard seeds. *Aspergillus* dominated with greater count of 9 species. Four species each of *Alternaria*, *Curvularia, Fusarium*; three of *Penicillium*; two of *Helminthosporium* & *Rhizopus* and single of remainings have been confined as seed surface contaminants with diverse frequencies (Table 1).

The standard blotter test proved superior over others was used for periodic detection of seed surface contaminants to record seasonal diversity in storage climate. Considering diverse fungal count and variable infection level in monthly period of season, the fungal isolates may be categories into *four* types viz., prevailing (a) throughout a year; (b) in winter only; (c) in summer only and (d) rare without showing any specificity to a time of recurrence.

(a) *Fungal flora prevailing throughout a year*:

A fungal population of forty one diverse isolates was seemed to be prevailing as seed surface contaminants in storage. Of the total forty one isolates, a population of 17 species of 11 genera was encountered throughout a year of storage on seeds included *Absidia corymbefera*, *Alternaria alternata, A. brassicicola, A. brassicae A. solani, Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Fusarium miniliformae, F. solani Mucor pusillus, Nigrospora sp., Penicillium oxalicum, Phytophthora infestans, Rhizoctonia solani* and *Rhizopus stolonifer*. Among these, *Rhizopus stolonifer* was appeared to be predominant, exhibiting 214.5 per cent cumulative incidence followed by *Aspergillus fumigatus* (198.0%), *Mucor pusillus* (174.5%), *Aspergillus flavus* (150.5%); *Absidia corymbifera* (123.0%); *Aspergillus niger* (89.5%); *Nigrospora sp* (81.5%). Moderate level of cumulative incidence, ranged between 51-77% has been recorded for *Alternaria alternata, A. solani, A. brassicicola, A. brassicae*; *Curvularia lunata, Phytophthora infestans* and *Penicillium oxalicum* while two isolates, *Fusarium moniliformae* and *Rhizoctonia solani* had little incidence (Table 1).

Table 1 : Periodic distribution of seed mycoflora in storage of one year on seeds of *Brassica campestris* L from various geographical locations of divisions of Nagpur District

S. N	Name of fungal isolates	Frequency (%) of fungal incidence												Total frequency
		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
A	**Oomycota**	**2.0 (0.09)[1]**	**2.5 (0.12)**	**1.0 (0.05)**	**1.0 (0.05)**	**0.5 (0.02)**	**3.5 (0.17)**	**3.5 (0.17)**	**6.5 (3.11)**	**9.5 (0.45)**	**15.0 (0.72)**	**9.0 (0.43)**	**7.0 (0.34)**	**61.0 (2.92)**
1.	*Phytophthora infestans* de Bary	2.0 (0.09)[1]	2.5 (0.12)	1.0 (0.05)	1.0 (0.05)	0.5 (0.02)	3.5 (0.17)	3.5 (0.17)	6.5 (3.11)	9.5 (0.45)	12.0 (0.57)	5.5 (0.26)	4.5 (0.21)	52.0 (2.49)
2.	*Pythiumaphanidermatum* (Edson) Fitzp..	-	-	-	-	-	-	-	-	-	3.0 (0.14)	3.5 (0.17)	2.5 (0.12)	9.0 (0.43)
B	**Zygomycota**	**35.0 (1.68)**	**35.5 (1.70)**	**30.0 (1.44)**	**27.0 (1.29)**	**32.5 (1.56)**	**34.5 (1.65)**	**49.5 (2.37)**	**52.5 (2.51)**	**58.5 (2.80)**	**66.5 (3.18)**	**83.0 (3.98)**	**71.5 (3.42)**	**576.0 (27.6)**
3.	*Absidia corymbifera* (Cohn) Sacc. & Trotter	16.0 (0.77)	18.5 (0.89)	17.0 (0.81)	14.0 (0.67)	13.0 (0.62	13.5 (0.65)	2.5 (0.12)	2.5 (0.12)	2.0 (0.09)	2.0 (0.09)	9.0 (0.43)	13.0 (0.62)	123.0 (5.89)
4	*Mucor pusillus* Lindt.	10.0 (0.48)	4.0 (0.19)	6.5 (0.31)	8.0 (0.38)	10.5 (0.50)	10.5 (0.50)	14.0 (0.67)	17.0 (0.81)	21.0 (1.01)	24.0 (1.15)	28.0 (1.34)	21.0 (1.01)	174.5 (8.36)
5	*Cunninghamella elegans* Lendner	-	-	-	-	-	-	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	6.0 (0.29)	2.5 (0.12)	3.5 (0.17)	21.50 (10.3)
6.	*Rhizopus nigricans Demelius*	-	-	-	-	-	-	9.5 (0.45)	7.5 (0.36)	6.0 (0.29)	5.5 (0.26)	9.5 (0.45)	4.5 (0.21)	42.5 (2.04)
7	*Rhizopus stolonifer* Eh. Ex.Rr.)Lind.	9.0 (0.43)	13.0 (0.62)	6.5 (0.31)	5.0 (0.24)	9.0 (0.43)	10.5 (0.50)	20.0 (0.96)	23.0 (1.10)	26.0 (1.25)	29.0 (1.39)	34.0 (1.63)	29.5 (1.41)	214.5 (10.3)
C	**Ascomycota**	**48.5 (2.32)**	**48.0 (2.30)**	**58.5 (2.80)**	**66.0 (3.16)**	**54.0 (2.59)**	**48.5 (2.32)**	**40.5 (1.94)**	**59.0 (2.83)**	**77.0 (3.69)**	**91.0 (4.36)**	**79.5 (3.81)**	**94.5 (4.53)**	**765.0 (36.6)**
8	*Aspergillus amstelodomi (Mang) Thom & Church*	-	-	-	-	-	-	-	-	-	3.0 (0.14)	4.5 (0.22)	6.5 (0.31)	14.0 (0.67)
9	*A. flavus* Link.	5.0 (0.24)	4.5 (0.21)	6.0 (0.29)	9.5 (0.45)	12.0 (0.57)	14.0 (0.67)	14.0 (0.67)	17.0 (0.81)	21.0 (1.01)	22.5 (1.08)	17.0 (0.81)	8.0 (0.38)	150.5 (7.21)
10	*A. fumigatus* Fres.	18.5 (0.89)	17.0 (0.81)	20.0 (0.96)	21.5 (1.03)	16.0 (0.77)	15.0 (0.72)	11.0 (0.53)	14.0 (0.67)	13.5 (0.65)	12.0 (0.57)	18.5 (0.89)	21.0 (1.01)	198.0 (9.48)
11	*A. nidulans* (Eidam) Winter	-	-	-	2.5 (0.12)	-	-	-	-	-	1.5 (0.07)	-	3.5 (0.17)	7.5 (0.36)
12	*A. niger* Van Tieghen	5.5 (0.26)	3.0 (0.14)	3.5 (0.17)	6.0 (0.29)	5.0 (0.24)	2.5 (0.12)	3.5 (0.17)	6.5 (0.31)	12.0 (0.57)	18.0 (0.86)	16.0 (0.77)	8.0 (0.38)	89.5 (4.29)

Table 1: Continued..

S. N	Name of fungal isolates	Frequency (%) of fungal incidence												Total Frequency
		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
13	*A. ochracious* Wihelm	6.0 (0.29)	4.5 (0.21)	5.0 (0.24)	3.5 (0.17)	1.5 (0.07)	-	-	-	-	-	1.5 (0.07)	-	22.0 (1.05)
14	*A. sulphureus* (Fres.)Thom & Church	-	-	-	-	2.5 (0.12)	-	-	-	-	2.0 (0.09)	-	4.5 (0.21)	9.0 (0.43)
15	*A. terreus* Thom.	3.5 (0.17)	8.0 (0.38)	7.5 (0.36)	5.5 (0.26)	3.5 (0.17)	-	-	-	-	-	-	3.5 (0.17)	31.5 (1.51)
16	*Aspergillus versicolor* (Vuill.) Tiraboschi	-	-	-	-	- -	-	-	-		2.5 (0.12)	-	2.5 (0.12)	5.0 (0.24)
17	*Botrytis cinera* Pets.	-	-	-	-	-	-	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	4.0 (0.19)	-	2.5 (0.12)	16.0 (0.77)
18	*Chaetomium glabosum Kunze & Schm*	7.0 (0.34)	9.0 (0.43)	12.0 (0.57)	14.0 (0.67)	9.5 (0.45)	10.5 (0.50)	-	-	-	-	-	8.5 (0.41)	70.5 (3.38)
19	*Cladosporium fulvum* Cooke.	-	-	-	-	-	2.5 (0.12)	3.0 (0.14)	7.0 (0.34)	6.5 (0.31)	3.0 (0.14)	4.5 (0.21)	8.5 (0.41)	35.0 (1.68)
20	*Penicillium oxalicum* Currie & Thom.	3.0 (0.14)	2.0 (0.09)	4.5 (0.21)	3.5 (0.17)	4.0 (0.19)	4.0 (0.19)	5.5 (0.26)	7.5 (0.36)	10.5 ((0.50)	9.0 (0.43)	13.0 (0.62)	10.0 (0.48)	76.5 (3.66)
21	*Penicillium pallidum* (Cruick & Shank) Pitt.	-	-	-	-	-	-	-	-	3.5 (0.17)	4.5 (0.21)	-	-	8.0 (0.38)
22	*Penicillium digitatum* (Pers. Ex. Fr.) Sacc.	-	-	-	-	-	-	-	-	-	1.5 (0.07)	-	2.5 (0.12)	4.0 (0.19)
23	*Phoma glomerata* (Corda) Wr. & Hocha	-	-	-	-	-	-	-	4.5 (0.21)	6.5 (0.31)	7.5 (0.36)	4.5 (0.21)	5.0 (0.24)	28.0 (1.34)
D	**Basidiomycota**	-	-	-	-	-	-	-	-	-	-	-	-	**-**
E	**Deuteromycota**	**30.0 (1.44)**	**30.0 (1.44)**	**31.5 (1.51)**	**23.5 (1.13)**	**30.0 (1.44)**	**20.5 (0.98)**	**49.5 (2.37)**	**64.5 (3.09)**	**107.5 (5.15)**	**133.0 (6.37)**	**89.5 (4.29)**	**76.5 (3.66)**	**686.0 (32.9)**
24	*Alternaria alternata* (Fr.) Keissler	4.0 (0.19)	3.5 (0.17)	3.5 (0.17)	2.5 (0.12)	2.5 (0.12)	1.5 (0.07)	2.5 (0.12)	6.5 (0.31)	10.5 (0.50)	12.0 (0.57)	6.5 (0.31)	4.5 (0.21)	60.0 (2.87)
25	*Alternaria solani (E & M) Jones & Grout*	3.5 (0.17)	2.5 (0.12)	2.0 (0.09)	3.0 (0.14)	3.5 (0.17)	2.5 (0.12)	3.5 (0.17)	5.5 (0.26)	8.5 (0.41	14.0 (0.67)	7.5 (0.36)	5.5 (0.26)	61.5 (2.95)
26	*Alternaria brassicicola* (Schweinitz, Wiltshire)	3.5 (0.17)	4.0 (0.19)	3.5 (0.17)	3.5 (0.17)	3.0 (0.14)	1.0 (0.05)	3.5 (0.17)	4.0 (0.19)	6.5 (0.31)	12.5 (0.60)	4.5 (0.21)	4.0 (0.19)	53.5 (2.56)
27	*Alternaria brassicae*	5.5 (0.26)	0.5 (0.02)	4.5 (0.21)	3.0 (0.14)	3.5 (0.17)	1.5 (0.07)	3.0 (0.14)	4.5 (0.21)	7.5 (0.36)	14.5 (0.69)	5.5 (0.26)	4.5 (0.21)	62.5 (2.99)

Table 1: Continued…

S. N	Name of fungal isolates	Frequency (%) of fungal incidence												Total Frequency
		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
28	*Curvularia clavata* Jain	-	-	-	-	-	-	8.5 (0.41	-	7.5 (0.36)	6.5 (0.31)	4.0 (0.19)	3.0 (0.14)	29.5 (1.41)
29	*Curvularia ovoidea* (H & W) Munt.	-	-	-	-	-	-	-	5.5 (0.26)	7.0 (0.34)	-	5.0 (0.24)	3.5 (0.17)	21.0 (1.01)
30	*Curvularia lunata* (Wakker) Boedijn	1.5 (0.07)	3.0 (0.14)	3.0 (0.14)	2.5 (0.12)	7.0 (0.34)	5.0 (0.24)	7.5 (0.36)	8.0 (0.38)	10.0 (0.48)	9.0 (0.43)	7.5 (0.36)	7.0 (0.34)	7.0 (0.34)
31	*Curvularia intermedia* (Tracy & Barle) Boedjin	-	-	-	-	-	-	8.5 (0.41)	-	7.5 (0.36)	6.5 (0.31)	-	-	22.5 (1.08)
32	*Fusarium miniliformae* Sheldom	3.0 (0.14)	0.5 (0.02)	1.5 (0.07)	1.5 (1.07)	2.5 (0.12)	2.0 (0.09)	4.0 (0.19)	4.5 (0.21)	5.5 (0.26)	9.5 (0.45)	7.0 (0.34)	6.0 (0.29)	47.5 (2.27)
33	*Fusarium oxysporum* Schlecht	-	-	0.5 (0.02)	-	-	2.0 (0.09)	-	4.5 (0.21)	2.5 (0.12)	7.5 (0.36)	6.5 (0.31)	2.5 (0.12)	26.0 (1.25)
34	*Fusarium semitectum* Berk & Rav.	-	-	-	-	-	-	-	-	-	-	5.5 (0.26)	6.5 (0.31)	12.0 (0.57)
35	*Fusarium solani* (Mert.) APP. & Wollenw	3.5 (0.17)	1.5 (0.07)	3.5 (0.17)	5.0 (0.24)	2.0 (0.09)	2.5 (0.12)	3.5 (0.17)	5.0 (0.24)	7.5 (0.36)	9.5 (0.45)	7.5 (0.36)	4.5 (0.21)	51.0 (2.44)
36	*Helminthosporium* spiciferum (Bain.) Nicol	-	-	-	-	-	-	-	-	4.5 (0.21)	7.0 (0.34)	4.0 (0.19)	4.5 (0.21)	20.0 (0.96)
37	*Helminthosporium tetramera* G & A	-	-	-	-	-	-	-	8.5 (0.41	7.5 (0.36)	13.5 (0.65)	5.0 (0.24)	6.5 (0.31)	41.0 (1.96)
38	*Nigrospora sp.*	1.5 (0.07)	1.5 (0.07)	1.5 (0.07)	2.0 (0.09)	2.5 (0.12)	2.0 (0.09)	2.5 (0.12)	3.0 (0.14)	6.5 (0.31)	4.0 (0.19)	3.5 (0.17)	3.0 (0.14)	81.5 (3.90)
39	*Paecilomyces variotii* Bainier	2.5 (0.12)	7.5 (0.36)	6.5 (0.31)	3.5 (0.17)	2.5 (0.12)	-	-	-	-	-	-	2.5 (0.12)	35.0 (1.68)
40	*Rhizoctonia solani* Kuhn.	1.5 (0.07)	1.0 (0.05)	1.5 (0.07)	1.5 (0.07)	1.0 (0.05)	2.5 (0.12)	2.5 (0.12)	5.0 (0.24)	8.5 (0.41)	7.0 (0.34)	6.0 (0.29)	3.5 (0.17)	41.5 (1.99)
41	*Trichothecium roseum* Link	-	-	-	-	-	-	-	-	-	-	4.0 (0.19)	5.0 (0.24)	9.0 (0.43)
	Total frequency	**115.5 (5.53)**	**116.0 (5.55)**	**121.0 (5.80)**	**117.5 (5.63)**	**117.0 (5.60)**	**107.0 (5.12)**	**143.0 (6.85)**	**182.5 (8.74)**	**252.5 (12.1)**	**305.5 (14.6)**	**261.0 (12.5)**	**249.5 (11.9)**	**2088**

1. Values in parenthesis indicates per cent fungal incidence over total frequency of incidence.

(b) *Fungal flora prevailing in summer season only*:
Of the total isolates, a population of 4 species representing 4 genera namely *Aspergillus ochracious, A. terreus, Chaetomium glabosum,* and *Paecilomyces varioti* were remained prevailing throughout summer in varying degree of infestation. The isolate, *Chaetomium glabosum* was appeared to be predominant with cumulative 70.50 per cent incidence followed by *Paecilomyces variotii* (35.0%) and *Aspergillus terreus* (31.0%). Least level of incidence has been recorded for *Aspergillus ochracious* (Table 1).

(c) *Fungal flora prevailing in winter season only*:
Winter season (October to March) contributed a population comprising total of 18 species belonging to 12 genera, included *Aspergillus amstelodomi, A. versicolor, Botrytis cinera, Cladosporium fulvum, Cunninghamela elegans, Curvularia clavatus, C. ovoidea, C. intermedia, Fusarium oxysporum, F. semitectum, Helminthosporium specifectum, H. tetramera, Penicillium digitatum, P. pallidum, Phoma glomerata, Pythium aphanidermatum, Rhizopus nigricans* and *Trichothecium roseum* in varying level of infestation (Table 1). An isolate of Zygomycota, *Rhizopus nigricans* was seemed to be major components in the winter season, contributing cumulative 42.5 per cent incidence followed by *Helminthosporium tetramera* (41.0%) and *Cladosporium fulvum* (35.0%)., Moderate level of incidence varied between 20.0 to 29.5 per cent has been detected for *Cunninghamela elegans, Phoma glomerata, Curvularia clavatus, C. ovoides, C. intermedia, F. oxysporum* and *Helminthosporium tetramera* while it was recorded at low level for *Aspergillus amstelodomi* and *Botrytis cinera*. An isolate of Oomycota, *Pythium aphanidermatum* had least incidence (Table 1).

(d) *Fungal flora of rare occurrence without showing any specificity to a time of recurrence*.
A population of only two isolates belongs to single genus categorize to Ascomycota has been detected as seed surface contaminants, but did not exhibit any consistence of their recurrence in relation to changing and fluctuating climate. An isolate, *A. sulphureus* was appeared with higher level of infestation over *Aspergillus nidulans* (Table 1).

A population of all fungal seed borne isolates encountered to seeds for a year of storage in a set of environment has been categorized under various fungal divisions and their count as well as per cent incidence for individual division is presented in table 2.

Oomycota contributed maximum 2 genera and 2 species to a month of January to March. The greater level of cumulative incidence, 15 % has been detected in the month of January while other each month period had single isolate. Incidence level varied between, 3.5 – 15.0 per cent during the winter while it was detected 0.5-3.5 per cent in summer (Table 2).

Zygomycota contributed 4 species categorize to 3 genera in winter while only 3 species, each representing single genus confined to seeds in summer. Level of incidence varied between 50-83%has been recorded in winter while it was 27-35 per cent in summer, exhibited doubling of incidence in winter over summer. Higher incidence, 83.0% has been detected in February while it was 71.5% and 66.5% for March and January respectively.

A population of 14 fungal species and 6 genera of Ascomycota was seemed to be appear on seeds with cumulative 94.5 per cent incidence in March followed by January and February contributing 91.0 and 79.5 per cent incidence respectively. An isolate count of 6-8 species and 3-4 genera has been confined to seeds in summer against 6-14 species and 5-6 genera in winter. Winter dominates with heavy infestation ranged between 40.5 – 94.5 per cent while it was recorded 48.0%-81.8 per cent during summer (Table 2).

Similar trend was observed for Deuteromycota, contributing higher count of 16 species and 7 genera to a month of January with significant level of infestation, varied between 49.0 to 133.0 per cent in winter against 10-12 isolates representing 6 genera with 20.5 to 31.5 per cent incidence in summer. Greater cumulative incidence (133%) has been confined to seeds in January followed by December (107.8%), February (89.5%) and March (76.5%). Basidiomycota did not contribute any isolate throughout storage period (Table 2).

Fungal spore concentration on seed surface varies with seasonal climate. Prevalence of higher count of isolates, contributing greater incidence during winter elicited response to climate of this season. Greater count of isolates comprising of 35 species and 17 genera was detected to a month of January followed by March, contributing 33 species and 18 genera while December and February had 28 and 26 species fall under 16 genera respectively (Table 2). A population of an isolates and their level of infestation was observed decline during summer. Heavy infestation was confined in middle period of winter season, estimated maximum, 305.5 per cent to a month of January followed by February (261.0%), December (252. 5%) and March (249.5%) while moderate, total 143.5 and 182.5 per

cent in October and November respectively. It was observed decline, in summer to the months of June (121.0%); April (115.5); July (117.5) & August (117.0) ; March (152.75%) and detected low in May (107.0%). It was again enhanced to 143.5 per cent in an initiation period (October) of the winter season (Table 2).

The fungal flora associated with seeds includes a very large, diverse and heterogeneous group of microfungal contaminants that occupy position of great economic importance in agriculture, exhibiting an enormous diversity in life-history strategies Saskatchewan (2013). Post-harvest and stored seeds of mustard are highly infested by fungal contaminants (Ghugal and Thakre, 2014). The standard blotter technique is applied in routine seed health test to periodic/monthly isolation of seed mycoflora for longer storage period of a year as this technique is inevitable for getting a complete picture of seed surface contaminants (Ramesh *et al*, 2013; Bhajbhuje, 2014). A population of total 41 fungal species classified under 20 genera has been encountered on blotter paper from composite seed sample of *Brassica campestris* L. as surface contaminants for area of 14 taluka belongs to five sub-divisions of Nagpur district. Deuteromycota contributed with highest, 44% fungal count followed by Ascomycota (39%). Zygomycota contributed moderate while Oomycota *had* least count of isolates (Fig. 1).

Table 2: Periodic count of seed borne fungal pathogens and their infestation on seeds of *Brassica campestris* L from various geographical locations of sub-divisions of Nagpur District.

SN	Division	Parameter	Seed borne fungal isolates and their frequency(%) of incidence												Total
			Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
A	Oomycota	Genera	1	1	1	1	1	1	1	1	1	2	2	2	
		Species	1	1	1	1	1	1	1	1	1	2	2	2	
		Percent incidence	2.0 (0.09)[1]	2.5 (0.12)	1.0 (0.05)	1.0 (0.05)	0.5 (0.02)	3.5 (0.17)	3.5 (0.17)	6.5 (3.11)	9.5 (0.45)	15.0 (0.72)	9.0 (0.43)	7.0 (0.34)	61.0 (2.92)
B	Zygomycota	Genera	3	3	3	3	3	3	3	3	3	3	3	3	
		Species	3	3	3	3	3	3	4	4	4	4	4	4	
		Percent incidence	35.0 (1.68)	35.5 (1.70)	30.0 (1.44)	27.0 (1.29)	32.5 (1.56)	34.5 (1.65)	49.5 (2.37)	52.5 (2.51)	58.5 (2.80)	66.5 (3.18)	83.0 (3.98)	71.5 (3.42)	576.0 (27.6)
C	Ascomycota	Genera	3	3	3	3	3	4	4	5	5	5	4	6	
		Species	7	7	7	8	8	6	6	7	8	13	8	14	
		Percent incidence	48.5 (2.32)	48.0 (2.30)	58.5 (2.80)	66.0 (3.16)	54.0 (2.59)	48.5 (2.32)	40.5 (1.94)	59.0 (2.83)	77.0 (3.69)	91.0 (4.36)	79.5 (3.81)	94.5 (4.53)	765.0 (36.6)
D	Basidiomycota	Genera	-	-	-	-	-	-	-	-	-	-	-	-	-
		Species	-	-	-	-	-	-	-	-	-	-	-	-	-
		Percent incidence	-	-	-	-	-	-	-	-	-	-	-	-	-
E	Deuteromycota	Genera	6	6	6	6	6	6	6	7	7	7	7	7	
		Species	10	10	11	10	10	11	12	15	13	16	14	13	
		Percent incidence	30.0 (1.44)	30.0 (1.44)	31.5 (1.51)	23.5 (1.13)	30.0 (1.44)	20.5 (0.98)	49.5 (2.37)	64.5 (3.09)	107.5 (5.15)	133.0 (6.37)	89.5 (4.29)	76.5 (3.66)	686.0 (32.9)
Total genera			13	13	13	13	13	14	14	16	16	17	16	18	
Total species			21	21	22	22	22	21	23	27	26	35	28	33	
Cumulative frequency			115.5 (5.53)	116.0 (5.55)	121.0 (5.80)	117.5 (5.63)	117.0 (5.60)	107.0 (5.12)	143.0 (6.85)	182.5 (8.74)	252.5 (12.1)	305.5 (14.6)	261.0 (12.5)	249.5 (11.9)	2088
Per cent seed viability			85.0	85.0	84.0	82.0	82.0	73.0	61.0	52.0	46.0	37.0	33.0	28.0	

1. Values in parenthesis indicates percent fungal incidence over total frequency of incidence.

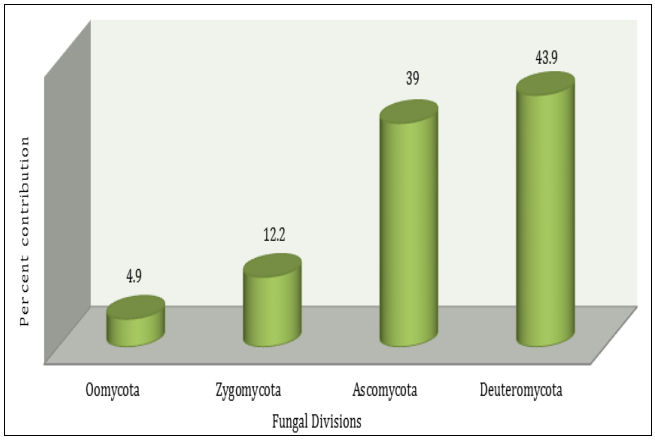


Fig. 1: Division wise distribution of seed borne fungal flora of *Brassica compestris* L.

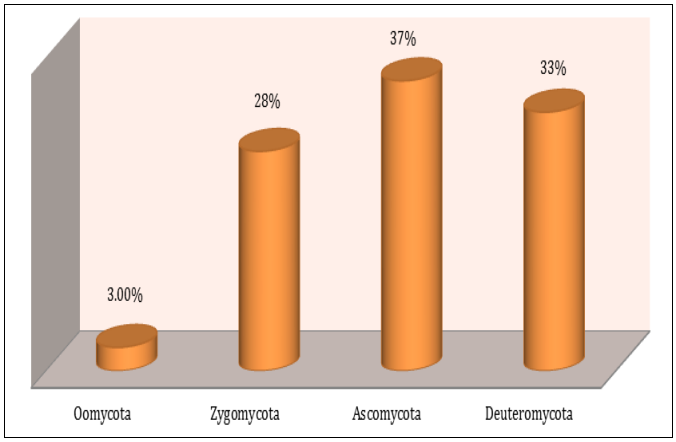


Fig. 2: Division wise distribution of incidence seed borne fungal flora of *Brassica compestris* L.

These results confirmed with earlier finding involving seed of *Coriandrum sativum* L (Ramesh *et al*., 2013); *Solanum melongena* L. (Bhajbhuje, 2013). Recently, Madavi and Bhajbhuje (2014) reported greater count of fungal flora categorized under Deuteromycota from stored seeds of *Brassica oleracea* var. botrytis by blotter paper test. Majority species confined to genus, *Aspergillus* contributing greatest percent incidence over total. This finding is in consistent with Ramesh *et al*., (2013) who reported huge population of *Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. parasiticus, A. sydowi, A. terreus* in addition to predominant occurrence of *Alternaria solani, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramera* and *Trichoderma viride* from coriander seeds. *Aspergillus, Alternaria, Penicillium, Cladosporium* and *Fusarium* were reported in higher frequency from seeds of *Brassica oleracia var botrytis* (Madavi and Bhajbhuje, 2014).

The composite seed sample of mustard was heavily infested with fungal flora. Of the cumulative per cent incidence recorded periodically in storage for a year, Ascomycota dominated with greatest, 36.6 per cent exhibiting higher cumulative incidence against Deuteromycota (32.9%) and *Zygomycota* (27.6%). *Oomycota* had little incidence (Fig.2). These results are in conformity with an earlier finding in brinjal (Bhajbhuje, 2013).

The fungal isolates belong to genera, *Aspergilli* and *Penicilli* of *Ascomycota* as well as *Alternaria, Curvularia, Fusarium, Helminthosporium* of Deuteromycota contributed as major components on *Brassica campestris* L. seed, represented a group of taxa of cosmopolitan distribution that can exploit virtually any organic substrate provided favourable storage environment of oxygen, temperature & relative humidity and accumulates toxic secondary metabolites (Saskatchewan, 2013). It was interesting to record that Deuteromycota had comparative higher count of isolates may possibly attributed to heavy infestation to seed coat by conidia and other propagules of *Hyphomycetes* during post-harvest period of crop. Ascomycota contributed higher per cent incidence may be due to rapid proliferation and sporulation of mycoflora on nutrient rich seed surface in response to ambient storage climate of slight low temperature, high humidity and seed moisture content (Jyoti and Malik, 2013; Bhajbhuje, 2014). Moreover, members of this group are known facultative parasites on crop plants as well as involved as saprophyte in deterioration of seeds and debris of plant & animal origin (Ismael, 2010). Under storage, in moist climate, seeds form an ideal organic substrate to storage fungal flora (Stephan, 2013). Deuteromycota members are mitosporic, sporulated rapidly and complete their life cycle asexually producing numerous resistant, long persisting thick walled conidia which may remain viable for longer period in adverse climatic storage environment (Ramesh *et al*., 2013). Even at warmer climate in summer and low humidity, the conidia *Alternaria, Cladosporium, Helminthosporium, Curvularia, and Paecilomyces* are remained prevailing abundantly (Niaz *et al*, 2011). *Basidiomycotina* members did not persist on stored seeds of mustard may be possibly attributed to mode of nutrition as majority of fungal organisms of this group are obligate parasites of other crop plants.

The climatological factors of geographical area understudy determine the seasonal and regional occurrence of plant diseases (Bhajbhuje, 2013). An existence of viable fungal propagules on seed surface depends on their ability to survive under extremes of dry condition of seed storage (Ramesh *et al*, 2013). Certain fungal flora survived and majority of them prevalent during winter and others during summer

(Swer, 2010; Bhajbhuje, 2014). An existence of fungal flora in certain season can be attributed to host, pathogen as well as seasonal fluctuation in climate. The hosts are available in a particular season and therefore a season is usually favourable for propagations of fungal pathogens. It is proved that temperature and humidity existing before or after a particular season may be unfavourable for survival of dormant propagules of pathogen (Nazim *et al*, 2013). An experiment has been designed for an establishment of seasonal variation pattern as well as the possible correlations with the climatological factors. To establish same, storage climate of laboratory and Nagpur city's (M.S.) highest and lowest temperature in degree Celsius and humidity in percentage have been taken into consideration for a storage period. There was no marked variation in the climate of laboratory and Nagpur city (Fig. 5). Periodic/monthly isolation of seed surface contaminants was made by usual blotter paper test under laboratory climate and their count as well as per cent incidence has been recorded (Table 1).

Results of present investigation revealed that fungal population of seed surface contaminants varied significantly in response to seed quality as substrate, fluctuating climatological conditions and storage period. Of the total population recorded during storage period for a year, the winter season is dominated by 44 per cent; nearly half of total population of isolates while 41 per cent isolates survived luxuriously throughout the storage period. Summer season had 10 per cent while population of 3 per cent isolates were prevailed rarely without exhibiting any specificity to a time of recurrence (Fig.3). Moreover, greatest fungal count was confined in the months of *January* followed by *December* and *February* while moderate count in *October* and *November*. Declining of fungal count began from the end of the winter and remained throughout the summer (Fig. 4). These results are confirmed with earlier finding (Nazim *et al*, 2013). Similar trend has been reported concerning fungal infestation to seed coat that was confined significant in the mid-period of the winter season. Heavy infestation was estimated to a storage period in *January* followed by *February* and *December* while it was reported moderate in *November and March*. Infestation level had declined with an initiation of summer, reached to minimum level between the months of *April* to *September* and again enhanced gradually at the onset of winter season (Fig. 5). Heavy fungal infestation to seeds of diverse crops in winter season at slightly low temperature and high humidity over summer has been reported by Swer *et al*, (2010); Ramesh *et al* (2013) and Bhajbhuje (2014).

Fungal population of total 17 species classified under 11 genera were significantly prevailing throughout a storage of a year with greater per cent incidence to a period of *January* except *Absidia corymbifera, Aspergillus fumigatus, Penicillium oxalicum Nigrospora sp*. Among these exceptional isolates, *Absidia corymbifera* was detected on mustard seeds with low per cent incidence in winter season but their degree of infestation was confined significant over others between the months of *April* to *September*. Depletion in level of infestation correlated with gradual increased temperature of storage environment (Fig.5 & 6). Among the seed borne fungal flora remained prevalent throughout a year on seeds surface, majority of them exhibited low level of infestation during summer are obviously more versatile in a food requirement and capacity of tolerance for varying environment (Swer *et al*, 2010) . Moreover, infestation level of these isolates varied in relation to a variation in temperature and humidity of storage climate may be attributed to their variable existence on seed coats. It is in agreement with earlier finding of Bhajbhuje (2014) who studied seasonal variation of seed mycoflora of *Solanum melongena* L and came to same conclusion. The isolated fungal flora is mostly mesophilic, rapidly proliferates and sporulated at 25-28^{0}C. Moreover, *Absidia corymbifera* and *Aspergillus fumigatus* are thermo-tolerants, able to propagate in moderate humidity at 30-35^{0}C (Swer *et al*., 2010; Stephan, 2013). *Aspergillus niger*, *Penicillium oxalicum* and *Rhizopus stolonifer* were seemed to be appear with higher level of incidence in December on seed surface where a storage temperature ranged between 22-28^{0}C (Fig.6). These results are confirmed to earlier findings from infested seeds involving solanaceous vegetables (Ismael, 2010); cabbage (Gupta *et al*., 2012); coriander (Ramesh *et al*., 2013) *Solanum melongena* L.(Bhajbhuje, 2014) Joshi and Kareppa (2010) reported optimum temperature 25-27^{0}C for rapid mycelial growth and sporulation of *Aspergillus flavus, A. niger, Alternaria alternata, Curvularia lunata, Rhizopus nigricans Fusarium oxysporum* and 30-35^{0}C for *Aspergillus fumigatus.* Seed health testing recommended temperature range between 20-28^{0}C and 30-35^{0}C for better sporulation of meophiles and thermo-tolerant fungal flora (CMI, 2010; Stephan, 2013).

In winter season only, a population of altogether 18 species categorize under 12 genera have been detected with heavy infestation to a period of *January* followed *December* and *February* at storage temperature ranged between 22-28^{0}C (Table 1). They did not exist in summer season. This is in agreement with the finding of Swer *et al*., (2011) and Gupta *et al*., (2012), who

reported optimum temperature 25^{0}C for rapid proliferation of mesophilic seed mycoflora. Majority of mesophilic fungal flora remains dormant above 30-35^{0}C or had a very slow or negligible rate of proliferation and sporulation (Stephan , 2013). This is confirmed to finding of Nazim *et al* (2013) that winterly occurring fungal population reflects greater sensitivity to a storage temperature.

Fungal isolates dominated only in the summer season included a population of 4 species representing 4 genera. Excepting *Chaetomium glabosum* an isolates *Aspergillus ochracious, A. terreus, Chaetomium glabosum,* and *Paecilomyces varioti* , had significant level of per cent incidence to a period of *April*, *May* and *July* at a storage average maximum temperature 32-38^{0}C, but it was declined at 41-44^{0}C in *June* and *July* (Fig. 6). These results are conformed to earlier findings (Niaz *et al*., 2011; Gupta *et al*., 2012; Nazim *et al*., 2013; Bhajbhuje, 2014). Ramesh *et al* (2013) reported heavy infestation by *Ascomycota* group including some species of *Aspergillus* and *Chaetomium* at 35-40^{0}C. *Paecilomyces varioti* sporulated rapidly in warm summer (Bhajbhuje, 2014). This is in agreement with the finding of Joshi and Kareppa (2010) who reported zero sporulation of thermo-tolerant fungal isolates at low range of temperature indicating higher sensitivity to a storage temperature. However, two isolates, *Aspergillus nidulans* and *A. sulphureus,* were confined as seed surface contaminants, but did not exhibit any consistence of their recurrence in relation to changing and fluctuating environment (Fig.4). It is possibly due to non-availability of substrate with proportional nutrients for propagation of these isolates.

In nature, temperature is decisive climatic factor that determines dissemination and geographical distribution of biotic components including fungal flora (Nazim *et al*, 2013). The fungal pathogens have to face considerable temperature fluctuation under natural conditions (Bhajbhuje, 2014). Fig. 6 represents a record of storage climate for temperature and humidity. Heavy infestation, 14.6 per cent of a total frequency was confined to a period of *January* at average mean temperature 21.8^{0}C., followed by February (12.5%) and December (12.1%) at 25.2^{0}C and 21.4^{0}C respectively. The infestation level declined to the extent of 5.12 to 5.8% in summer at temperature range between 31.5-44.2^{0}C for *April* to *September* (Fig. 5 & 6).

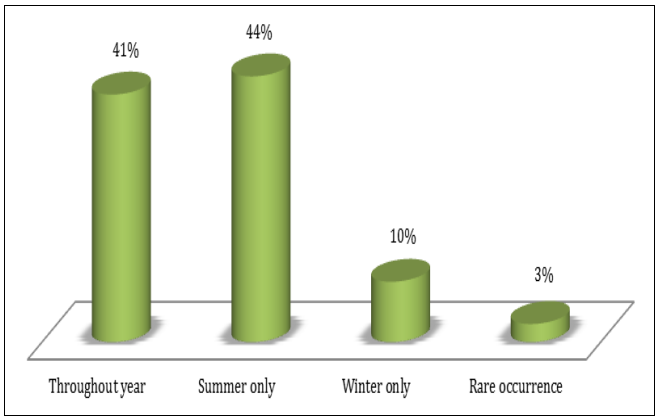


Fig. 3: Periodic fungal count of seed surface contaminants of *Brassica compestris* L.

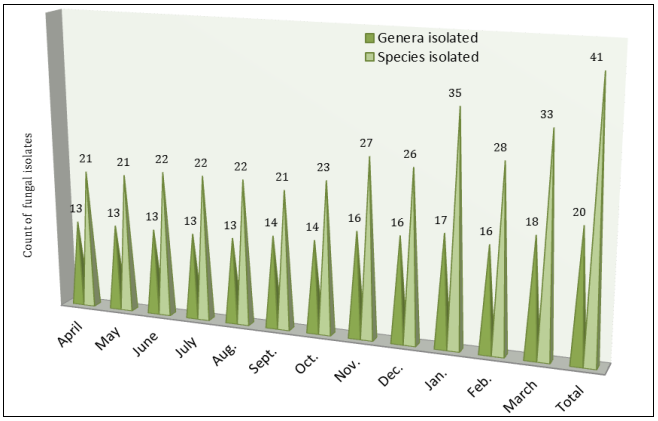


Fig. 4: Periodic count of fungal isolates recorded as seed borne pathogens of *Brassica compestris* L.

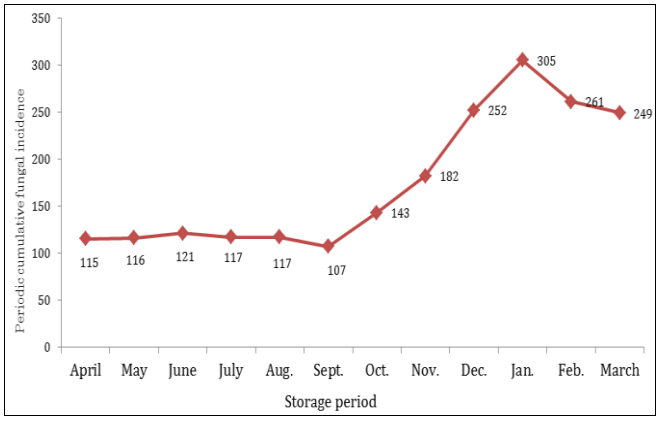
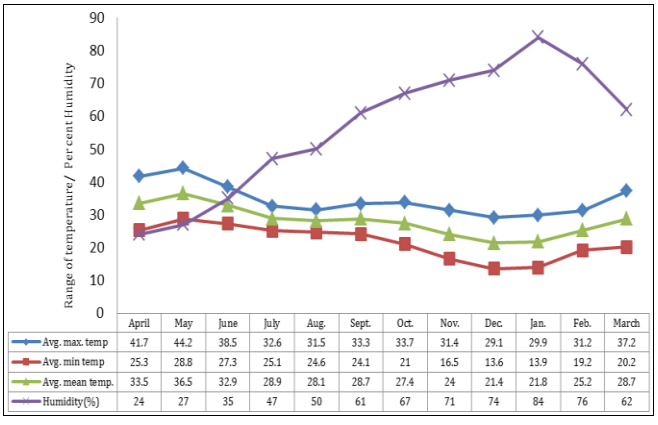


Fig. 5: Periodic cumulative incidence of seed borne fungal flora of seed of *Brassica compestris* L.



	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
Avg. max. temp	41.7	44.2	38.5	32.6	31.5	33.3	33.7	31.4	29.1	29.9	31.2	37.2
Avg. min temp	25.3	28.8	27.3	25.1	24.6	24.1	21	16.5	13.6	13.9	19.2	20.2
Avg. mean temp.	33.5	36.5	32.9	28.9	28.1	28.7	27.4	24	21.4	21.8	25.2	28.7
Humidity(%)	24	27	35	47	50	61	67	71	74	84	76	62

Fig.6: Record of average temperature in degree Celsius & per humidity of seed storage environment

It is in conformity with an earlier finding of Gupta *et al*., (2012) who reported declining trend of seed mycoflora of cabbage and their low level of incidence in warmer climate in combination with low humidity but rate of sporulation becomes higher at optimum temperature and high humidity. On the contrary, maximum storage temperature above 41^0C was recorded to a period of *April* and *May* while it was 31-38 for remaining period of summer (Fig. 5) but level of incidence enhanced over other period of summer season may attributed to rapid proliferation of thermo-tolerant. These results confirmed with earlier findings of Joshi and Kareppa (2010) who reported higher count and greater frequency of some thermo-tolerant on oil seeds. Nazim *et al*., (2013) studied seasonal decomposition of naturally growing mangrove population by soil fungal flora in association with major bacterial group and reported higher rate of decomposition to a period of *June*, maximum accumulation in *August* while minimum litter accretion in *January*. These results are contrary to a present study, may possibly attributed to quite variation in weather condition in Pakistan over Nagpur (M.S., India) and secondly involvement of bacteria in association with fungi for decomposition. Average mean temperature curve of a storage period coincides with level of infestation, revealed that enormous isolates required optimum value ranged between 25-28^0C for sporulation while thermo-tolerant propagates at 30-35^0C (Fig. 5 & 6). Relatively narrow range of temperature permitting sporulation suggested that this phase involves some physiochemical processes, may not seemed essential for mycelial growth but more exacting their temperature requirement than those which suffice for vegetative phase (Stephan, 2013).

The rare prevalence of seed mycoflora reflects their more demanding nature of nutrients and environmental condition. It is also quite likely that such organisms are missed mostly during examination and thus they become more important than others. Inconspicuous seed-borne inoculum may be produce scarcely detectable symptoms to quite destructive epiphytotic in a field (Stephan, 2013). Pathogenic fungal flora did not cause any severity to crop plants. The prevalence of *certain* dominant *Aspergilli* and *Penicilli* species might have aid in antagonizing pathogenic species and reduce disease severity where these fungi can inflict on seed surface (Chanu and Chhetry, 2012; Ramesh *et al*., 2013).

Heavy infestation to seeds under storage by a large population of diverse fungal isolates, mostly in the winter season, reflects faster rate of fungal activity, growth and sporulation provided organic nutrient rich substrate and favourable storage climate. It seems possibly that storage climate with optimum temperature and ambient humidity for period of the winter resulted in greater fungal population over warmer summer. Significant correlations between fungal populations and storage climate proved that temperature and humidity are the two major climatological factors which play pivotal role in establishing rate of fungal propagation and sporulation (Chanu and Chhetry, 2012). Seed nutrient content in storage may serve as organic rich substrate can be viewed as an excellent way in harboring higher fungal populations (Nazim *et al*., 2013; Bhajbhuje, 2014). It is proposed that optimum temperature of storage in the winter of Nagpur (M.S.), high nutritive & moisture content of seed moisture creates favourable microclimates for a profuse growth and sporulation of isolates resulted enormous fungal flora on seeds coats in winter season (Stephan, 2013). Deuteromycota comprises mostly cellulose decomposing saprophytes, proliferates at faster rate on readily available organic rich substrate. This could be one explanation for dominance of Deuteromycota isolates in an organically rich substrate. Inconsistent diversity and monthly/periodic variation in fungal flora in storage is attributed to different stages of fungal growth, the types, availability & a degree of deterioration of an organic substrate (Niaz, *et al*, 2011; Chanu and Chhetry, 2012; Nazim *et al*, 2013; Bhajbhuje, 2014). Steady nutrient uptake from substrate by saprophytes during various stages of propagation resulted in depletion of nutrient availability hence count of fungal flora reduces when a crop growth is at its peak. In present study, lower fungal count in summer is attributed to lack of supportive storage climate, fluctuation in temperature and lowering of humidity level during the post- harvest could be another factor for seasonal diversity of seed borne fungal flora associated with *Brassica campestris* L in storage.

CONCLUSION

Seasonal and regional variation of fungal population is determined by climatological factors of the geographical area. Results from the present investigation revealed that slightly low temperature in combination of high humidity for period of winter season resulted in maximum fungal population over summer season. Diversity in seed mycoflora throughout a year of storage on *Brassica campestris* L. seeds from diverse geographic location of Nagpur district revealed possible correlation of temperature and humidity for an establishment of seasonal diversity

of seed borne fungal population. A climate of *December, January* and *February* of winter favours to heavy infestation by mesophilic mycoflora to seeds over very warm summer. It is correlated to the climatological pattern for areas under Nagpur district where summer is extremely hot, reaching average temperature above 44^0C and winter is pleasant with 22-25^0C. Inconsistent monthly variation of fungal population coincides with fluctuating temperature & humidity of storage environment and degree of deterioration of stored seeds as substrate. Only surface sterilized healthy seeds respond better to all inputs thus seeds can be stored under ambient temperature and relative humidity to reduce deterioration. Research on seasonal fungal diversity provides a basis for estimating the functional role of fungi in an ecosystem.

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