INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD ISSN: 2455-0620 Volume - 6, Issue - 11, Nov – 2020
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value: 86.87 Impact Factor: 6.719
Received Date: 13/11/2020 Acceptance Date: 27/11/2020 Publication Date: 30/11/2020

Studies on mycoflora associated with seeds of *Sesamum indicium* L. from Nagpur District

M. N. Bhajbhuje
Associate Professor
Department of Botany
Jawaharlal Nehru Mahavidyalaya, Wadi, Nagpur-440 023 (M.S.) India.
Email - dr_mnbhajbhuje@rediffmail.com

Abstract: *Sesamum indicum L. is one of the major kharif season oilseed crops in India. A count of 26 fungal isolates of diverse groups classified under 14 genera has been isolated from infested stored seeds. Altogether 21 isolates belongs to 14 genera confined to blotter paper as well as agar plates. Altogether nine isolates representing six genera restricted to blotter paper as external seed borne only while 5 genera with single species confined only to agar plate as internal seed borne contaminants. Ascomycota dominated with nearly more than three-fourth of the total incidence exhibiting higher level of infestation on seed surfaces, followed by Deuteromycota and Zygomycota. Oomycota had least incidence. A single Ascomycetous genus Aspergillus contributed more than one quarter of the total incidence recorded. Ascomycota contributed greatest incidence followed by Deuteromycota. The dominant micro-fungal genera representing Ascomycota included Aspergillus, Penicillium and Cladosporium. Deuteromycota dominated with Alternaria, Fusarium, Helminthosporium, and Trichoderma. Moreover, higher count of isolates as well as significant level of fungal incidence was encountered on blotter paper over agar plate.*

Key Words: *Aspergillus, Deuteromycota, mycoflora, fungal isolates.*

1. INTRODUCTION:

Seed is the custodian of the genetic potential and most vital input for enhancing productivity hence quality of the seed determines the limits of productivity to be realized in a cropping system. The seeds associating with microbe proved hazardous for the seed or new plant created from it. Seed borne pathogen causes both. pre- and post-emergence losses at various stages of crop growth. Seeds are regarded as highly effective means of transporting plant pathogen over long distance. Besides these, mold fungi growing on the seed substratum produce mycotoxins. Such contaminated seeds are reported unsuitable for germination into normal seedlings and hazardous to human and animal. The consumption of such infested seeds is considered hazardous and poses several disorders to consumers (1).

Sesamum indicum L. is well known oldest, sixth oilseed crop plant, grown in 63 countries of the world belonging to the family Pedaliaceae, largely cultivated in India, China, Sudan, Burma and Mexico (2). It is grown in all major crops growing seasons for pearl shaped ovate very small protein rich seeds. The seed is also a rich source of linoleic acid, vitamin E, A, B_1 and minerals including calcium and phosphorus. The oil from seeds is beneficial to human health due to its ability to improve hair and skin health. It is a plant of medicinal value, having ability to prevent diabetes, lower blood pressure, prevent a wide variety of cancers, build strong bones, protect again radiation etc. It is also used as topping for bread and grain products, sushi cakes and other applications (3).

Sesamum indicum is prone to attack by diverse group of fungal pathogens causing several seed borne diseases which was reported to grow on stored seeds as internal seed borne and causes physiological damage to the seeds. During seedling emergence, the pathogen transmits from seed to seedling (4). The transmission and spread of diseases from seed to seedlings causing premature defoliation, multi-fold loss to both pre- and post-harvest crop, that adversely affect economy of poor farmers. Prevalence of seed borne mycoflora concern to this crop has been highlighted by researcher (5). Literature survey suggest that a little is known from the Nagpur region of Maharashtra state concerning to biodiversity of fungal flora adhering to seed surfaces of *Sesamum indicum.* It seems worthwhile considered that data on the diversity of fungal species of Nagpur region would be a great importance for predicting the extent of pre-and post-infections and might be of some use in future architecting bio-control mechanism to avert the seed deterioration and storage loss.

2. MATRIAL AND METHODS:

A composite seed sample of *Sesamum indicum* L. from different cultivators, retailers and stockiest of Nagpur region have been screened for isolation of fungal flora employing standard blotter paper as well as agar plate technique

(6). The colonies developed on the untreated and pre-treated seeds were counted, isolated and identified after sub-culturing on tube slants containing Czapek's nutrient media. The species were identified on the basis of micro- & macro morphology; reverse and surface coloration of colonies grown on Czapek's medium and finally authenticated by authority. Fungal infestation level has been recorded as a percentage of infested seeds (7).

3. RESULTS AND DISCUSSION:

Seed constitute the basic input for crop production in phanerogams, are known to be contaminated with diverse fungal micro-propagules under storage as a result of environmental conditions of high relative humidity, suitable temperature and high level of seed moisture content causing manifold losses to the crop (8). Planting infected seeds resulted in a widespread distribution of diseases within the crop, and an increased count of initial infection sites causing significant seedling infection in the field (9). The deterioration of seeds nutrients under storage by seed mycoflora resulted to loss of seed germ inability; alteration in physio-chemical properties of seed with secretion of toxic metabolites in seeds, contributing seed losses to the greater extent. The toxic metabolites are reported to pose serious health hazard to consumers including man (10).

Prevalence of a population of total 26 fungal isolates of diverse groups classified under 14 genera on the seed surfaces from the seed sample indicated high level of infestation of seeds by fungal propagules in storage (Table 1). Ascomycota dominated with 69.2% exhibiting highest count of fungal isolates on seed surfaces of sesame followed by Deuteromycota, contributed 26.9% of the total isolates. Zygomycota contributed 3.8% fungal isolates while *Oomycota* had least count of isolates. Fungal spores from Basidiomycota did not persist on the seed surface of sesame. Zygomycota represents single genera, *Rhizopus* with single species, *R. stolonifer*. (Table 1).

Ascomycota represents a fungal population of 18 species belonging to 7 fungal genera. Out of these 18 isolates, greatest count of eight species belongs to single genus *Aspergillus*, three species each representing genus *Penicillium*; one species belongs to *Chaetomium* and other four genera had single species.

Deuteromycota had a fungal population of 7 species representing 6 genera. Out of these 7 isolates, a count of two species representing genus *Fusarium*; one species belongs to genera *Alternaria* and *one* isolates belongs to genus *Helminthosporium* while other isolates viz., *Botrytis cinerea*, *Nigrospora*, and *Trichoderma* have been isolated with single species representing single genera (Table 1).

Both blotter and agar plate techniques are recommended for seed health testing and these two techniques are standardized time to time (6) for accuracy. A fungal population of total 17 isolates belongs to 14 genera of diverse fungal groups confined to sesame seeds on blotter paper as well as agar plates as both external and internal seed borne pathogens. Altogether 5 isolates representing 4 genera restricted to blotter paper as external seed borne pathogens while 9 genera representing 7 fungal species, each representing only to agar plate as internal seed borne pathogens (Table 1).

Amongst the total 26 isolates belongs to 14 genera associated with seed coat of Sesame the isolates belongs to genera *Aspergillus* dominated with a count of 10 species, exhibiting higher count of species for this single genera compared to other genera encountered to seed surfaces of sesame. The genus *Penicillium* is sub-dominant with a count of three species. A count of two species contributed by genera *Fusarium*, *Alternaria* and *Colletotrichum* while *Chaetomium*, *Helminthosporium* had one species each. Remaining seed borne genera contaminating sesamum seeds are represented by single species (Table 1).

The seed borne fungi include a very large and heterogeneous group of organisms that occupy position of great economic importance in agriculture. They exhibit an enormous diversity in life-history strategies. The seeds of sesame from composite sample were highly infested by fungal pathogens. Ascomycota dominated with 76 per cent infestation indicating highest level of infestation followed by Deuteromycota, contributed 14.4 per cent incidence. Least level of incidence has been recorded for Zygomycota (Fig.3)

Ascomycota exhibited higher level of infestation on seed surfaces of sesame. *Aspergillus flavus* was dominated with 18.5% incidence exhibiting greatest fungal incidences followed by *Aspergillus niger*, contributing 11.9% incidence over total incidence of this fungal group. The isolates, *Penicillium oxalicum* had 6.3% incidence. Other isolates of Aspergillus with two species had moderate 3.6-5.6% fungal incidence. Remaining isolates of Ascomycota had minimum level of incidence (Table 1).

Deuteromycota had second higher level of infestation over total incidence recorded for fungal flora associated with sesame seeds. *Fusarium oxysporum* was dominated with 5.6% incidence, exhibiting highest fungal incidence

compared to other isolates of this group. The infestation level varied between 0.5-4.0%. was significant with *Alternaria alternata, Botrytis cinerea, Fusarium solani, Helminthosporium tetramera, Nigrospora sphaerica* and *Trichoderma viride*.

The seed coats of the sesame seeds seemed to be heavily infested with viable fungal propagules. The high level frequency of incidence was confined to sesame seeds. The incidence of all the fungal pathogens detected by standard blotter and agar plate techniques was summarized to be 205 per cent. Out of the total, 53.3% fungal incidence was recorded on blotter paper while 46.7% incidence was observed on agar plates. Ascomycota are dominated with 41.4% and 34.7% followed by Deuteromycota with 8.5% and 5.8%; Zygomycota contributed 5.9% and 6.1% *on* blotter paper and agar plate respectively (Fig. 4).

Moreover, greater incidence of isolates was noticed on blotter paper from composite seed sample of sesame over agar plate. These results coincide with the findings of earlier reports from other region of the country. Recently researchers (9) recorded higher frequency of fungal pathogens from stored seeds of pulses on blotter paper over agar plate. Several other investigators reported similar findings by blotter test from infested stored seeds involving oil seeds (4), solanaceous vegetables (11); Niger (12); brinjal (13).

A single Ascomycetous genus *Aspergillus* contributed 55.5% incidence, which was more than one quarter of the total incidence followed by *Rhizopus*, contributing 9.2% incidence. *Fusarium* and *Penicillium* had nearly infestation level, accounting 6.5% and 12% respectively. The isolates of genera, *Alternaria, Chaetomium, Cladosporium, Botrytis, Helminthosporium, Nigrospora, Trichoderma,* exhibited moderate infestation while others has little to mild infestation.

Ascomycota contributed greatest, 76.0% fungal incidence over total incidence followed by Deuteromycota (14.4%). The dominant microfungal genera of this group include *Aspergillus* and *Penicillium.* The prevalence of maximum species confined to genus, *Aspergillus* contributing greatest percent incidence over total infestation. *Rhizopus stolonifer* retrieved from external as well as internal seed borne contaminant. It is in agreement with researcher (7) who reported comparable higher count of species of *Aspergillus* such as *A. niger, A. terreus, A. fumigatus, A. flavus* from maize seeds. These results are in confirmation with earlier findings in *Solanum melongena* L. (14).

The dominant microfungal genera of Deuteromycota included *Alternaria, Fusarium, Helminthosporium, Nigrospora, Botrytis and Trichoderma* (15) reported predominant occurrence of *Alternaria solani, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum,* and *Helminthosporium tetramera* on maize seeds. The isolates of genera *Aspergillus, Alternaria, Penicillium, Cladosporium, Fusarium* and *Stachybotrys atra* were reported in higher frequency from seeds of *Bixa orellana* (16).

The efficacy of both standard blotter and agar plate tests varied with nature of fungal flora. The members of Zygomycota developed more profusely on agar plate possibly because they require softer medium rich in moisture for their establishment and growth. Among the seed health test techniques, standard blotter method was proved comparative superior over agar plate method to the fungal pathogens isolation. Researcher (7) pointed out that in using the agar plate method, the quick growing saprophytes adhering to the outer seed coat may be troublesome to detect internal slow growing pathogen.

The fungal isolates belong to genera, *Aspergilli* and *Penicilli* of *Ascomycotina* as well as *Alternaria, Fusarium, Helminthosporium, Nigrospora and Trichoderma* of Deuteromycota contributed as major components on sesame seeds represented a group of taxa of cosmopolitan fungal organisms that can exploit virtually any organic substrate provided favorable storage environment of oxygen, temperature & relative humidity and accumulates toxic secondary metabolites (9 &17).

Ascomycota has greatest frequency of fungal incidence on sesame seeds followed by Deuteromycota. It may possibly due to prevalence of greater count of fungal propagules associated with seed coat with their higher incidence. Moreover, members of this group are known facultative parasites on crop plants as well as involved as saprophyte in biodegradation of seeds, and debris of plant and animal origin (10 & 11).

The report of the present study revealed that Ascomycota genera, *Aspergilli* and *Penicilli* which are the highly predominant on sesame seeds, are among the most abundant and widely distributed organisms on the globe (16 & 17). Members of the genus *Aspergillus* are known obligate saprophyte and survive in the environment without causing disease (10). *Aspergillus fumigatus, A. niger. A. terreus, A. flavus* had the highest count of occurrence. These ubiquitous

species are commonly isolated from seeds and other substrates such as soil, plant litter, dried fruits and nuts (18); oil seeds (19) and brinjal seeds (13).

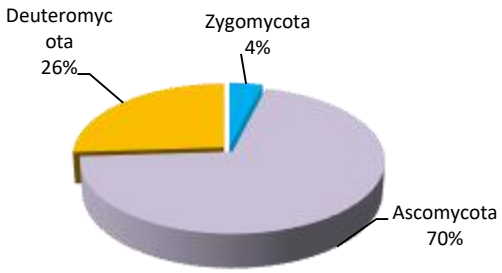
The results of prevent study indicated that sesame seeds harbour arrays of fungal contamination. Some of these fungi had been reported in various stored seeds. The mold infestation recorded in this study may be due to contamination as a result of improper storage environment (18). The practices associated with the quality of seeds at the time of storage, environmental factors during pre- and post-harvest stages, moisture content or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents pre- and post- harvest, processing and handling of seeds may be responsible for its contamination.

Majority of fungal isolates involved in seed deterioration of sesame are xerophilic moulds such as *Aspergilli* and *Penicilli* of Ascomycotina as well as *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium* of *Deuteromycotina* (13). After planting of deteriorated seeds, seedling emergence may be poor and increases chances of pathogen transmission to a new crop. The toxic metabolites from 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 (10).

The result of present study exhibited prevalence of several seed surface contaminants on seeds under storage that cause various diseases in sesame. On the other hand, these fungi are known to produce mycotoxins which are harmful do human health. *Aspergillus flavus* produce aflatoxin B1, B2, G1, G2 which are carcinogenic and mutagenic toxins *Aspergillus niger* attacks human skin and is a parasitic for human ear. Thus there is a need for the control of these pathogens by employing various management techniques to ensure improvement of seed health which ultimately increases crop quality and human health (5).

4. CONCLUSION:

The result obtained in present survey indicated that seeds samples are highly infected with pathogens that cause various diseases in sesame. Moreover, these fungal seed contaminants are known to produce mycotoxins which are harmful to human health. Only high quality seeds respond better to all inputs thus seeds can be stored under ambient temperature and relative humidity at very low cost, without deterioration in quality for periods over one or more season is of immense importance for farmers. The farmers are advised to use improved scientific methods of storage to discourage proliferation of these organisms on seeds.



Fi.1: Division wise distribution of fungal isolates of *sesamum indicum L.*

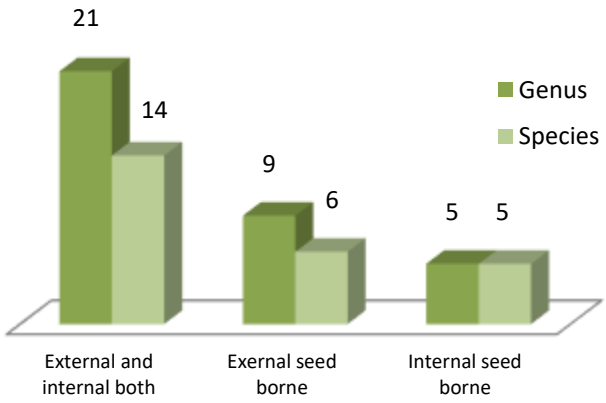


Fig. 2: Distribution of fungal flora associated with seed of Sesamum indicum L.

Table 1 : Distribution of seed borne mycoflora of *Sesmum indicum* L from Nagpur District

S. No	Name of fungal isolate	Frequency (%) of fungal incidence		Total Frequency	% over total incidence	
		Blotter	Agar		Species	Genus
A	**Oomycota**					
B	**Zygomycota**	6.5	12.5	19	9.2	9.2
1	*Rhizopus stolonifer (Ehrarb.* Ex.Fr. Lind.	6.5	12.5	19	9.2	9.2
C	**Ascomycota**	85	71	156	76.0	76.0
2	*Aspergillus amstelodami* (Thom & Church)	1.5	1.5	3.0	1.4	55.5
3	*A. candidus* Link	2.0	1.5	3.5	1.7	
4	*A. japonicus* Saito, Bot.Mag.	4.5	3.0	7.5	3.6	
5	*A. flavus* Link	22.5	15.5	38.0	18.5	
6	*A. fumigatus* Fres.	4.5	6.0	10.5	5.1	
7	*A. niger* Van Tieghen	14.5	10.0	24.5	11.9	
8	*A. ochracious* Wihelm	3.0	1.0	4.0	1.9	
9	*A. sulphureus* (Fres.) Thom & Church	6.5	2.5	9.0	4.4	
10	*A. terreus* Thom	2.0	1.0	3.0	1.4	
11	*Aspergillus versicolor* (Vuill.) Tirab	6.0	5.5	11.5	5.6	
12	*Chaetomium sp.* Kunze.	3.0	-	3.0	1.4	1.4
13	*Cladosporium cladosporoides* Fresen.	1.0	4.0	5.0	2.4	2.4
14	*Colletotrichum lindemuchianum* Sacc.& Magnus	4.0	-	4.0	1.9	1.9
15	*Diplodia sp.*	-	2.5	2.5	1.2	1.2
16	*Gleocladium penicullatus* Pers.	-	2.0	2.0	0.9	0.9
17	*Penicillium citrinum* Thom	6.5	4.5	11	5.3	12
18	*Penicillium sp.* Link.	-	1.0	1.0	0.4	
19	*P. oxalicum* Currie & Thom	3.5	9.5	18.0	6.3	
D	**Basidiomycota**	-	-	-	-	-
E	**Deuteromycota**	17.5	12	29.5	14.4	14.4
20	*Alternaria alternata* (Fr.) Keissl.	2.0	-	2.0	0.9	0.9
21	*Botrytis cinera* Pers.	-	1.0	1.0	0.4	0.4
22	*Fusarium oxysporum* Schlecht	5.0	6.5	11.5	5.6	0.9
23	*F. solani* (Mert.) APP. & Wollenw	-	2.0	2.0	0.9	
24	*Helminthosporium tetramera* Mc Kinney	2.5	1.0	3.5	1.7	1.7
25	*Nigrospora sphaerica* (Sacc.)	1.5	-	1.5	0.7	0.7
26	*Trichoderma viride* Pers.	6.5	1.5	8.0	3.9	3.9
	Total fungal incidence	109.5	95.5	204.5		99.9
	Percent total incidence	53.5%	46.7%			

ACKNOWLEDGEMENT

Author gratefully acknowledges the facilitation of this work by Dr .R.P. Thakre, Ex- Professor & Mycologist and Head, P.G. Deptt. of Botany, RTM, Nagpur University, Nagpur.

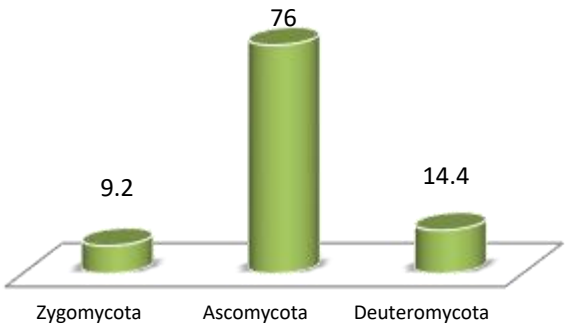


Fig.3: Division wise percent fungal incidence on seeds of *Sesamum indicum* L.

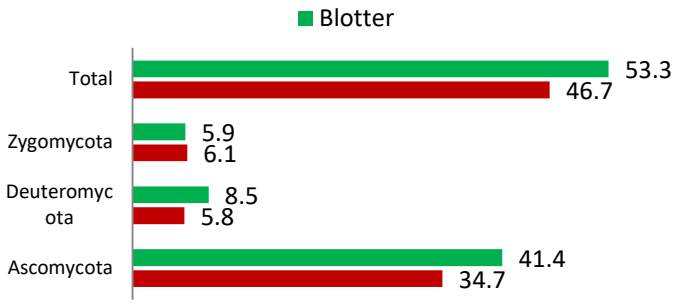


Fig. 4: Compsrison of percent incidence of seed myclfora of *Sesamum indicum* L. by both blotter and agar plate technique

REFERENCES:

1. Uma and E.G. Wesely (2013) seed borne fungi of rice from Tamil Nad Tirunelveli-627012.
2. Bailey, LH (1949), Manual of cultivated plant and classification of sesame. Macmillan co. New York.
3. Vaidehi BK (2002), seed mycoflora of sunflower-a perspective, frontiers in micro biotic plant pathology, 25-40.
4. Anwar et al, (2013) seed borne fungi associated with cauliflower seeds and their role in seed germination, Pakistan J. phytopathogenic.24(1):26-31.
5. Nayyar, Abida Akram, Muhammad Arshad, S.M. Mughal Shaista Akhund and Saira Mushtaq,(2013), Mycoflora were detected from seed of sesame in Sailkot Pakistan, PP-99109
6. ISTA (2019). International Seed Testing Association), 2019 international rules for seed testing rules
7. Chukunda FR, Osakwe JA and RE Boraka (2013) Control of seed borne fungi of stored maize from Nigerian Stored Products Research Institute Port. Harcount. *Web Pub J. Agric. Res.*, 98-21
8. Lew Smith J(2013) seed borne diseases & its control, Mowing organiseeds.76,Quarry Road: :Wolcott, VT 05680 ph- 802-472-6174, fax-802-472-3101 (Retrieved on Nov 20, 2020).
9. Saskatchewan (2013) Guideline for seed borne diseases of pulse crops. Agricultural Knowledge Centre at 1-866-457-2377,wwww.agriculture.gov.sk.ca/seed testing, labs. (Retrieved April 22, 2014).
10. Jyoti and CP Malik (2013) Seed deterioration : A review. International Journal of Life Science Biotechnology & Pharma Res., 2(3) : 373-386
11. Ismael JHS (2010) Isolation and identification of some fungi from certain solanaceous seeds in Sulaimania and Germian regions and their exudate effects on germination rate. Agriculture and Biology Journal of North America, 1(4) : 615-619.
12. Nagaraja, O and M. Krishnappa (2011) Seed borne mycoflora of Niger (Guizotia Abyssinia Cass) and it effect on germination. Indian phytopathology, 62(4) : 210-214
13. Bhajbhuje, M.N. (2014). Seasonal diversity of seed borne micro-fungal flora in storage on *Solanum melongena* L.. *Int. J. of Life Sciences*, 2 (1) : 31-43.
14. Bhajbhuje, M.N. (2013). Distribution of micro-fungal propagules in storage on seeds of *Lycopersicon esculentum* Mill. *Int. J. of Lif Sciences*, 1(4): 248-263
15. Srivastava, M., Gupta, S.K, Saxena, A.P., Shittu, L.A.J. and Gupta, S.K., (2011). review of occurrence of fungal pathogens on significant Brassicaceou vegetable crops and their control measures. *Asian Jour. Agric. Sci*. 3(2):70-79
16. Venugopalan A and P Giridhar (2012) Mycoflora associated with seeds of *Bixa orellana*. African *Journal of Microbiology Res*., 6(9):2091-2094.
17. Gayatri, D.A. and Madhuri, V. (2014). Seed mycoflora of safflower and its control by using botanical Bioagents & fungicides-a reviewe *Int. Jour Appl Biol and Pharmaceu Technol*, 5(1) : 208-215.
18. Jain, P.C. (2008) Microbial degradation of grain, oil seeds, wood corrosion of metal and bioleaching of ores. Appl Microbiol nsdl.niscair,res.in/Microbial Degradation pdf (retrieved 25th Oct. 2020)
19. Kakde, R.B, Badar, Pawar, S.M. and Chavan. A.M. (2013) Storage mycoflora of oilseeds : A review. *International Multidisciplinary Res. J.* 2(3) : 39-42.