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Biodiversity of mycoflora in storage of *Solanum melongena* L. seeds.

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ABSTRACT

Solanum melongena L., a highly preferred vegetable amongst the weight-conscious consumers and a cash crop for poor farmers originated from healthy seed, is vulnerable to attack by diverse group of fungal pathogens in storage and spread of diseases in crop plants that adversely affect economy of poor farmers. Fungal isolations were made from twenty stored mixed seed samples of five different cultivars and individual cultivar collected form eggplant growing farmers of Vidarbha, yielded total 54 fungal species of 30 genera, including eleven species of individual genus *Aspergillus,* four of *Fusarium,* three each of *Alternaria, Curvularia, Helminthosporium* and *Penicillium,* two each of *Chaetomium, Rhizopus* and *Trichoderma while* others had single species*. Deuteromycota* dominated with 44.7% isolates followed by *Ascomycota* (39.9%). *Basidiomycota* had single isolate. A total of 33 to 36 isolates were confined to seeds of individual cultivar. *Aspergillus nidulans, A. sulphureus, A. sydowi, A. terreus, Sporotrichum pulverulentum, Stachybotrys atra* and *Syncephalastrum racemosus* have been recorded for first time as new records in India. *Aspergilli* encountered in seeds with 4 – 14% incidence excluding *Aspergillus fumigatus* (28%) *and A. parasiticus* (18%). *Curvularia, Alternaria, Drechslera and Helminthosporium* were detected as predominant pathogens with 11-13% incidence while *Aureobasidium, Colletotrichum, Pythium* and *Trichothecium* had little count of incidence. Of the total count of incidence, *Deuteromycota* dominated with 47.2% followed by *Ascomycota* (42.1%). Greatest count of fungal isolates and frequency incidence was recorded on blotter paper against agar plates. The standard blotter technique proved superior over agar plate. Cultivars *Pusa Purple Cluster* and *Vaishali* are moderately susceptible to fungal diseases.

KEYWORDS

Seed borne pathogen, Solanum melongena L*, isolates, incidence, frequency, cultivars.*

INTRODUCTION

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 embryos of a new generation and vehicle for the spread of new life. It was recognized in the ancient times about the importance of pathogen free, healthy seed and the way that cultivated the crops, had given a clear picture of their glorious golden era (Saskatchewan, 2013).

A warm-season, non-tuberous, low price, summer vegetable of *Solanaceae* family, the *Solanum melongena* L. (*brinjal, eggplant, melongene, guinea squash or aubergine*) is cultivated quite extensively worldwide for its fleshy fruits. The cultivars display wide range of fruit shapes ranging from oval or egg-shaped to club-shaped; and colour from white, yellow, green through degrees of purple pigmentation to almost black. The eggplant is native to southern India. It was introduced to America in 1806 initially as ornamental but with development of improved bitter less-tasting cultivars makes an integral part of US disc. Presently, it is widely cultivated in America, China, India, Japan, Bangladesh, Pakistan, Egypt, France, Italy and Philippines. In India, eggplant is grown throughout the country except on higher altitude (Anonymous, 2011).

A fleshy fruit of eggplant is rich source of dietary fiber, vitamins, potassium, and calcium; it has 92.7% water, 4% carbohydrate, 1.4% protein, 0.8% mineral matters, low fat, zero cholesterol, and very low calorie content hence make an integral part of a healthy diet and preferred food among weight-conscious consumers. It is a vital ingredient in French ratatouille, Italian melanzane alla parmigiana, and Middle Eastern baba ghanuj dishes. In Indian cuisines, fruit can be boiled, stir-fried, deep-fried, steamed, roasted, or baked and used in preparation of curries, chutneys etc. Baigun bhurta is a most popular, delicious eggplant dish of India. Aside from being used as food, the plant parts have great demand in pharmaceutical industries for medicine formulation in China, Southeast Asia, and Philippines. Roots are employed to cure syphilis and skin diseases. The decoction preparation from roots, dried stalk, and leaves is used as an astringent for hemorrhages. Leaves infusion is employed as an anodynes and remedy for throat and stomach troubles. Burnt fruit is light in digestion, purgative, slightly bilious, and proved beneficial in phlegm and obesity. Long fruit is phlegmatic and generative of phthisis, coughs, and loss of appetite. Tender fruit is antiphlegmatic and alleviative of wind and a ripe fruit is bilious. It is an excellent remedy for liver complaints. Fruits bruised with vinegar, and employed as a poultice for abscesses and cracked nipples. Peduncle is used in intestinal hemorrhages, piles, and toothache. The seeds are used as a stimulant but are apt to lead to dyspepsia and constipation (FTRNR, 2012).

In India, eggplant is an important cash crop for poor farmers provides a stable income round a year. It ranks second highest consumed vegetable after potato along with tomato and onion. A total of 1.6 million Indian farmers grow eggplant as one of the first vegetable crops on an area of 6,80,000 hectares in 2011-12. According to FAO, March 2012, India produces 1,18,96,000 metric tons eggplant annually, equivalent to one quarter of the global production(IHD, 2012). India ranks second leading producer contributing around 27.55% of the global annual harvest after China (55.75%); other top producing countries include Egypt, Iran, Turkey, Indonesia, Iraq and Japan. There is conflicting evidence to support the concept that the eggplant yield has increased over the past decades. Productivity has increased from 12.6 metric tons in 1987-88 to 17.5 metric tons per hectare in 2011-12. This suggests that changes in yield potential are associated with lodging resistance, tolerance of high plant density, pest tolerance and other vegetable traits but the existing productivity becomes insufficient to growing mouths in India (IHD, 2012).

Vidarbha is the eastern region, occupies 31.6% of total area and holds 21.3% of total population of Maharashtra. Vidarbha economy is primary agricultural. Asides from being growing cotton, oranges and soya beans as the main cash crop, the poor farmers cultivate eggplants round a year. Among various factors responsible for low yield are diseases and use of poor quality seeds play an important role. The climate of Vidarbha supports the fungal pathogen to cause diseases, ultimately reduced the productivity to a greater extend.

Eggplant is prone to attack by several fungal pathogens that cause enormous loss both to pre- and post-harvest crop. The deterioration of seeds under storage involves succession of seed borne fungal pathogens resulting in loss of seed nutrients, alteration of the physio-chemical properties of the seeds, loss the seed weight, seed viability & vigour, medicinal properties, aesthetic changes including discoloration, heating & mustiness, cracking and abnormal odours contributing losses of seeds to the extent of 24% (Ismael, 2011). The consequences deterioration leading to series of deteriorative changes include membrane degradation, accumulation of toxic metabolites, decreased enzymatic activity, lipid autoxidation, failure of repair mechanisms, genetic degradation, reduced yield, finally loss of germinability or death of seed (Jyoti and Malik, 2013). Some fungal propagules may bring about certain biochemical changes and secrete toxic metabolites (mycotoxins) that elicit a toxic response such as carcinogenicity, genotoxicity, terrotogenicity, hepatotoxicity, immunesuppression etc. Secondary fungal metabolites are reported to be toxic to man, animals and pose serious health hazard (Brakhage and Schroeckh, 2011).

Planting infected seeds result in a widespread distribution of diseases within the crop, and an increased count of initial infection sites from which the disease can spread. High rate of seed-to-seedling transmission of seed borne pathogens create alarming situation, even a small percentage of infected seed can result in significant seedling

infection in the field (Saskatchewan, 2013). Majority genera of plant-pathogenic seed borne fungi infect eggplant seedlings, some of them causing severe diseases. They limits the ability of plants to produce healthy fruit bearing shoots, causing damping-off, collor rot, stem canker, leaf blight and fruit rot resulting in premature defoliation, reduction in size & quality of fruits, thereby reducing potential yield to the extent of 20-30% (Grafware, 2013).

Prevalence of seed borne mycoflora on stored seeds of *Solanum melongena* L. has been highlighted by Nishikawa, et al., (2006); Habib et al., (2007); Jain (2008); Ismael (2010); Kuri et al., (2011), Grafware (2013). Although considerable research information has been gathered from various regions of India, a little is known from the Vidarbha concerning to seed borne fungi of eggplant. It seems worthwhile considered that a report of Vidarbha region on fungal flora on stored seeds might be of some use in future architecting bio-control mechanism to avert the seed deterioration and storage loss. The present paper reports the detail results of studies on biodiversity of fungal flora encountered in seeds under storage of *Solanum melongena* L. from Vidarbha region of Maharashtra state.

MATERIALS AND METHODS

During present investigation, after collection of twenty seed samples of five commonly grown cultivars of eggplant (viz., *Pusa purple long, Pusa Purple cluster, Vaishali, Vaibhav, and Black round*) of different cultivators from all districts of Vidarbha were screened preliminary for apparent deformities or discolouration employing dry examination technique. The collected seed samples of each cultivar and all cultivars were mixed together and screened for prevalence of seed-borne fungal pathogens employing standard blotter and agar plate test as recommended by International Seed Testing Association (ISTA, 2012).

The randomly selected four hundred seeds from mixed seed samples of all cultivars and individual cultivar were screened for fungal isolation. Of these, two hundred seeds without pretreatment were placed in ten sterilized petri plates equidistantly containing three layered sterile moistened absorbent blotter papers for isolation of external seed borne fungal flora. The remaining two hundred seeds were pretreated with 1% mercuric chloride solution for a minute, rinsed with sterile distilled water for five consecutive times and screened for isolation of internal seed borne fungal flora by agar plate technique. The pretreated twenty seeds were transferred aseptically to each 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. After incubation for seven days in B.O.D incubator at 25±2°C under alternating cycles of 12 hours light and darkness, all these seeds in petri plates were examined directly under stereoscopic microscope. Fungal growth appearing on seeds surfaces was identified with the help of colony colour and sporulation type. The count of fungal isolates and their incidence on the seeds was recorded. The seed borne isolates were purified, sub-cultured and maintained on Czapek's Dox agar nutrient medium.

RESULT AND DISCUSSION

Seed comprises an essential component of agricultural strategy and healthy seeds may act as catalyst for realizing the potential of all other inputs. Health of seeds can be affected by direct infection of pathogen or through contaminated seeds by pathogenic propagules as contamination in, on or with the seeds or as concomitant contamination (Saskatchewan, 2013). Infection of seed by seed borne pathogenic fungal organisms and prevalence of propagules of pathogen in seed lot is vitally important because infected seed(s) may fail to germinate, cause infection to seedlings and reduce health of growing plants (Chukunda et al., 2013). The seasonal climate of Vidarbha and improper storage conditions contribute to make the storage environment extremely supportive for fungal attack to the nutrient rich eggplant seeds (Bhajbhuje, 1989).

Mycological analysis of mixed seed samples of five different commonly grown cultivars obtained from farmers of Vidarbha revealed the prevalence of total 54 fungal pathogens fall under 30 genera in varying incidence. Of these, isolates belong to *Deuteromycota* are most predominant ones, represented by 14 genera and 24 species. *Ascomycota* are represented by 8 genera and 21 species. *Zygomycota* had 5 genera and 6 species.

Oomycota are represented by 2 genera and 2 species while *Basidiomycetes* had only one genus and single species. Individual genus, *Aspergillus* dominated with 11 species, followed by *Fusarium* (4). Three species of genera *Alternaria, Helminthosporium, Curvularia* and *Penicillium*; two species of *Rhizopus, Chaetomium,* and *Trichoderma* have been recorded while others had single species. A total of seven isolates, *Aspergillus nidulans, A. sulphureus, A. sydowi, Aspergillus terreus, Sporotrichum pulverulentum, Stachybotrys atra* and *Syncephalastrum racemosus* were reported as seed borne pathogens on eggplant seeds for the first time from eggplant seeds in India (Table 1 & 2).

Percent incidence of seed borne fungal pathogens from mixed seed samples of all cultivars was presented in Table 1. A total 25 fungal species of 13 genera have been isolated on both blotter paper and agar plate included *Absidia corymbifera, Alternaria porri, A. tenuis, Aspergillus amstelodomi, A. candidus, A. flavus, A. fumigatus, A. parasiticus, A. niger, Botryodiplodia theobromae, Cladosporium fulvum, Curvularia lunata, C. ovoidea, Drechslera rostrata, Fusarium miniliformae, F. oxysporum, F. semitectum, Helminthosporium anomalus, H. tetramera, Paecilomyces varioti, Penicillium oxalicum, Penicillium sp., Phytophthora infestans, Trichoderma lignorum and T. viride.* Of the total isolates, *Curvularia lunata* (47.5%) and *Aspergillus fumigatus* (41%) were appeared to be predominant exhibiting higher incidence. The isolates recorded subdominant had incidence between 19.5 to 19.0% included *Aspergillus parasiticus, Penicillium sp., Alternaria porri, A. tenuis, Cladosporium fulvum, Helminthosporium tetramera* and *Drechslera rostrata* while others were detected with 9.0 to 28.0% incidence. Low count of fungal incidence was detected for *Fusarium semitectum* and *Trichoderma viride* by both health testing techniques.

A total 23 fungal species belongs to 17 genera, *Aspergillus nidulans, A. ochracious, A. sulphureus, A. terreus, A. sydowi, Aureobasidium pullulans, Chaetomium glabosum, Chaetomium sp., Curvularia intermedia, Cunninghamela elegans, Helmintho-sporium spiciferum, Mortierella sp Myrothecium roridum, Nigrospora sp., Penicillium pallidum, Pythium sp. Rhizopus stolonifer, R. nigricans, Sporotrichum pulveulentum, Stachybotrys atra, Stemphylium botryosum, Syncephalastrum recemo-sum* and *Trichothecium roseum* were confined to blotter test only. Among these, *Aspergillus nidulans* was appeared to be most dominant with 14% incidence. The frequency of incidence, 8% was detected for *Rhizopus nigricans, Chaetomium glabosum, Cunninghamela elegans, Myrothecium roridum* and *Nigrospora* sp. while moderate 5% fungal incidence was recorded for *Aspergillus terreus, Stachybotrys atra, Stemphylium botryosum* and *Syncephalastrum recemosum*. Low frequency of incidence was recorded for *Aureobasidium pullulans* and *Pythium* sp. while remaining isolates had frequency of incidence varies between 2.5-4.5% on blotter paper only. The fungal isolates from mixed seed samples of all cultivars, restricted only to agar plate included six genera, *Alternaria solani*, *Colletotrichum dematium, Fusarium solani, Geotrichum candidum, Phoma glomerata* and *Phomopsis sp.* Excepting *Colletotrichum dematium*, others were detected with incidence frequency varies between 4.5 -9.5% (Table 1).

The seed sample of cv. Br-1(*Pusa purple long*) was infested with total 33 fungal pathogens of 19 genera (Table 2 & 3). Of them, 18 isolates of 10 genera were detected in varying frequencies of incidence as both external and internal seed borne on both blotter paper and agar plate included *Alternaria pori, Aspergillus candidus, A. flavus, A. fumigatus, A niger, A. parasiticus, A. terreus, Cladosporium fulvum, Curvularia intermedia, C. lunata, C. ovoides, Drechslera rostrata, Fusarium semitectum, Paecilomyces varioti, Penicillium oxalicum, Penicillium sp., Trichoderma lignorum and T. viride.* Only four isolates were restricted to agar plate in the frequency of incidence varies from 1-6%, included *Aspergillus nidulans, Colletotrichum dematium, Phytophthora infestans and Phoma glomerata* whereas 11 isolates representing eight genera were confined to blotter paper only in frequency of incidence varying from 2.5-18% included *Absidia corymbifera, Alternaria tenuis, Aspergillus amstelodomi, A. sydowi, Chaetomium glabosum, Cunninghamela elegans, Mortierella sp. Phomopsis sp., Rhizopus stolonifer, R. nigricans and Trichothecium roseum.* The isolates, *Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Drechslera rostrata, Paecilomyces varioti Penicillium sp., Rhizopus nigricans* were appeared to be most predominant with 14.5-34% incidence whereas low frequency, 2-4% was recorded for *Colletotrichum dematium, Phoma glomerata, Trichoderma lignorum and T. viride* (Table 1).

Table 1: Percent fungal incidence in storage on *Solanum melongena* L. seeds

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			Br-1 cv. PPL[3]		Br-2 cv. PPC[4]		Br-3 cv. Vaishali		Br-4 cv. Vaibhav		Br-5 cv. Black Round			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
A	Oomycota	6.0(0.9)	3.5 (0.6)	9.5 (1.6)	-	3.5 (0.9)	-	6.0 (1.6)	-	3.5 (0.9)	-	4.0 (1.3)	2.0 (0.6)	5.5 (1.8)	24.5 (1.35)	1.35
1	*Phytophthora infestans* de Bary.	4.0 (0.65)[1]	3.5 (0.57)	7.5 (1.22)	-	3.5 (0.94)	-	6.0 (1.60)	-	3.5 (0.88)	-	4.0 (1.27)	1.0 (0.31)	5.5 (1.75)	23.5 (1.29)	1.29
2	*Pythium sp*	2.0 (0.33)	-	2.0 (0.33)	-	-	-	-	-	-	-	-	1.0 (0.31)	-	1.0 (0.06)	0.06
B.	Zygomycota	40.5 (6.6)	7.0 (1.1)	47.5 (7.7)	46.5 (11.4)	-	51.0 (13.6)	-	48.0 (12.1)	12.0 (3.0)	16.5 (5.3)	3.0 (0.9)	31.5 (9.7)	2.5 (0.8)	211.0 (11.61)	11.61
3.	*Absidia corymbifera* (Cohn) Sacc. & Trotter	2.5 (0.41)	7.0 (1.18)	9.5 (1.55)	4.0 (0.98)	-	6.5 (1.73)	-	4.0 (1.01)	-	4.0 (1.27)	-	6.0 (1.85)	-	24.5 (1.35)	1.35
4	*Cunninghamella elegans* Lendner	8.0 (1.30)	-	8.0 (1.30)	3.0 (0.74)	-	-	-	-	-	-	-	5.5 (1.69)	2.5 (0.77)	11.0 (0.61)	0.61
5	*Mortierella sp.*	9.0 (1.46)	-	9.0 (1.46)	13.5 (3.32)	-	8.0 (2.13)	-	6.0 (1.52)	-	2.5 (0.80)	-	3.0 (0.92)	-	33.0 (1.82)	1.82
6	*Rhizopus nigricans Demelius*	8.0 (1.30)	-	8.0 (1.30)	18.0 (4.42)	-	8.5 (2.26)	-	14.0 (3.54)	12.0 (3.03)	2.0 (0.64)	3.0 (0.96)	4.5 (1.38)	-	62.0 (3.41)	6.52
7	*Rhizopus stolonifer (Ehrarb.* Ex.Fr. Lind.	8.0 (1.30)	-	8.0 (1.30)	8.0 (1.97)	-	14.0 (3.72)	-	18.0 (4.55)	-	8.0 (2.55)	-	8.5 (2.62)	-	56.5 (3.11)	
8	*Syncephalastrum recemosum* (Cohn.) Sch.	5.0 (0.81)	-	5.0 (0.81)	-	-	14.0 (3.72)	-	6.0 (1.52)	-	-	-	4.0 (1.23)	-	24.0 (1.32)	1.32
C	Ascomycota	177.0 (28.8)	82.0 (13.3)	259.0 (42.1)	144.0 (35.4)	89.0 (21.9)	113.5 (30.2)	84.5 (22.8)	94.0 (23.7)	82.5 (20.8)	114.5 (36.5)	93.5 (29.8)	105.5 (32.5)	86.0 (26.5)	1007.0 (55.33)	55.3
9	*Aspergillus amstelodomi (Mang) Thom & Church)*	13.0 (2.11)	6.0 (0.96.)	19.0 (3.09)	2.0 (0.49)	-	-	4.0 (1.06)	-	5.0 (1.26)	-	4.5 (1.43)	-	6.0 (1.85)	21.5 (1.18)	43.5
10	*Aspergillus candidus* Link	6.5 (1.06)	5.5 (0.89)	12.0 (1.95)	8.0 (1.97)	8.0 (1.97)	8.0 (2.13)	4.5 (1.20)	-	-	9.5 (3.03)	6..0 (1.91)	-	-	44.0 (2.42)	
11	*Aspergillus flavus Link*	10.5 (1.71)	8.0 (1.30)	18.5 (3.01)	24.0 (5.90)	18.0 (4.42)	17.5 (4.65)	11.5 (3.06)	14.0 (3.54)	14.0 (3.54)	24.5 (7.80)	19.0 (6.05)	22.5 (6.92)	20.0 (6.15)	185.0 (10.18)	
12	*Aspergillus fumigatus Fres.*	28.0 (4.71)	13.0 (2.19)	41.0 (6.67)	34.0 (8.35)	14.0 (3.44)	19.0 (5.05)	18.0 (4.79)	22.5 (5.68)	12.0 (3.03)	18.5 (5.89)	18.0 (5.73)	28.0 (8.62)	14.0 (4.31)	197.0 (10.84)	
13	*Aspergillus nidulans (Eldam) Winter*	14.0 (2.28)	-	14.0 (2.28)	-	6.0 (1.47)	7.0 (1.86)	1.0 (0.27)	-	5.5 (1.39)	-	-	-	-	519.5 (1.07)	
14	*Aspergillus niger Van Tieghen*	13.0 (2.11)	6.0 (0.96.)	19.0 (3.09)	2.0 (0.49)	-	-	4.0 (1.06)	-	5.0 (1.26)	-	4.5 (1.43)	-	6.0 (1.85)	21.5 (1.18)	

Table 1: Continued…

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			Br-1 cv. PPL[3]		Br-2 cv. PPC[4]		Br-3 cv. Vaishali		Br-4 cv. Vaibhav		Br-5 cv. Black Round			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
15	*Aspergillus ochracious Wihelm*	4.5 (0.73)	-	4.5 (0.73)	-	-	-	-	-	-	-	-	2.0 (0.62)	-	2.0 (0.11)	
16	*Aspergillus parasiticus Speare.*	18.0 (2.93)	10.0 (1.63)	28.0 (4.55)	14.5 (3.56)	8.0 (1.97)	14.0 (3.72)	12.0 (3.19)	12.0 (3.03)	9.5 (2.40)	9.5 (3.03)	5.0 (1.59)	11.0 (3.38)	10.5 (3.23)	106.0 (5.83)	
17	*Aspergillus sulphureus* (Fres.) Thom & Church	4.0 (0.65)	-	4.0 (0.65)	-	-	-	-	-	-	-	-	4.0 (1.23)	-	4.0 (0.22)	
18	*Aspergillus sydowi (Bein. & Sartory) Thom & Church*	4.0 (0.65)	-	4.0 (0.65)	6.0 (1.47)	-	8.0 (2.13)	-	4.5 (1.34)	-	6.0 (1.91)	-	2.0 (0.62)	-	26.5 (1.46)	
19	*Aspergillus terreus* Thom	5.0 (0.81)	-	5.0 (0.81)	6.0 (1.47)	1.0 (0.25)	-	4.0 (1.06)	-	4.5 (1.34)	-	6.0 (1.91)	-	9.5 (2.92)	31.0 (1.71)	
20	*Aureobasidium pullulans* Vala & Boyer	1.0 (0.16)	-	1.0 (0.16)	-	-	-	4.0 (1.06)	-	-	-	-	-	-	4.0 (0.22)	0.22
21	*Botryodiplodia theobromae* Pat.	6.5 (1.06)	2.5 (0.41)	9.0 (1.46)	-	-	-	-	5.5 (1.39)	-	-	-	-	-	5.5 (0.30)	0.30
22	*Chaetomium glabosum Kunne & Schm*	8.0 (1.30)	-	8.0 (1.30)	2.5 (0.61)	-	14.5 (3.86)	-	-	-	8.0 (2.55)	-	-	-	25.0 (1.38)	1.71
23	*Chaetomium sp.*	4.0 (0.65)	-	4.0 (0.65)	-	-	-	-	-	-	6.0 (1.91)	-	-	-	6.0 (0.33)	
24	*Cladosporium fulvum Cooke*	12.5 (2.03)	7.5 (1.22)	20.0 (3.25)	6.5 (1.60)	2.5 (0.61)	4.5 (1.20)	2.5 (0.66)	6.0 (1.52)	4.5 (1.34)	12.0 (3.82)	6.5 (2.07)	12.0 (3.69)	6.0 (1.85)	63.0 (3.46)	3.46
25	*Penicillium oxalicum Currie & Thom*	5.5 (0.89)	5.5 (0.89)	11.0 (1.78)	6.0 (1.47)	3.5 (0.86)	5.0 (1.33)	1.0 (0.27)	4.5 (1.34)	2.0 (0.51)	5.0 (1.59)	2.0 (0.64)	6.0 (1.84)	-	35.0 (1.93)	4.19
26	*Penicillium pallidum* (Cruick & Shank) Pitt.	2.5 (0.41)	-	2.5 (0.41)	-	-	-	-	-	-		4.5 (1.43)	-	-	4.5 (0.25)	
27	*Penicillium* sp	16.5 (2.68)	9.0 (1.46)	25.5 (4.15)	12.5 (3.07)	12.0 (2.95)	-	5.0 (1.33)	-	1.5 (0.38)	1.5 (0.48)	-	1.5 (0.46)	2.5 (0.77)	36.5 (2.01)	
28	*Phoma glomerata* (Corda) Wr. & Bochapfal	-	4.5 (0.73)	4.5 (0.73)	-	2.0 (0.49)	-	4.5 (1.20)	-	5.0 (1.26)	-	6.0 (1.91)	-	1.0 (0.31)	18.5 (1.02)	1.02
29	*Phomopsis sp*	-	6.0 (0.96.)	6.0 (0.96)	3.5 (0.86)	-	-	-	-	4.0 (1.01)	-	6.5 (2.07)	-	4.5 (1.38)	18.5 (1.0)	1.0
D.	Basidiomycota	9.0 (1.46)	-	9.0 (1.46)	-	-	-	-	4.0 (1.01)	-	-	-	-	-	4.0 (0.22)	0.22
30	*Sporotrichum pulverulentum* Nov…Cain & Grover	9.0 (1.46)	-	9.0 (1.46)	-	-	-	-	4.0 (1.01)	-	-	-	-	-	4.0 (0.22)	0.22

Table 1: Continued…

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			Br-1 cv. PPL[3]		Br-2 cv. PPC[4]		Br-3 cv. Vaishali		Br-4 cv. Vaibhav		Br-5 cv. Black Round			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
E.	Deuteromycota	172.5 (28.1)	117.5 (19.1)	290.0 (47.2)	76.0 (18.7)	48.0 (11.8)	78.5 (20.9)	42.5 (11.4)	102.0 (30.3)	50.0 (12.6)	45.5 (14.5)	37.0 (11.8)	77.5 (23.9)	14.5 (4.5)	571.5 (31.44)	31.44
31	*Alternaria porri* (Ell.)	6.5 (1.06)	15.5 (2.52)	22.0 (3.58)	8.0 (1.97)	2.0 (0.49)	-	-	-	4.0 (1.01)	-	-	16.0 (4.92)	4.0 (1.23)	34.0 (1.87)	4.24
32	*Alternaria solani (Elis & Mart.) Jones & Grout*	-	8.0 (1.30)	8.0 (1.30)	-	-	3.0 (0.80)	2.0 (0.53)	2.5 (0.63)	1.5 (0.38)	-	-	-	-	9.0 (0.50)	
33	*Alternaria tenuis Auct.*	11.0 (1.79)	9.5 (1.55)	20.5 (3.33)	8.0 (1.97)	-	2.0 (0.53)	-	6.0 (1.52)	-	4.0 (1.27)	-	14.0 (4.31)	-	34.0 (1.87)	
34	*Colletotrichum dematium* Pers. Ex Fr.	-	2.5 (0.41)	2.5 (0.41)	-	1.0 (0.25)	-	1.5 (0.40)	-	4.5 (1.14)	-	6.5 (2.07)	-	6.5 (2.0)	20..0 (1.10)	1.10
35	*Curvularia intermedia* (Tracy & Barle) Boedjin	9.0 (1.46)	-	9.0 (1.46)	8.5 (2.09)	6.0 (1.47)	14.0 (3.72)	6.0 (1.60)	-	-	-	3.5 (1.11)	-	-	38.0 (2.09)	9.64
36	*Curvularia lunata* (Wakker) Boedijn	28.0 (4.55)	19.5 (3.17)	47.5 (7.72)	14.5 (3.56)	14.5 (3.46)	16.0 (4.26)	15.0 (3.99)	44.0 (11.11)	5.0 (1.26)	5.5 (1.75)	4.0 (1.27)	12.5 (3.85)	-	131.0 (7.21)	
37	*Curvularia ovoidea* (Hirosa & Watan) Munt.	12.5 (2.03)	4.0 (0.65)	16.5 (2.68)	3.5 (0.86)	2.5 (0.61)	-	-	-	-	-	-	-	-	6.0 (0.33)	
38	*Drechslera rostrata* (Drechsler) Rich. & Fraser	11.0 (1.79)	9.5 (1.55)	20.5 (3.33)	4.5 (1.11)	3.5 (0.86)	4.0 (1.06)	3.5 (0.93)	14.0 (3.54)	12.5 (3.16)	9.0 (0.50)	6.0 (1.91)	8.5 (2.62)	-	65.5 (3.60)	3.61
39	*Fusarium miniliformae* Sheldom	6.0 (0.96.)	4.0 (0.65)	10.0 (1.62)	-	-	12.5 (3.32)	5.5 (1.46)	-	-	3.5 (1.11)	-	-	-	21.5 (1.18)	3.85
40	*Fusarium oxysporum Schlecht*	8.0 (1.30)	5.0 (0.81)	13.0 (2.11)	-	-	6.0 (1.60)	2.5 (0.66)	7.5 (1.89)	2.5 (0.63)	-	-	1.0 (0.31)	-	19.5 (1.07)	
41	*Fusarium semitectum* Berk & Rav.	4.0 (0.65)	1.0 (0.16)	5.0 (0.81)	2.5 (0.61)	2.5 (0.61)	-	-	6.0 (1.52)	4.0 (1.01)	3.5 (1.11)	2.5 (0.78)	-	-	21.0 (1.16)	
42	*Fusarium solani* (Mert.) APP. & Wollenw	-	8.0 (1.30)	8.0 (1.30)	-	-	-	-	2.0 (0.51)	6.0 (1.52)	-	-	-	-	8.0 (0.44)	
43	*Geotrichum candidum* Link ex Fr.	-	9.5 (1.55)	9.5 (1.55)	-	-	-	2.0 (0.66)	-	2.0 (0.51)	-	4.5 (1.43)	-		8.5 (0.47)	0.47
44	*Helminthosporium anomalus* Gilman & Abbott	9.0 (1.46)	5.0 (0.81)	14.0 (2.28)	-	-	-	-	-	-	2.5 (0.78)	1.5 (0.48)	-	-	4.0 (0.22)	0.94

Table :1 Continued…

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			Br-1 cv. PPL[3]		Br-2 cv. PPC[4]		Br-3 cv. Vaishali		Br-4 cv. Vaibhav		Br-5 cv. Black Round			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
45	*Helminthosporium spiciferum* (Bain.) Nicol	4.5 (0.73)	-	4.5 (0.73)	-	-	4.0 (1.06)	-	-	-	-	-	-	-	4.0 (0.22)	0.94
46	*Helminthosporium tetramera* Mc Kinney	13.5 (2.19)	6.5 (1.06)	20.0 (3.25)	-	-	-	2.0 (0.66)	5.0 (1.26)	2.0 (0.51)	-	-	-	-	9.0 (0.50)	
47	*Myrothecium roridum* Tode ex Fr.	8.0 (1.30)	-	8.0 (1.30)	-	-	-	-	-	-	-	-	2.5 (0.77)	-	2.5 (0.14)	0.14
48	*Nigrospora sp.*	8.0 (1.30)	-	8.0 (1.30)	-	-	2..0 (0.66)	-	-	-	-	-	-	-	2.0 (0.11)	0.11
49	*Paecilomyces variotii* Bainier	8.0 (1.30)	6.0 (0.96.)	14.0 (2.28)	18.0 (4.42	13.0 (3..19)	-	-	8.5 (2.15)	-	-	4.0 (1.27)	-	-	43.5 (2.40)	2.40
50	*Stachybotrys atra* Corda	5.0 (0.81)	-	5.0 (0.81)	-	-	4.0 (1.06)	-	-	-	5.0 (1.59)	-	-	-	9.0 (0.50)	0.50
51	*Stemphylium botryosum* Waller.	5.0 (0.81)	-	5.0 (0.81)	-	-	-	-	-	-	-	-	3.0 (0.92)	-	3.0 (0.17)	0.17
52	*Trichoderma lignorum* (Tochi & Shimada) Pidolp	8.5 (1.38)	2.5 (0.41)	11.0 (1.73)	2.5 (0.61)	1.5 (0.04)	4.5 (1.20)	2.5 (0.66)	-	2.5 (0.63)	3.0 (0.96)	-	4.5 (1.38)		21.0 (1.16)	2.18
53	*Trichoderma viride* Pers. Ex.Fr.	4.5 (0.73)	1.5 (0.24)	6.0 (0.96)	1.5 (0.04)	1.5 (0.04)	-	-	-	3.5 (0.88)	-	4.5 (1.43)	6.0 (1.85)	1.5 (0.46)	18.5 (1.02)	
54	*Trichothecium roseum* Link	2.5 (0.41)	-	2.5 (0.41)	4.5 (1.11)	-	6.5 (1.73)	-	6.5 (1.52)	-	9.5 (0.53)	-	9.5 (2.92)	2.5 (0.77)	39.0 (2.18)	2.15
	Total fungal incidence	**405.0 (65.8)**	**210.0 (34.2)**	**615.0 (100.0)**	**266.5 (65.5)**	**140.5 (34.5)**	**243.0 (64.6)**	**133.0 (35.4)**	**248.0 (62.7)**	**148.0 (37.4)**	**176.5 (56.2)**	**137.5 (43.8)**	**216.5 (66.6)**	**108.5 (33.4)**	**1818.0**	99.64
	Sun total incidence	**615.0**			**407.0**		**376.0**		**396.0**		**314.0**		**325**			

1. Values in parenthesis calculated in terms of percent incidence over total incidence
2. Values in parenthesis indicate percent fungal isolates over total isolates recorded
3. PPL - Pusa Purple Long; 4. PPC – Pusa Purple Cluster

Table 2: Diversity of fungal isolates and their incidence on *Solanum melongena* L. seeds

Sr. No.	Fungal genera isolates	Seed samples of *Solanum melongena* L.																		Sum total of incidence
		Mixed seed samples			Br-1 cv. PPL			Br-2 cv. PPC			Br-3 cv. Vaishali			Br-4 cv. Vaibhav			Br-5 cv Black round			
		Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	
A	**Oomycota**	2	9.5	1.55	1	3.5	0.86	1	6.0	1.60	1	3.5	0.88	1	4.0	1.27	2	7.5	2.31	24.5
1	*Phytophthora*	1	7.5	1.22	1	3.5	0.86	1	6.0	1.60	1	3.5	0.88	1	4.0	1.27	1	7.5	2.31	23.5
2	*Pythium sp*	1	2.0	0.33	-	-	-	-	-	-	-	-	-	-	-	-	1	1.0	0.31	1.0
	Total genera	**2 (6.67)**			**1 (5.26)**			**1(4.55)**			**1 (4.76)**			**1 (4.76)**			**2 (9.52)**			
B.	**Zygomycota**	6	47.5	7.71	5	46.5	11.4	5	51.0	13.6	5	60.0	15.2	4	19.5	6.2	6	34.0	10.5	211.0
3.	*Absidia*	1	9.5	1.54	1	4.0	0.98	1	6.5	1.73	1	4.0	1.01	1	4.0	1.27	1	6.0	1.85	24.5
4	*Cunninghamela*	1	8.0	1.30	1	3.0	0.74	-	-	-	-	-	-	-	-	-	1	8.0	2.46	11.0
5	*Mortierella*	1	9.0	1.46	1	13.5	3.32	1	8.0	2.13	1	6.0	1.52	1	2.5	0.80	1	3.0	0.92	33.0
6	*Rhizopus*	2	16.0	2.60	2	26.0	6.39	2	22.5	5.98	2	44.0	11.11	2	13.0	4.14	2	13.0	4.0	118.5
7	*Syncephalastrum*	1	5.0	0.81	-	-	-	1	14.0	3.72	1	6.0	1.52	-	-	-	1	4.0	1.23	24.0
	Total genera	**5 (16.67)**			**4 (21.05)**			**4 (18.18)**			**4 (19.05)**			**3 (14.29)**			**5 (23.81)**			
C	**Ascomycota**	21	259.0	42.1	15	233.0	57.2	15	198.0	52.7	15	176.5	44.6	16	208.0	66.2	14	191.5	57.7	1007.0
8	*Aspergillus*	11	167.5	27.23	9	182.0	44.72	9	157.0	41.76	8	143.5	36.24	8	150.0	47.77	8	158.0	46.61	790.5
9	*Aureobasidium*	1	1.0	0.16	-	-	-	1	4.0	1.06	-	-	-	-	-	-	-	-	-	4.0
10	*Botryodiplodia*	1	9.0	1.46	-	-	-	-	-	-	1	5.5	1.39	-	-	-	-	-	-	5.5
11	*Chaetomium*	2	12.0	1.95	1	2.5	0.61	1	14.5	3.86	1	-	-	2	14.0	4.46	-	-	-	31
12	*Cladosporium*	1	20.0	3.25	1	9.0	2.21	1	7.0	1.86	1	10.5	2.65	1	18.5	5.89	1	18.0	5.84	63
13	*Penicillium*	3	39.0	6.34	2	34.0	8.35	2	11.0	2.93	2	8.0	2.02	3	13.0	4.14	3	10.0	3.08	76
14	*Phoma*	1	4.5	0.73	1	2.0	0.49	1	4.5	1.20	1	5.0	1.26	1	6.0	1.91	1	1.0	0.31	18.5
15	*Phomopsis*	1	6.0	0.98	1	3.5	0.86	-	-	-	1	4.0	1.01	1	6.5	2.07	1	4.5	1.38	18.5
	Total genera	**8 (26.67)**			**6 (31.58)**			**6 (37.27)**			**6 (28.57)**			**6 (28.57)**			**5 (23.81)**			
D.	**Basidiomycota**	1	9.0	1.46	-	-	-	-	-	-	1	4.0	1.01	-	-	-	-	-	-	4.0
16	*Sporotrichum*	1	9.0	1.46	-	-	-	-	-	-	1	4.0	1.01	-	-	-	-	-	-	4.0
	Total genera	**1 (3.33)**						**-**			**1 (4.76)**			**-**			**-**			

Table 2: Continued...

Sr. No.	Fungal genera isolates	Seed samples of *Solanum melongena* L.																		Sum total of incidence
		Mixed seed samples			Br-1 cv. PPL			Br-2 cv. PPC			Br-3 cv. Vaishali			Br-4 cv. Vaibhav			Br-5 cv Black round			
		Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	Sp	Total incidence	% of total	
E.	**Deuteromycota**	24	290.0	47.15	12	124.0	30.5	15	121.0	32.2	14	152.0	38.4	13	82.5	26.3	11	92.0	28.3	571.5
17	*Alternaria*	3	50.5	8.21	2	18.0	4.42	2	7.0	1.86	3	14.0	3.54	1	4.0	1.27	2	34.0	10.46	77.0
18	*Colletotrichum*	1	2.5	0.41	1	1.0	0.25	1	1.5	0.40	1	4.5	1.34	1	6.5	2.07	1	6.5	2.0	20.0
19	*Curvularia*	3	73.0	11.87	3	49.5	12.16	2	51.0	13.56	1	49.0	12.37	2	13.0	4.14	2	12.5	3.85	175.0
20	*Drechslera*	1	20.5	3.33	1	8.0	1.97	2	7.5	1.99	1	26.5	6.69	1	15.0	4.78	1	8.5	2.62	65.5
21	*Fusarium*	4	36.0	5.85	1	5.0	1.23	1	26.5	7.05	3	28.0	7.07	2	9.5	3.03	1	1.0	0.31	70.0
22	*Geotrichum*	1	9.5	1.54	-	-	-	1	2.0	0.53	1	2.0	0.51	1	4.5	1.43	-	-	-	8.5
23	*Helminthosporium*	3	38.5	6.26	-	-	-	2	6.0	1.60	1	7.0	1.77	1	4.0	1.27	-	-	-	17.0
24	*Myrothecium*	1	8.0	1.30	-	-	-	-	-	-	-	-	-	-	-	-	1	2.5	0.77	2.5
25	*Nigrospora*	1	8.0	1.30	-	-	-	1	2.0	0.53	-	-	-	-	-	-	-	-	-	2.0
26	*Paecilomyces*	1	14.0	2.28	1	31.0	7.62	-	-	-	1	8.5	2.15	1	4.0	1.27	-	-	-	43.5
27	*Stachybotrys*	1	5.0	0.81	-	-	-	1	4.0	1.06	-	-	-	1	5.0	1.59	-	-	-	9.0
28	*Stemphylium*	1	5.0	0.81	-	-	-	-	-	-	-	-	-	-	-	-	1	3.0	0.92	3.0
29	*Trichoderma*	2	17.0	2.76	2	7.0	1.72	1	7.0	1.86	2	6.0	1.52	2	7.5	2.39	1	12.0	3.69	39.5
30	*Trichothecium*	1	2.5	0.41	1	4.5	1.11	1	6.5	1.73	-	6.5	1.64	1	9.5	3.03	1	12.0	3.69	39.0
	Total Genera	**14 (46.67)**			**8 (42.11)**			**11 (50.0%)**			**9 (42.86)**			**11 (52.38)**			**9 (42.86)**			
Sum total species		**54**			**33**			**33**			**36**			**35**			**33**			
Sum total Genera		**30**			**19**			**22**			**21**			**21**			**21**			
Sum total frequency		**615.0**			**407.0**			**376.0**			**396.0**			**314.0**			**325.0**			**1818**

1. Values in parenthesis calculated in terms of percent incidence over total incidence
2. Values in parenthesis indicate percent fungal isolates over total isolates recorded
3. PPL - Pusa Purple Long; 4. PPC – Pusa Purple Cluster; 5. BR – Black Round

Table 3: Distribution of external and internal seed borne flora with frequency of incidence.

Sr. No.	Cultivars	Number of 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 of all cultivars	25 (46.3)	23 (76.7)	23 (42.6)	17 (56.7)	06 (11.11)	06 (20.0)	30	54	405 (65.8)	210 (34.2)	615
2	Br.-1 cv. PPL	18 (52.9)	10 (52.6)	11 (33.3)	08 (42.1)	04 (11.8)	04 (21.1)	19	33	266.5 (65.5)	140.5 (34.5)	407
3	Br.-2 cv. PPC	15 (41.7)	08 (36.4)	12 (33.3)	11 (50.0)	09 (25.0)	08 (36.4)	22	36	243.0 (64.6)	133.0 (35.4)	376
4	Br.-3 cv. Vaibbhav	14 (40.0)	09 (42.9)	10 (47.6)	10 (46.3)	12 (43.3)	09 (42.9)	21	36	248.0 (62.7)	148 (37.4)	396
5	Br.-4 cv. Vaishali	12 (43.3)	07 (33.3)	12 (43.3)	11 (52.4)	11 (31.4)	10 (47.6)	21	35	176.5 (56.2)	137.5 (46.8)	314
6.	Br.-5 cv. Black Round	11 (33.3)	08 (38.1)	17 (51.5)	14 (66.7)	15 (45.5)	05 (23.8)	21	33	216.5 (66.5)	108.5 (33.5)	325

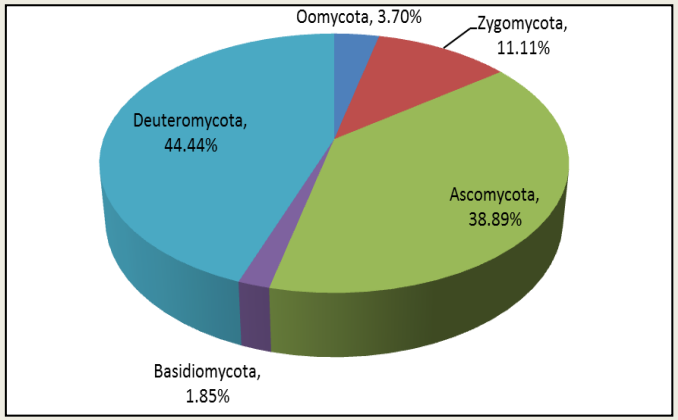

Fig.1: Distribution of fungal flora on stored seed of *Solanum melongena* L.

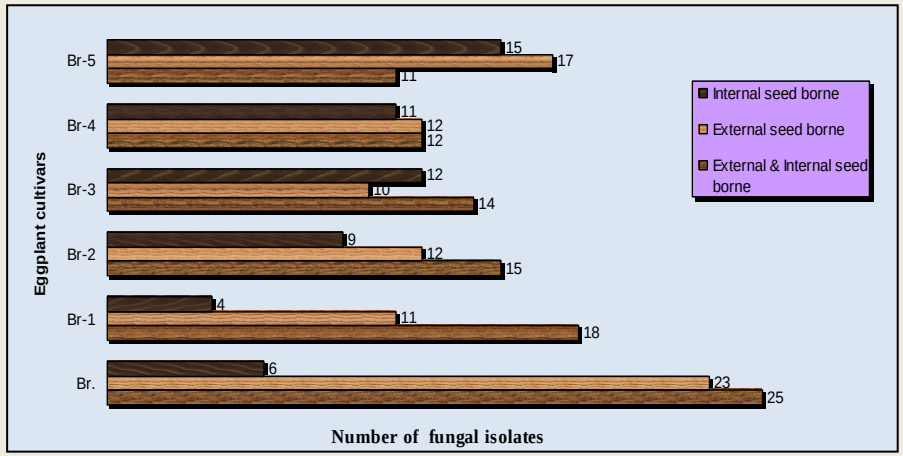

Fig.2 :Seed borne fungal pathogens associated with stored seed samples of individual & all cultivars of *Solanum melongena* L.

Altogether, total 36 fungal pathogens belongs to 22 genera were encountered in seeds of cv. Br-2 (*Pusa purple cluster*), of them 15 isolates of 8 genera, *Alternaria solani, Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans A. niger, A. parasiticus, Cladosporium fulvum, Curvularia intermedia, C. lunata, Drechslera rostrata, Fusarium miniliformae, F. oxysporum, Penicillium oxalicum and Trichoderma lignorum* were confined to both standard blotter and agar plate test. Of the 11 isolates encountered on blotter paper, *Chaetomium glabosum and Syncephalastrum racemosus* were appeared to be most dominant with incidence ranged from 14-14.5% while others had 2.0-8.5% incidence. Nine isolates belongs to eight genera, *Aspergillus amstelodomi, Aspergillus terreus, Aureobasidium pullulans, Colletotrichum dematium, Geotrichum candidum, Helminthosporium tetramera, Phoma glomerata, Penicillium palladium and Phytophthora infestans* were confined to agar plate only with 2-6% occurrence. Among these, greatest frequency of incidence, 12.5-19% was recorded for *Aspergillus flavus, A. fumigatus, A. parasiticus, A. niger, Curvularia intermedia, C. lunata* and *Fusarium moniliformae* whereas *Aspergillus nidulans, Cladosporium fulvum, Fusarium oxysporum, Penicillium oxalicum and Trichoderma lignorum* were appeared to be sub-dominant with 4-7% incidence (Table 1).

Eggplant cv. Br-3 (*Vaishali*) seeds favored the association of 36 fungal pathogens of 21 genera (Table 2). Of them, ten isolates, *Absidia corymbefera, Alternaria tenuis, A. sydowi, Botryodiplodia theobromae, Paecilomyces varioti, Mortierella sp.,Rhizopus stolonifer, Sporotrichum pulveulentum, Syncephalastrum recemosum, Trichothecium roseum* were recorded as seed surface contaminants. Excluding *Rhizopus stolonifer*, others were detected with 4-8% occurrence. Twelve fungal isolates of nine genera, *Alternaria porri, Aspergillus amstelodomi A. nidulans, A. terreus, A, Colletotrichum dematium, Geotrichum candidum, Penicillium sp.,Phoma glomerata Phomopsis sp., Phytophthora infestans, Trichoderma lignorum and T. viride* confined to agar plate, were appeared in frequency of incidence varies between 1.5-5.5%. Of the total, 14 isolates of 9 genera has been recorded by both seed health tests. Excepting C. lunata, greatest percent incidence, 14-25% was recorded for *Aspergillus fumigatus, A. niger, Drechslera rostrata and Rhizopus nigricans* whereas *Alternaria solani, Cladosporium fulvum, Fusarium solani, F. semitectum F. oxysporum and Penicillium oxalicum* were appeared to be sub-dominant with 2-6% incidence (Table 1).

Total 35 fungal pathogens fall under 21 genera were encountered in seeds of cv. Br-4 (*Vaibhav*). Of the 11 isolates of 10 genera, encountered on agar plate, the greatest incidence, 6.0-6.5% was recorded for *Aspergillus terreus, Phoma glomerata and Phomopsis* sp. whereas it ranged between 3.5 to 4.5% for *Aspergillus amstelodomi, Colletotrichum dematium, Curvularia intermedia, Geotrichum candidum, Paecilomyces varioti, Penicillium pallidum, Phytophthora infestans* and *Trichoderma viride*. Twelve isolates belongs to 11 genera were restricted only to blotter test (Table 2). Of them, greatest incidence, 4-8% was recorded for *Absidia corymbifera, Alternaria tenuis, Aspergillus sydowi, Chaetomium sp., C. glabosum, Rhizopus stolonifer, Stachybotrys atra* and *Trichothecium roseum* whereas 1.5-3.5% incidence was noticed for *Fusarium miniliformae, Penicillium sp., Trichoderma lignorum* and *Mortierella sp.* Prevalence of 12 isolates of 7 genera, *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. parasiticus, Cladosporium fulvum, Curvularia lunata, Drechslera rostrata, Fusarium semitectum, Helminthosporium anomalus, Penicillium oxalicum and Rhizopus nigricans* were recorded by both tests. *Aspergillus flavus, Cladosporium fulvum* and *Drechslera rostrata* were seemed predominant. *Curvularia lunata, Helminthosporium anomalus, Fusarium semitectum* and *Penicillium oxalicum were* recorded to be subdominant with 3.5-5.5% incidence.

Prevalence of 33 fungal isolates fall under 21 genera was detected from seed samples of cv. Br.-5 (*Black Round*) (Table 2). Of these, 11 species belongs to 8 genera encountered on both blotter paper and agar plate, with maximum frequency of incidence for *Alternaria porri, Aspergillus flavus, A. fumigatus, A. parasiticus A. niger and Cladosporium fulvum* whereas *Cunninghamela elegans Penicillium sp., Phytophthora infestans, Trichoderma lignorum and T. viride* had percent incidence. Total 17 species of 14 genera detected on blotter paper. Frequency of incidence, 6-14% was recorded for *Absidia corymbifera, Alternaria tenuis Curvularia lunata, Drechslera rostrata, Penicillium oxalicum, Rhizopus. Stolonifer* and *Trichothecium roseum* whereas *Aspergillus sulphureus, A. ochracious, A.*

sydowi, Fusarium oxysporum, Mortierella sp., Myrothecium roridum, Pythium sp., Rhizopus nigricans, Stemphylium botryosum, Syncephalastrum recemosum were dominated by1-4% incidence*. Of the* five isolates confined only to agar plate, exhibiting their incidence varies between 6.0-9.5% for *Aspergillus amstelodomi, A. terreus, Colletotrichum dematium Fusarium solani, F. moniliformae* whereas *Fusarium semitectum, Phoma glomerata and Phomopsis* were appeared to be sub-dominant (Table 1).

The samples of five cultivars of eggplant representing the cv. Br-1, Br-2, Br-3, Br-4 and Br-5 were obtained from different cultivators from Vidarbha region of Maharashtra States to ascertain the degree of fungal association with the seeds. An isolation was made by standard blotter and agar plate technique (ISTA, 2012). Altogether 54 isolates belong to 30 genera were confined to mixed seed samples. *Aspergillus fumigatus, A. flavus, A. parasiticus, A. niger, Cladosporium fulvum, Curvularia lunata, Drachslera rostrata and Penicillium oxalicum* were seemed to be common on the seeds of all cultivars, and detected by both seed health tests (Table 1). *Absidia corymbifera, Alternaria tenuis, Aspergillus amstelodomi, A. sydowi Mortierella sp., Rhizopus stolonifer and Trichothecium roseum* were confined to blotter test only while *Colletotrichum dematium, Phoma sp*. were restricted to agar plate only.

Majority of the fungal pathogens were confined to both seed health test. Moderately high frequency of fungal incidence was detected from the mixed seed samples of all the cultivars of eggplant for *Aspergillus fumigatus, A. flavus, A. niger, A. parasiticus,* and *Cladosporium fulvum*. The isolate, *Fusarium oxysporum* from seeds of cv Br-2,3 and *Fusarium solani* from Br-3 were detected as both external and internal seed borne pathogens. *Aspergillus nidulans, A. terreus, Curvularia intermedia, C. ovoides, Fusarium miniliformae, F. semitectum, Paecilomyces varioti, Penicillium sp., Phytophthora infestans, Trichoderma lignorum, T. viride* were frequently isolated from some cultivars by both seed heath test in one cultivar and either blotter or agar test in others (Table 1).

Some fungal pathogens remain restricted to specific cultivar in storage environment. The isolates confined to blotter paper only belongs to species, *Aureobasidium pullulans, Helmintho-sporium spiciferum* and *Nigrospora sp*. (Br-2); *Botrydiplodia theobromae* and *Sporotrichum pulveulentum* (Br-3); *Chaetomium* sp., (Br-4); *Aspergillus ochracious*, *A. sulphureus, Myrothecium roridum, Stemphylium botryosum and Pythium sp.*(Br-5); *Chaetomium glabosum* (Br-1,2,4), *Syncephalastrum recemosum* (Br-2,3,5); *Stachy-botrys atra* (Br-2,4), and *Alternaria tenuis* (Br. 1-5) were detected as surface contaminant. However, *Geotrichum candidus* (Br-2,3,4); *Colletotrichum dematium* (Br. 1-5), and *Penicillium pallidum* (Br-4) were observed by agar plate test. *Alternaria porri, Aspergillus flavus, A. niger, A fumigatus, A. parasiticus, Curvularia lunata, Rhizopus stolonifer and R. nigricans* were recorded in higher frequency of incidence over other isolates (Table 1).

The blotter and agar plate technique are applied in routine seed health test for detection of seed-borne fungal pathogens as these two tests are inevitable for getting a complete picture of the fungal infection/association with the seeds (Saskatchewan, 2013). A total 54 fungal isolates classified under 30 genera have been recorded as external and internal seed borne pathogen from the seeds of mixed samples of five different cultivars. *Deuteromycota* dominated with 44.4% exhibiting highest fungal isolates followed by *Ascomycota,* contributed 38.9% of the total pathogens isolated. Zygomycota are represented by 11.1% fungal isolates. The frequent count of isolates was recorded with *Oomycota* (3.7%) and *Basidiomycota* of the total pathogens recorded (Fig. 1).

The seed borne fungi include a very large and heterogeneous group of organisms that occupy position of great economic importance in agriculture. They show an enormous diversity in life-history strategies. The eggplant stored seeds samples were highly infested by fungal pathogens. *Deuteromycota* contributed nearly half of the total fungal incidence, represented by 47.2%. *Ascomycota* contributed 42.1%, followed by Zygomycota (7.7%) and Oomycota (1.6%). Basidiomycota contributed little incidence (Fig. 3). Of the total, 65.85% fungal incidence was recorded on blotter paper while 34.15% incidence was observed on agar plates. *Ascomycota* are dominated with 28.78% and 13.13% followed by *Deuteromycota* with 28.05% and 19.11%; *Zygomycota* contributed 6.59% and 1.13% while 0.98% and 0.57% fungal incidence was recorded

for *Oomycota* on blotter paper and agar plate respectively. Little fungal incidence 1.46% was encountered for *Basidiomycota* on blotter paper only. Moreover, higher incidence of isolates was noticed on blotter paper from mixed seed samples of individual cultivars over agar plate (Fig. 2). These results coincide with the data obtained earlier from other region of the country. Recently Saskatchewan (2013) 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 (Jain. 2008), solanaceous vegetables (Ismael, 2010); Niger (Nagaraja and Krishnappa, 2011); *Sorgham* (Yago et al., 2011); maize (Chukunda et al., 2013).

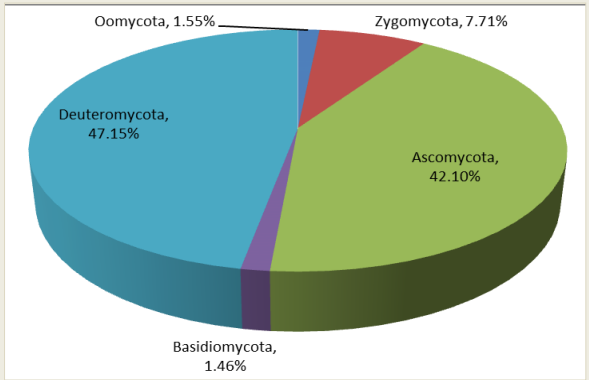

Fig. 3 Percent fungal incidence on stored seed of *Solanum melongena* L.

Deuteromycota contributed greatest, 47.15% fungal incidence over total count followed by Ascomycota (42.1%) (Fig.3). The dominant microfungal genera of this group include *Alternaria, Curvularia, Helminthosporium, Fusarium, Drechslera, Trichoderma, and Paecilomyces* (Fig. 4). The prevalence of maximum species confined to genus, *Aspergillus* contributing greatest percent incidence over total count. Both species of *Rhizopus* retrieved from all samples as seed-surface contaminant. These results are in confirmation with earlier findings. Shirurkar and Wahegaonkar (2012) most frequently isolated comparable higher count of species of *Aspergillus* such as *A. niger, A. terreus, A. fumigatus, A. flavus, A. parasiticus* from maize seeds. Kulkarni et al., (2012) reported predominant occurrence of *Alternaria solani, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramera* and *Trichoderma viride* 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* (Venugopalan and Giridhar, 2012).

Among the fungal isolates, seed borne nature of *Aspergillus nidulans, A. sulphureus, A. sydowi, Aspergillus terreus, Sporotrichum pulverulentum, Stachybotrys atra* and *Syncephalastrum racemosus* was recorded for first time in eggplant as new records in India. Prevalence of these fungal isolates on seed of other crop confirmed their seed borne nature. Yago et al., (2011) reported *Aspergillus nidulans* from *Sorghum* seeds. *Aspergillus terreus* from infested maize seeds (Shirpurkar and Wahegaonkar, 2012), *Aspergillus sydowi* from *okra* seeds (Bhajbhuje, 1989), *Sporotrichum pulverulentum* from oil seeds (Jain, 2008), *Stachybotrys atra* from Niger seeds (Nagaraja and Krishnappa, 2011), *Syncephalastrum recemosum* was associated with seeds of *Bixa orellana* (Venugopalan and Giridhar, 2012).

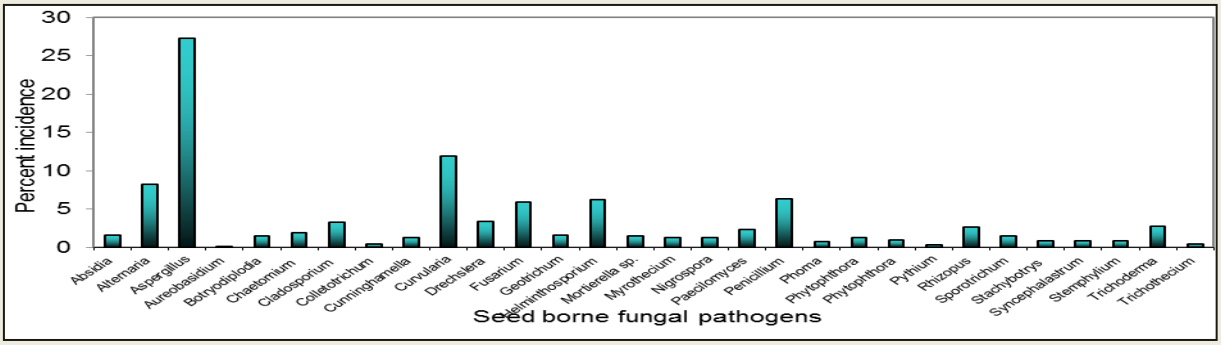

Fig. 4: Percent incidence of fungal flora from mixed seed samples of different cultivars of *Solanum melongena* L

These reports are in conformity of seeds borne nature for first time isolated fungal pathogen from mixed seed samples of eggplant.

The efficacy of both standard blotter and agar plate tests varied with nature of fungi. The members of *Oomycota* and *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, standard blotter method was proved comparative superior over agar plate method to the fungal pathogens isolation. Chukunda et al., (2013) 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. These variations may possibly attribute to the prolonged incubation that might lead to the development of deep seated infection (Jain, 2008). The physiochemical nature of the seed as well as agricultural practices and storage environment provided for the different crop seeds are also possibly responsible to variation in two methods (Yago et al., 2011; Venugopalan and Giridhar, 2012). Other possibility for such divergence might be attributed to comparable rapid growth of the saprophytes adhering to the seed surface and making of growing pathogenic forms (Nagaraja and Krishnappa, 2011;

Chukunda et al., 2013). Mycological analysis of disinfected and non-disinfected seeds gave only general information about inner seed infection, with assuming that fungal propagules exist in non-disinfected seeds and absent in disinfected seeds and that fungi were contaminated their surface and they did not penetrate the inner tissues. This information, although not very precise, can be a starting point to determine proper strategies of seed treatment.

The fungal isolates belong to genera, *Aspergilli* and *Penicilli* of *Ascomycotina* as well as *Alternaria, Curvularia, Fusarium, Helminthosporium* of *Deuteromycotina* contributed as major components on eggplant seeds (Fig. 4); 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* had comparatively maximum count of isolates (Fig. 1) and greatest frequency of fungal incidence on eggplant seeds followed by Ascomycota (Fig. 3). It may possibly due to prevalence of maximum count of fungal flora 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 (Ismael, 2010). Under storage, in moist environment the seeds form the ideal organic substrate to the development of storage fungi (Jain, 2008). Members of *Deuteromycota* complete their life cycle asexually producing numerous resistant, thick walled conidia which may remain viable for longer duration in adverse climatic environment (Jyoti and Malik, 2013). The conidia *Cladosporium, Alternaria, Helminthosporium, Trichothecium, and Curvularia* were remained in greatest abundance under storage even at low humidity, generally during warmer climate (Jain, 2008). It was interesting to record that members of *Basidiomycotina* rarely persist on eggplant 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.

The report of the present study revealed that *Ascomycota* genera, *Aspergilli and Penicilli* which were the highly predominant on eggplant seeds are among the most abundant and widely distributed organisms on the globe (Venugopalan and Giridhar, 2012). Members of the genus *Aspergillus* are known obligate saprophyte and survive in the environment without causing disease (Jyoti and Malik, 2013). *Aspergillus fumigatus, A. parasiticus, A. niger. A. amstelodomi, 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 (Jain, 2008). *Aspergillus niger* has potential to produce *ochratoxin-A*. *Aspergillus flavus* secretes aflatoxin B_1, B_2, G_1 & G_2 and other toxic compounds including *strigmatocystin, cyclopiazonic acid, kojic acid, β-nitropropionic acid, aspertoxin, aflatrem, gliotoxin* and *aspergillic acid*. *Penicillium* have been reported as a common opportunistic pathogen, 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). Members of *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. *Fusarium* contributed with 5.9% incidence, 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 the eyes and fingernails (EFSA, 2011). Several species of *Alternaria* are reported to secrete *Altersolarol-A* and *alternaric acid dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III* (Brakhage and Schroeckh, 2011).

The susceptible cultivars of *Solanum melongena* L are affected with several fungal diseases including Damping off (*Pythium* spp, *Phytophthora* spp, *Rhizoctonia* spp.), Phomopsis blight (*Phomopsis vexans*), leaf spot (*Cercospora melongenae*) Alternaria leaf spot (*Alternaria melongenae*), Alternaria early blight (*Alternaria solani*), fruit rot (*Phytophthora nicotianae*) and Verticillium wilt (*Verticillium dahlia*). Among them *Alternaria* early blight is a serious, caused by a seed borne fungal pathogen, *Alternaria solani* 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. It caused lesion formation on leaves and fruits resulting in premature defoliation, reduction in size & quality of fruits, and reported to reduce the productivity to the extent of 20-30% (Nishikawa et al., 2006; Bhajbhuje, 2013).

The data obtained in the present survey indicated that eggplant seeds harbor 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 (Jain, 2008). 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. The growth of the isolated fungi results in changes associated with various cellular, metabolic and chemical alterations, including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, enzymes degradation & inactivation, loss of membrane integrity, lowering of ATP, decline in sugar and protein content, inability of ribosomes to dissociate, changes in nutritive quality, starvation of meristematic cells, increase in seed leaches and fatty acid content, reduced respiration and accumulation of toxic substances which 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 (ESFA, 2011).

Mutagenic and carcinogenic effect of mycotoxins has been highlighted by ESFA (2011). The mycotoxins are known to cause chromosomal breakage, create disturbances in normal mitotic cell division, alter regular metabolism & cell membrane permeability and also induced physiological and biochemical changes in host cells, resulting in the rapid increase of electrolyte loss and decrease in the membrane potential of metabolically active meristematic cells of the plant system (Bhajbhuje, 2013). They induced micro-mutation, cause carcinogenic disorders in experimental animals and also pose variety of health hazards in domestic animals and human beings (ESFA, 2011). It was interesting to record that primary metabolites secreted by *Alternaria alternata* at early stages of its growth, act as growth promoter, and induced vigorous growth of eggplant seedlings by stimulating phosphorylation in the tissues (Bhajbhuje, 2013). More than 300 fungal metabolites are reported to be toxic to man, animals and pose serious health hazard (Brakhage and Schroeckh, 2011).

Majority of fungal isolates involved in seed deterioration of eggplant are xerophilic moulds such as *Aspergilli* and *Penicilli* of *Ascomycotina* as well as *Alternaria, Curvularia, Fusarium, Helminthosporium* of *Deuteromycotina* (Bhajbhuje, 1989). After planting of deteriorated seeds, seedling emergence may be poor and increases chances of pathogen transmission to a new crop.

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

Seeds are important input for crop production hence pathogen free healthy seeds are considered as the vital factor for desired plant population and good economic harvest. On the basis of present observations, it has been concluded that all the seed lots of eggplant are more vulnerable to fungal attack and carried greater count of fungal propagules on seed surface, leads to deterioration of nutritional components and may posed damage to the seeds. The internal seed borne pathogens may involve in eggplant diseases in Vidarbha. 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.

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REFERENCES

Anonymous (2011) Small fruit and vegetable. IPM-advisory – Integrated Pest Management Utah State University cooperative extension.

Bhajbhuje MN (1989) Investigation of the seed mycoflora associated with vegetable seeds from Vidarbha region. Ph.D. Thesis RTM Nagpur University, Nagpur.

Bhajbhuje MN (2013) Karyotoxicity of fungal metabolites in *Solanum melongena* L . *Journal. Science. Information*, 6 : 1-5.

Brakhage AA and Schroeckh V (2011) Fungal secondary metabolites in strategies of activate silent gene of clusters. *Fungal Gene. Biol.,* 48(1) : 15-22.

Chukunda FR, Osakwe JA and RE Boraka (2013) Control of seed borne fungi of stored maize from Nigerian Stored Products Research Institute Port Harcount. *WebPub J. Agric. Res*., 1920 : 98-21.

EFSA (2011) Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal*, 9(10):2407.

FTRNR (2012) Uses and medicinal benefits of eggplant. Food Technologies Resource, News & Review http/www.foodrecap.net/health/talisary-benefits, Aug, 2011) (accessed August 5, 2013)

Grafware (2013) Seed mycoflora of brinjal *Solanum melongena* Natural Science reporting htm. (accessed August 5, 2013)

Habib A, Sahi ST, Ghazanfar MU and S Ali (2007) Location of seed-borne mycoflora of eggplant (*Solanum melongena* L.) in different seed components and impact on seed germinability. *International Journal of Agricultural Biology*, 9(3) : 514-516.

IHD (2012) Indian Horticulture Database. Source FAO website March 2012 & for India – Indian H. Database (accessed August 5, 2013).

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.

ISTA (2012) Seed Testing International ISTA News Bulletin No. 143. Zurich, Switzerland.

Jain PC (2008) Microbial degradation of grain, oil seeds, wood corrosion of metals and bioleaching of ores. Applied Microbiology nsdl.niscair,res.in/Microbial Dedradation pdf (assessed on25th July 2013)

Jyoti and CP Malik (2013) Seed deterioration: A review. *International Journal of Life Sci. Biotechnology & Pharma Res.*, 2(3) : 373-386

Kulkarni AU, Chauhan AM, Kesare, UT and SM Pawar (2012) Effect of nitrogen and phosphorus sources on amylase production in seed born fungi of maize. *Current Botany*, 3(4): 01-04.

Kuri SK, Islam MR and U Mondal (2011) Antifungal potentiality of some botanical extracts against important seed borne fungal pathogen associated with brinjal seeds, *Solanum melongena* L. *Jour. of Agricultural technology*, 7(4);1139-1153.

Nagaraja O and Krishnappa M (2011) Seed borne mycoflora of Niger (Guizotia Abyssinia Cass) and its effect on germination. *Indian Phytopathology,* 62(4) : 210-214.

Nishikawa J, Kobayashi T, Shirata K, Chibana T and K Natsuki (2006) Seed borne fungi detected on stored solanaceous berry seeds and their biological activities. *Journal Gen. Plant Pathology*, 72:305-313.

Saskatchewan (2013) Guideline for seed borne diseases of pulse crops. Agricultural Knowledge Centre at 1-866-457-2377 www.agriculture.gov.sk.ca/seed-testinglabs. (Accessed August 5, 2013)

Shirurkar DD and NK Wahegaonkar (2012) Antifungal activity of selected plant extracts derived oils and some fungicides against seed borne fungi of maize. *European Journal of Experimental Biology*, 2(5) : 1693-1396.

Venugopalan A and P Giridhar (2012) Mycoflora associated with seeds of *Bixa orellana*. *African Journal of Microbiology Res*. 6(9):2091-2094.

Yago JI, Roh JH, Bae SD, Yoon YN, Kim HJ, and MB Nam (2011) The effect of seed borne mycoflora from Sorghum and Foxtain Millets on germi

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