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Distribution of micro-fungal propagules in storage on seeds of *Lycopersicon esculentum* Mill

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ABSTRACT

Seed is 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. The high level seed moisture content, relative humidity and suitable temperature support the fungal microbes to contaminate essential seed components causing multi-fold losses. A total of twenty seed samples of five cultivars of *Lycopersicon esculentum* Mill. obtained from various locations of Central India (Vidarbha), were mycologicaly analyzed by standard seed health testing technique revealed the prevalence of 49 diverse fungal species belonging to 29 genera from mixed seed samples, with higher count of species and percent incidence for *Fusarium* and *Aspergillus* followed by *Curvularia* (55%), *Alternaria* (42%) *Rhizoctonia* (33%) while others had moderate to low incidence. *Deuteromycota* contributed highest, 46.9% fungal count followed *Ascomycota* (38.9%); *Zygomycota* and *Oomycota* (6.1%). A least count was recorded with *Basidiomycota*. *Aspergillus nidulans, A. sulphureus, A. ochracious, A. amstelodomi, Fusarium moniliformae, F. semitectum, Geotrichum candidus, Paecilomyces varioti, Penicillium oxalicum* and *Sporotrichum pulverulentum* have been recorded on tomato seeds for the first time in India. *Deuteromycota* contributed more than half, 52.1% incidence followed by *Ascomycota* (35.1%); *Zygomycota* (5.9%) and Oomycota (5.2%) while *Basidiomycota* contributed little incidence. A total of 28 to 36 isolates belongs to 19 to 23 genera were confined to individual cultivar seeds. *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici and Phytophthora infestans* were appeared to be common on seeds of all cultivars as external and internal seed borne pathogens. *Drachslera rostrata, Fusarium culmorum, F. equiseti, Rhizopus stolonifer, Rhizoctonia solani* were confined to blotter paper while *Chaetomium globosum, Curvularia ovoides, Fusarium solani, Geotrichum candidum* were restricted to agar plating. Greatest count of fungal isolates and percent incidence was recorded by blotter test against agar plating. The standard blotter technique proved superior over agar plating.

KEYWORDS

Lycopersicon esculentum Mill., *Seed borne pathogen, isolates, incidence, frequency, cultivars.*

INTRODUCTION

Seed constitute the basic input for crop production in phanerogams so that pathogen free healthy seeds are considered as the vital factor for desired plant population and good economic harvest. Seeds are known to be contaminated with diverse fungal micro-propagules, some of them attack the seeds in the field internally contaminating the seed sheaths, tissues of embryo, endosperm and others under storage as a result of environmental conditions of high relative humidity, suitable temperature and high level of seed moisture content. The seed carrying organism's causes manifold loses to the crop and reduced the agricultural productivity (Bhajbhuje, 2013). Seed-borne diseases are able to spread across international borders very easily and are often difficult to identify as their typical symptoms being rare on seed surfaces as economic impact and importance has increased in recent years with regards to many kinds of crop worldwide (Lew-Smith, 2013). Planting infected seeds result in a widespread distribution of diseases within the crop, and an increased count of initial infection sites from which 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 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% (Lew-Smith, 2013).

Seed deterioration is an inexorable, continuous and irreversible process, involves succession of seed borne fungal pathogens under storage resulting in loss of seed

nutrients, alteration of physio-chemical properties of seeds, loss in seed weight, seed viability & vigour, medicinal properties, aesthetic changes including discoloration, heating & mustiness, cracking and abnormal odors contributing seed losses to the extent of 24% (Bhajbhuje, 1989). The consequences deterioration leading to series of deteriorative changes include membrane degradation, toxic metabolites accumulation, loss of enzymatic activity, lipid autoxidation, failure of repair mechanisms, genetic degradation, reduced productivity, finally loss of germinability or death of seed (Debnath et al., 2012). Some fungal propagules may bring about certain biochemical changes and toxic metabolites that elicit a toxic response such as carcinogenicity, genotoxicity, terrotogenicity, hepatotoxicity, immunosuppression etc. Secondary fungal metabolites are reported to be toxic to man, animals and pose serious health hazard (Jain, 2008; Brakhage and Schroeckh, 2011; Shephard, 2012; Jyoti and Malik, 2013).

Lycopersicon esculentum Mill is low calorie fruit vegetable for human nutrition belongs to Solanaceae family, originated in Mexico and presently grown across all continents on the globe for its edible red fruits. The ripe fruit is rich source of carotene lycopene, flavonoids, anti-oxidants, dietary fibres, Vit-C; moderate level of vital B-complex such as folates, thiamin, niacin, riboflavin; Vit-E, biotin., citric acid, oxalic acid, maleic acid and essential minerals such as calcium, phosphorus, iron, sodium and potassium; very low fat content and zero cholesterol level. Literature review suggest that fresh ripe fruits are consumed as vegetable and widely used for salad, soups, pickles, sauces, ketchups and homemade skin remedy preparation. Fresh fruit juice induces vomiting in case of food poisoning; reduced risk of cancer of breast, head, neck and lower urinary tract infection; removes excess oil from skin when used in combination with cucumber. Topical dosages of mixture of fruit juice and honey produce glowing effect to skin. The lycopene content of ripe fruit juice has property to lower blood pressure reducing cardiovascular risk associated with diabetes; induce uric acid lowering effect; helps keeping the looking youthful and might be strongly protective against neurodegenerative diseases (Singh, 2013). Its pulp and juice is digestible, a mild aperient, a promoter of gastric secretion and a blood purifier hence considered useful for bronchitis and asthma patients. It stimulates torpid liver, is good remedy for chronic dyspepsia and intestinal antiseptic. It is used as febrifuge to prevent excessive bleeding from wound, to treat edema of pregnant women, as cathartic for kidney and liver complaints. Flavonoid compound *'Zea-xanthin'* helps to protect eyes by filtering harmful UV rays (Singh, 2013). A total of 1.8 million Indian farmers grow tomato on an area of 8,65,000 hectares in 2011-12. India ranks second leading producer contributing around 11% of the global annual harvest after China (28%); other top producing eight countries include USA, Turkey, Egypt, Italy, Iran, Spain, Brazil and Uzbekistan (Singh, 2013). Vidarbha is the eastern part of Maharashtra, located in the centre of India, occupying 31.6% of total area and holds 21.3% of total population of Maharashtra. Vidarbha economy is primary agricultural. Farmer cultivates this crop round a year as fruit vegetable crop in addition to traditional main cash crops viz, cotton, oranges, soya beans & oil seeds. Mostly, poor quality seeds, traditional storage environment and seasonal Vidarbha climate favours fungal micro-propagules proliferate to cause diseases leads to reduction of productivity to a greater extend.

Lycopersicon esculentum Mill., a highly preferred fruit cash crop for poor farmers originated from healthy seed, is prone to attack by diverse group of fungal pathogens and spread of diseases causing 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 Nishikawa et al., (2006); Ismael (2010); Zakaria (2011); Igjjatav et al., (2012) and Clemson (2013). Literature review suggest that a little is known from the Vidarbha concerning to biodiversity of fungal isolates adhering to *Lycopersicon esculentum* Mill. stored seeds. It seems worthwhile considered that data on the diversity of fungal species of Vidarbha 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. The present paper reports the detail findings of studies on distribution of fungal micro-propagules confined to stored seeds of *Lycopersicon esculentum* Mill from Vidarbha region of Maharashtra state.

MATERIALS AND METHODS

a. Selection of plant material: Seeds of common cultivars, *Pusa Rubi, Pusa Early Dwarf, Sioux Co.-1* and *ART-1* of *Lycopersicon esculentum* Mill. were selected as an experimental material.

b. Collection of plant material: A total of twenty seed samples of five selected cultivars were obtained from different cultivators from all districts of Vidarbha in polythene bags, brought to laboratory and immediately transferred aseptically to cloth bags.

c. Screening of seed samples: Preliminary seed samples were screened for apparent deformities or discoloration employing dry examination technique.

d. Isolation of fungal pathogens: Seed samples of individual and mixed cultivars were screened for prevalence of fungal pathogens employing standard seed health tests (ISTA, 2012).

Of the randomly selected four hundred seeds, 50% seeds without pretreatment were placed in ten sterilized petri plates containing three layered sterile moistened absorbent blotter papers for isolation of external seed borne fungal flora. The remaining 50% 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 plating technique. The pretreated twenty seeds were transferred aseptically to each sterile petri plate containing semi-solid agar nutrient sterile medium composed of peeled potato ($400gm^{-1}$), dextrose ($20gm^{-1}$**)** and agar ($20gm^{-1}$**)** in a liter of distilled water. All petri plates were allowed incubate for seven days in B.O.D incubator at 25±2°C under alternating cycles of 12 hours light and darkness.

e. Identification of fungal isolates: After examination of incubated seeds under stereoscopic microscope the fungal growth appearing on surfaces was identified on the basis of colony colour and sporulation type. Fungal isolate count 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.

RESULTS AND DISCUSSION

Seed is primary mean to transmission and the spread of seed borne disease in seed producing plants. Fungal micro-propagules infect healthy seed mostly when they either 'crawl' all the way to seed on the outside of the plant, or else send out spores that land on the seed. They tend to be restricted on outside, in layers of seed coat or reside inside embryonic cells of seed. Spores on seed coat are more to either dry up or die, or else to get sloughed off with seed coat during seed germination, initiate infection and proceed to spread by attacking cells of outer layers, thereby falling to cause disease on next generation of plants (Lew-Smith, 2013). Seed infection by fungal organisms and prevalence of their propagules in seed lots is vitally important because infected seed(s) may fail to germinate, cause infection to seedlings and make these unhealthy (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 *Lycopersicon esculentum* Mill. seeds (Bhajbhuje, 1989).

Mycological analysis of mixed seed samples of five different commonly grown cultivars of *Lycopersicon esculentum* Mill obtained from farmers of Vidarbha revealed the prevalence of total 49 fungal pathogens belongs to 29 genera on seed surfaces in varying incidence. Of these, isolates belong to *Deuteromycota* are most predominant ones, represented by 13 genera and 23 species followed by *Ascomycota*, contributing 11 genera and 19 species. *Oomycota* and *Zygomycota* were represented by 2 genera and 3 species while *Basidiomycetes* had only one genus and single species. Individual genus, *Fusarium* dominated with 8 species, while *Aspergillus* was represented by 7 species. Two species of *Alternaria, Chaetomium, Curvularia, Penicillium, Phytophthora, Rhizoctonia* and *Rhizopus* have been encountered while others had single species. Of the total isolates, *Aspergillus nidulans, Aspergillus sulphureus, Aspergillus ochracious, Aspergillus amstelodomi, Fusarium moniliformae, F. semitectum, Geotrichum candidus, Paecilomyces varioti* and *Penicillium oxalicum* has been recorded as seed borne fungal pathogens for the first time in India from stored seeds lots of *Lycopersicon esculentum* Mill (Table 1 & 2).

Percent incidence of seed borne fungal pathogens from mixed seed samples of all cultivars was presented in Table 1. A total 21 fungal species of 11 genera including *Alternaria porri, A. solani, A. flavus, A. fumigatus, A. niger, Aureobasidium sp., Botrytis cinera, Colletotrichum capsici,, Curvularia lunata, C. ovoides,, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, F. oxysporum, F. semitectum, F. solani, Fusarium sp., Penicillium oxalicum, P. nigricans*, *Pythium aphanidermatum and Phytophthora infestans* have been encountered in stored seeds as both external and internal seed borne by both seed health tests.

Of the total isolates, *Curvularia lunata* (33%) and *Aspergillus fumigatus* (26%) were appeared to be predominant exhibiting higher incidence followed by *Aspergillus flavus* (24%), *Alternaria porri* (23%), *Fusarium lycopersici* (22.5%), *Curvularia ovoides* (22%) and *Aspergillus niger* (21%). *Alternaria solani, Colletotrichum capsici, Fusarium semitectum, Penicillium oxalicum* and *Phytophthora infestans* were recorded subdominant with fungal incidence varied between 13.0 to 18.5% while others had 6.5 to 9.5% incidence. Low count of fungal incidence was detected for *Aureobasidium sp., Pythium aphanidermatum and Fusarium oxysporum* by both health testing techniques.

Prevalence of 19 fungal species belongs to 16 genera in varying frequency were confined to blotter test only. These isolates included *Chaetomium sp., Cladosporium tenuissium, Corynespora cassicola, Drachslera rostrata, Fusarium culmorum, F, equiseti,, Myrothecium roridum, Paecilomyces variotii, Phoma destructiva, Phytophthora parasitica, Rhizoctonia solani, R. bataticola, Rhizopus stolonifer, Rhizopus sp., Sporotrichum pulverulentum, Stachybotrys atra,, Syncephalastrum racemosus, Trichoderma viride, and Trichothecium roseum.* Among these, *Rhizoctonia solani* was appeared to be most dominant with 18% incidence followed by *Rhizopus stolonifer* and *Rhizoctonia bataticola* (15%).

Table 1: Percent fungal incidence in storage on *Lycopersicon esculentum* Mill. Seeds.

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			LE cv.-1		LE cv. - 2		LE cv. - 3		LE cv.- 4		LE cv. - 5			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
A	**Oomycota**															
1	*Phytophthora infestans* de Bary	10.0 (1.85)	8.0 (1.48)	18.0 (3.33)	4.0 (1.81)	2.5 (1.13)	2.5 (0.94)	2.0 (0.75)	5.0 (1.82)	2.0 (2.73)	5.0 (2.20)	2.5 (1.10)	8.0 (3.39)	2.0 (0.85)	35.5 (2.90)	4.86
2	*P. parasitica* Dastur	4.0 (0.74)	-	4.0 (0.74)	-	-	-	12.0 (4.38)	12.0 (4.38)	-	-	-	-	-	24.0 (1.96)	
3	Pythium aphanidermatum (Eis.) Fitz.	4.0 (0.74)	2.0 (0.37)	6.0 (1.11)	2.5 (1.13)	-		-							2.5 (0.20)	0.20
	Total frequency	**18.0 (3.33)**	**10.0 (1.85)**	**28.0 (5.18)**	**6.5 (2.94)**	**2.5 (1.13)**	**2.5 (0.94)**	**14.0 (5.26)**	**17.0 (6.20)**	**2.0 (2.73)**	**5.0 (2.20)**	**2.5 (1.10)**	**8.0 (3.39)**	**2.0 (0.85)**	**62.0 (5.07)**	
B.	**Zygomycota**															
4	*Rhizopus stolonifer* (Ehrarb. Ex.Fr.) Lind	15.0 (2.78)	-	15.0 (2.78)	18.0 (8.15)	-	20.0 (7.52)	-	11.0 (4.01)	-	8.5 (3.74)	-	10.0 (4.24)	-	67.5 (5.51)	9.44
5	*Rhizopus sp.*	14.0 (2.59)	-	14.0 (2.59)	18.0 (8.15)	-	18.00 (6.77)	-	12.0 (4.38)	-	-	-	-	-	48.0 (3.92)	
6	*Syncephalastrum racemosus* (Cohn.) Schroet.	3.0 (0.56)	-	3.0 (0.56)	7.0 (3.17)	-	-	-	-	-	-	-	3.0 (1.27)	-	10.0 (0.82)	0.82
	Total frequency	**32.0 (5.93)**	**-**	**32.0 (5.93)**	**43.0 (19.46)**	**-**	**38.0 (14.29)**	**-**	**23.0 (8.39)**	**-**	**8.5 (3.74)**	**-**	**13.0 (5.51)**	**-**	**125.5 (10.25)**	
C	**Ascomycota**															
7	*Aspergillus amstelodomi (M) Thom & Church*	-	9.0 (1.67)	9.0 (1.67)	-	-	-	-	-	1.0 (0.36)	-	-	-	2.5 (1.06)	3.5 (0.29)	21.7
8	*A. flavus* Link	15.5 (2.87)	8.5 (1.57)	24.0 (4.44)	7.5 (3.39)	5.5 (2.49)	9.0 (3.38)	3.5 (1.32)	3.5 (1.28)	2.5 (0.91)	9.0 (3.96)	2.5 (1.10)	5.5 (2.66)	5.0 (2.12)	53.5 (4.37)	
9	*A. fumigatus* Fres.	20.5 (3.80)	5.5 (1.02)	26.0 (4.82)	8.5 (3.85)	8.5 (3.85)	8.0 (3.01)	4.5 (1.69)	14.0 (5.11)	4.5 (1.64)	15.0 (6.61)	7.5 (3.30)	13.5 (5.72)	5.0 (2.12)	89.0 (7.27)	
10	*A. nidulans* (Eidem) Winer	-	5.5 (1.02)	5.5 (1.02)	-	-	-	-	-	-	-	2.5 (1.10)	-	-	2.5 (0.20)	
11	*A. niger* Van Tieghan	14.5 (2.69)	6.5 (1.20)	21.0 (3.89)	9.5 (4.30)	7.5 (3.39)	17.5 (6.58)	8.0 (3.01)	18.5 (6.75)	7.5 (2.74)	15.0 (6.61)	7.5 (3.30)	13.5 (5.72)	5.0 (2.12)	109.5 (8.95)	
12	*A. ochracious* Wihelm	-	5.0 (0.93)	5.0 (0.93)	-	2.0 (0.90)	-	-	-	-	-	-	-	-	2.0 (0.16)	
13	*A. sulphureus (Fr.) Thom & Church*	-	5.0 (0.93)	5.0 (0.93)	-	1.0 (0.45)		5.0 (1.88)		-	-	-	-	-	6.0 (0.49)	
14	*Aureobasidium sp*	4.0 (0.74)	2.0 (0.37)	6.0 (1.11)	1.0 (0.45)	2.0 (0.91)	1.0 (0.38)	0.5 (0.19)	-	2.5 (0.91)	-	-	-	2.5 (1.06)	9.5 (0.78)	0.78
15	*Botrytis cinera* Pers.	8.0 (1.48)	2.0 (0.37)	10.0 (1.85)	2.0 (0.91)	1.0 (0.45)	3.0 (1.13)	0.5 (0.19)	2.5 (0.91)	1.5 (0.55)	1.5 (0.66)	1.5 (0.66)	2.5 (1.06)	0.5 (0.21)	16.5 (1.35)	1.35
16	*Chaetomium glabosum Kunze & Schm.*	-	4.5 (0.83)	4.5 (0.83)	-	2.5 (1.13)	-	4.5 (1.69)	-	2.5 (0.91)	-	3.5 (1.54)	-	3.0 (1.27)	16.0 (1.31)	1.51
17	*Chaetomium* sp	4.0 (0.74)	-	4.-0 (0.74)	-	-	-	-	-	2.5 (0.91)	-	-	-	-	2.5 (0.20)	

Table 1: Continued...

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			LE cv.-1		LE cv. – 2		LE cv. - 3		LE cv.- 4		LE cv. - 5			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
18	*Cladosporium tenuissium* Cooke	14.0 (2.59)	-	14.0 (2.59)	2.5 (1.13)	2.0 (0.91)	1.5 (0.56)	1.0 (0.38)	9.0 (3.28)	2.5 (0.91)	14.0 (6.17)	3.5 (1.54)	9.0 (3.81)	2.5 (1.06)	47.5 (3.88)	3.83
19	*Dendrophoma sp*	-	5.0 (0.93)	5.0 (0.93)	-	3.0 (1.36)	-	-	-	-	-	-		5.0 (2.12)	8.0 (0.65)	0.65
20	*Didymella lycopersici Kleb.*	4.5 (0.83)	2.0 (0.37)	6.5 (1.20)	2.5 (1.13)	2.5 (1.13)	3.5 (1.32)	3.0 (1.13)	4.0 (1.46)	1.0 (0.36)	0.5 (0.22)	0.5 (0.22)	2.5 (1.06)	1.0 (0.42)	21.0 (1.72)	1.72
21	*Myrothecium roridum* To de ex. Fr.	6.0 (1.11)	-	6.0 (1.11)	-	-	-	-	-	-	-	-	4.0 (1.69)	-	4.0 (0.33)	0.33
22	*Penicillium oxalicum* Currie & Thom	11.0 (2.04)	2.0 (0.37)	13.0 (2.41)	2.5 (1.13)	3.5 (1.58)			15.5 (5.66)	2.5 (0.91)	-	-	4.5 (1.91)	2.0 (0.85)	30.5 (2.49)	2.94
23	*Penicillium* sp	4.0 (0.74)	3.0 (0.56)	7.0 (1.30)	-	-	3.0 (1.13)	-	-	-	-	2.5 (1.10)	-	-	5.5 (0.45)	
24	*Phoma destructiva* Flower	14.0 (2.59)	-	14.0 (2.59)	2.0 (0.91)	-	-	-	-	-	-				2.0 (0.16)	0.16
25	*Ulocladium chartarum* (Ft.) Simmson	-	4.0 (0.74)	4.0 (0.74)	-	2.0 (0.91)	-	-	-	-	-	2.0 (0.88)	-		4.0 (0.33)	0.33
	Total frequency	120.0 (22.22)	69.5 (12.87)	189.5 (35.09)	38.0 (17.19)	43.0 (19.46)	46.5 (17.48)	30.5 (11.47)	67.0 (24.45)	30.5 (10.95)	55.0 (24.23)	33.5 (14.76)	55.0 (23.31)	34.0 (14.41)	433.0	
D.	**Basidiomycota**															
26	*Sporotrichum pulverulentum* Nov…Cain & Grover	9.0 (1.67)	-	9.0 (1.67)	-	-	-	-	4.5 (1.64)	-	-	-	-	-	4.5 (0.37)	0.37
	Total frequency	**9.0 (1.67)**	**-**	**9.0 (1.67)**	**-**	**-**	**-**	**-**	**4.5 (1.64)**	**-**	**-**	**-**	**-**	**-**	**4.5 (0.37)**	**0.37**
E.	**Deuteromycota**															
27	*Alternaria porri* (Ell.)	14.5 (2.69)	8.5 (1.57)	23.0 (4.26)	9.5 (4.30)	5.0 (2.26)	5.0 (1.88)	4.0 (1.50)	6.5 (2.37)	4.0 (1.46)	8.0 (3.52)	4.0 (1.76)	8.5 (3.60)	2.0 (0.85)	56.5 (4.62)	9.4
28	*Alternaria solani (E & M) Jones & Grout*	14.5 (2.69)	4.0 (0.74)	18.5 (3.43)	7.5 (3.39)	5.0 (2.26)	7.5 (2.82)	5.0 (1.88)	5.5 (2.01)	2.5 (0.91)	7.5 (3.30)	6.0 (2.64)	7.5 (3.18)	4.5 (1.91)	58.5 (4.78)	
29	*Colletotrichum dematium* (Pers.Ex zFr.) Grov.	8.0 (1.48)	6.0 (1.11)	14.0 (2.59)	5.0 (2.26)	2.5 (1.13)	6.0 (2.26)	2.5 (0.94)	3.0 (1.09)	2.5 (0.91)	3.5 (1.54)	3.0 (1.32)	3.0 (1.27)	2.0 (0.85)	33.0 (2.70)	2.70
30	*Corynespora cassiicola Gussow*	12.0 (2.22)	-	12.0 (2.22)	-	-	4.5 (1.69)	-	-	-	-	-	-	-	4.5 (0.37)	0.37
31	*Curvularia lunata* (Wakker) Boedijn	24.5 (4.54)	8.5 (1.57)	33.0 (6.11)	4.0 (1.81)	3.0 (1.36)	3.5 (1.32)	2.5 (0.94)	6.0 (2.19)	3.5 (1.28)	6.0 (2.64)	2.0 (0.88)	4.0 (1.69)	3.0 (1.27)	37.5 (3.06)	4.17
32	*Curvularia ovoidea* (Hirosa & Watan) Munt.	13.0 (2.41)	9.0 (1.67)	22.0 (4.07)	-	0.5 (0.23)	-	2.0 (0.75)	-	5.0 (1.82)	-	2.0 (0.88)	-	4.0 (1.69)	13.5 (1.10)	
33	*Drachslera rostrata* (Drechsler) Rich. & Fraser	4.0 (0.74)	-	4.0 (0.74)	2.0 (0.91)	-	4.0 (1.50)	-	3.0 (1.09)	-	5.0 (2.20)	-	4.0 (1.69)	-	18.0 (1.47)	1.47
34	*Fusarium culmorum* (Smith) Sacc.	4.0 (0.74)	-	4.0 (0.74)	2.0 (0.91)	-	14.0 (5.26)	-	12.0	-	2.0 (0.88)		4.0 (1.69)		34.0 (2.78)	
35	*F. equiseti* (Corda) Sacc.	6.0 (1.11)	-	6.0 (1.11)	4.0 (1.81)	-	12.0 (4.51)	-	6.0 (2.19)	-	12.0 (5.29)		4.0 (1.69)		38.0 (3.10)	

Table 1: Continued...

Sr. No.	Name of fungal isolate	Frequency (%) of fungal incidence													Total frequency	% over total incidence
		Mixed seed samples of all cultivars			LE cv.-1		LE cv. - 2		LE cv. - 3		LE cv.- 4		LE cv. - 5			
		Blotter	Agar	Total	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar	Blotter	Agar		
36	*F. moniliformae* Sheldom	5.0 (0.93)	2.0 (0.37)	7.0 (1.30)	2.5 (1.13)	2.5 (1.13)	1.5 (0.56)	1.5 (0.56)	4.5 (1.64)	6.5 (2.37)	6.5 (2.86)	6.5 (2.86)	4.0 (1.69)	2.0 (0.85)	38.0 (3.10)	
37	*F. lycopersici* Brushi	14.0 (2.59)	8.5 (1.57)	22.5 (4.16)	2.5 (1.13)	1.5 (0.68)	6.0 (2.26)	4.0 (1.50)	6.0 (2.19)	7.5 (2.74)	9.0 (3.96)	7.0 (3.08)	19.0 (8.05)	8.5 (3.60)	71.0 (5.80)	
38	*F. oxysporum* Sheldom	3.0 (0.56)	2.0 (0.37)	5.0 (0.93)	-	-	1.0 (0.38)	5.0 (1.88)	3.5 (1.28)	2.0 (2.73)	-	-	8.0 (3.39)		19.5 (1.59)	
39	*F. semitectum* Berk & Rav.	11.5 (2.13)	6.0 (1.11)	17.5 (3.24)	-	-	2.0 (0.75)	2.5 (0.94)	-	-	-	-	1.0 (0.42)	1.5 (0.64)	7.0 (0.57)	
40	*F. solani (Mert.) App &* Wollenw	4.0 (0.74)	2.5 (0.46)	6.5 (1.20)	-	2.0 (0.91)	-	2.0 (0.75)	-	4.0 (1.46)	-	4.0 (1.76)	-	2.0 (0.85)	14.0 (1.14)	
41	*Fusarium sp*	6.0 (1.11)	3.5 (0.65)	9.5 (1.76)	1.5 (0.68)	1.5 (0.68)	-	-	-	-	-	-	-	-	3.0 (0.25)	
42	*Geotrichum candidum* Link ex Fr.	-	7.0 (1.30)	7.0 (1.30)	-	1.0 (0.45)	-	2.0 (0.75)		3.5 (1.28)	-	3.5 (1.54)	-	1.5 (0.64)	11.5 (0.94)	0.94
43	*Helminthosporium tetramera* Mc Kirmey	-	9.0 (1.67)	9.0 (1.67)	-	-	-	-	-	4.5 (1.64)	-	5.5 (2.42)		-	10.0 (0.82)	0.82
44	*Paecilomyces variotii* Bainier	10.0 (1.85)	-	10.0 (1.85)	-	4.5 (2.04)	-	14.0 (5.26)	-	-	-	4.5 (1.98)	-	-	23.0 (1.88)	1.88
45	Rhizoctonia solani Kuehn	18.0 (3.33)	-	18.0 (3.33)	14.0 (6.33)	-	14.0 (5.26)	-	18.0 (6.57)	-	12.0 (5.29)	-	14.5 (6.14)	-	72.5 (5.92)	7.52
46	*R. bataticola (Trabu.) Butler*	15.0 (2.78)	-	15.0 (2.78)	2.0 (0.91)	-	-	-	6.0 (2.19)	-	-	-	11.5 (4.87)	-	19.5 (1.59)	
47	*Stachybotrys atra* Corda.	10.0 (1.85)	-	10.0 (1.85)	-	2.5 (1.13)	-	2.5 (0.94)	-	-	-	-	-		5.0 (0.41)	0.41
48	*Trichoderma viride* Pers. Ex.Fr.	4.0 (0.74)		4.0 (0.74)			2.0 (0.75)								2.0 (0.75)	0.16
49	Trichothecium roseum Link	4.0 (0.74)	-	4.0 (0.74)	-	-	2.0 (0.75)	-	4.5 (1.64)	-	-	3.0 (1.32)	-		9.5 (0.78)	0.78
	Total frequency	**205 (37.96)**	**76.5 (14.17)**	**281.5 (52.13)**	**56.5 (25.57)**	**31.5 (14.25)**	**85.0 (31.95)**	**49.5 (18.61)**	**84.5 (30.84)**	**45.5 (16.61)**	**71.5 (31.5)**	**51.0 (22.5)**	**93.0 (39.4)**	**31.0 (13.1)**	**599**	
	Total fungal incidence	**384 (71.1)**	**156 (28.9)**	**540**	**144 (65.2)**	**77 (34.8)**	**172 (64.7)**	**94 (35.3)**	**196 (71.5)**	**78 (28.5)**	**140 (61.7)**	**87 (38.3)**	**169 (71.6)**	**67 (28.4)**	**1224**	**99.64**
	Sum total incidence	**540.0**		**221.0**	**266.0**	**274.0**	**227.0**	**236.0**	**1224**							

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.

Table 2: Diversity of fungal isolates and their incidence on *Lycopersicon esculentum* Mill. seeds

Sr. No.	Fungal genera isolates	Seed samples of *Lycopersicon esculentum* L.																		Sum total of incidence
		Mixed seed samples			LE cv.-1			LE cv. - 2			LE cv. - 3			LE cv.- 4			LE cv. - 5			
		Sp	Total incidence	% over total	Sp	Total incidence	% over total	Sp	Total incidence	% over total	Sp	Total incidence	% over total	Sp	Total incidence	% over total	Sp	Total incidence	% over total	
A	**Oomycota**	**3**	**28.0**	**5.18**	**2**	**9.0**	**4.07**	**2**	**16.5**	**6.20**	**2**	**19.0**	**6.93**	**1**	**7.5**	**3.3**	**1**	**10.0**	**4.24**	**62.0**
1	*Phytophthora*	2	22.0	4.07	1	6.5	2.94	2	16.5	6.20	2	19.0	6.93	1	7.5	3.3	1	10.0	4.24	59.5
2	Pythium	1	6.0	1.11	1	2.5	1.33	-	-	-	-	-	-	-	-	-	-	-	-	2.5
	Total genera	**2 (6.9)**			**2 (8.7)**			**1 (4.8)**			**1 (5.3)**			**1 (5.3)**			**1 (5.3)**			
B.	**Zygomycota**	**3**	**32.0**	**5.93**	**3**	**43.0**	**19.46**	**2**	**38.0**	**14.29**	**2**	**23.0**	**8.39**	**1**	**8.5**	**3.71**	**2**	**13.0**	**5.51**	**125.5**
3	*Rhizopus*	2	29.0	5.37	2	36.0	16.3	2	38.0	14.29	2	23.0	8.39	1	8.5	3.71	1	10.0	4.24	115.5
4	*Syncephalastrum*	1	3.0	0.56	1	7.0	3.17	-	-	-	-	-	-	-	-	-	1	3.0	1.27	10.0
	Total genera	**2 (6.9)**			**2 (8.7)**			**1 (4.8)**			**1 (5.3)**			**1 (5.3)**			**2 (10.5)**			
C	**Ascomycota**	**19**	**189.5**	**35.09**	**14**	**81.0**	**36.65**	**10**	**77**	**28.95**	**11**	**97.5**	**35.58**	**10**	**88.5**	**38.98**	**12**	**89.0**	**37.7**	**433.0**
5	*Aspergillus*	7	95.5	17.69	5	50.0	22.62	4	55.5	20.86	4	51.5	18.8	4	59.0	25.99	4	50.0	21.19	266.0
6	*Aureobasidium*	1	6.0	1.11	1	3.0	1.36	1	1.5	0.56	1	2.5	0.91	-	-	-	1	2.5	1.06	9.5
7	*Botrytis*	1	10.0	1.85	1	3.0	1.36	1	3.5	1.32	1	4.0	1.46	1	3	1.32	1	3.0	1.27	16.5
8	*Chaetomium*	2	8.5	1.57	1	2.5	1.13	1	4.5	1.69	2	5.0	1.82	1	3.5	1.54	1	3.0	1.27	18.5
9	*Cladosporium*	1	14.0	2.59	1	4.5	2.04	1	2.5	0.94	1	11.5	4.2	1	17.5	7.71	1	11.5	4.87	47.5
10	*Dendrophoma*	1	5.0	0.93	1	3.0	1.36	-	-	-	-	-	-	-	-	-	1	5.0	2.12	8.0
11	*Didymella*	1	6.5	1.20	1	5.0	2.26	1	6.5	2.44	1	5.0	1.82	1	1.0	0.44	1	3.5	1.48	21.0
12	*Myrothecium*	1	6.0	1.11	-	-	-	-	-	-	-	-	-	-	-	-	1	4.0	1.69	4.0
13	*Penicillium*	2	20.0	3.71	1	6.0	2.71	1	3.0	1.13	1	18.0	6.57	1	2.5	1.10	1	6.5	2.75	36.0
14	*Phoma*	1	14..0	2.59	1	2.0	0.91	-	-	-	-	-	-	-	-	-	-	-	-	2.0
15	*Ulocladium*	1	4.0	0.74	1	2.0	0.91	-	-	-	-	-	-	1	2.0	0.88	-	-	-	4.0
	Total genera	**11 (37.9)**			**10 (43.5)**			**7 (33.3)**			**7 (36.8)**			**7 (36.8)**			**9 (47.4)**			
D.	**Basidiomycota**	**1**	**9.0**	**1.67**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**4.5**	**1.64**	-	-	-	-	-	-	4.5
16	*Sporotrichum*	1	9.0	1.67	-	-	-	-	-	-	1	4.5	1.64	-	-	-	-	-	-	4.5
	Total genera	**1 (3.5)**									**1 (5.3)**			**-**						
E.	**Deuteromycota**	**23**	**281.5**	**52.13**	**17**	**88.0**	**39.82**	**20**	**134.5**	**50.56**	**17**	**133.0**	**47.44**	**16**	**122.5**	**53.94**	**16**	**124.0**	**52.54**	**599.0**
17	*Alternaria*	2	41.5	7.69	2	27.0	12.22	2	21.5	8.08	2	18.5	6.75	2	25.5	11.23	2	22.5	9.53	115.0
18	*Colletotrichum*	1	14.0	2.59	1	7.5	3.39	1	8.5	3.20	1	5.5	2.01	1	6.5	2.86	1	5.0	2.12	33.0
19	*Corynespora*	1	12.0	2.22	-	-	-	1	4.5	1.69	-	-	-	-	-	-	-	-	-	4.5
20	*Curvularia*	2	55.0	10.18	2	7.5	3.39	2	8.0	3.01	2	14.5	5.29	2	10.0	4.40	2	11.0	4.66	51.0
21	*Drachslera*	1	4.0	0.74	1	2.0	0.91	1	4.0	1.5	1	3.0	1.09	1	5.0	2.20	1	4.0	1.69	18.0
22	*Fusarium.*	8	78.0	14.44	6	20.0	9.05	7	51.5	1.36	6	52.0	19.17	5	47.0	20.7	7	54.0	22.88	224.5
23	*Geotrichum*	1	7.0	1.3	1	1.0	0.45	1	2.0	0.75	1	3.5	1.28	1	3.5	1.54	1	1.5	0.64	11.5
24	*Helminthosporium*	1	9.0	1.67	-	-	-	-	-	-	1	4.5	1.64	1	5.5	2.42	-	-	-	10.0
25	*Paecilomyces*	1	10.0	1.85	1	4.5	2.04	1	14.0	5.26	-	-	-	1	4.5	1.98	-	-	-	23.0
26	Rhizoctonia	2	33.0	6.11	2	16.0	7.29	1	14.0	5.26	2	24.0	8.76	1	12.0	5.29	2	26.0	11.02	92.0
27	*Stachybotrys*	1	10.0	1.86	1	2.5	1.13	1	2.5	0.94	-	-	-	-	-	-	-	-	-	5.0
28	*Trichoderma*	1	4.0	0.74	-	-	-	1	2.0	0.75	-	-	-	-	-	-	-	-	-	2.0
29	*Trichothecium*	1	4.0	0.74	-	-	-	1	2.0	0.75	1	4.5	1.64	1	3	1.32	-	-	-	9.5
	Total genera	**13 (44.8)**			**9 (39.1)**			**12 (57.1)**			**9 (47.4)**			**10 (52.6)**			**7 (36.8)**			**1224**
	Total Genera	**29**			**23**			**21**			**19**			**19**			**19**			
Sum total species		**49**			**36**			**34**			**33**			**28**			**31**			

1. Values in indicate fungal isolates over total isolates recorded ; 2. Values calculated in terms of percent incidence over total incidence'
3. LE - *Lycopersicon esculentum* L. cv-1 – Pusa Rubi; cv-2 – Pusa Early Dwarf; cv-3 – Sioux; cv-4 – Co-1; cv.- 5 – ART-1

Table 3: Division wise distribution of seed borne fungal flora in storage of *Lycopersicon esculentum* Mill . seeds.

Cultivars	Divisions[4]	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					
Mixed seed samples of all cultivars	A	2 (4.1)[1]	2 (6.9)	1 (2.1)	1 (3.5)	-	-	2 (6.9)	3 (6.1)	18.0 (3.3)[2]	10.0 (1.9)	28.0 (5.8)
	B	-	-	3 (6.1)	2 (6.9)	-	-	2 (6.9)	3 (6.1)	32.0 (5.5)	-	32.0 (5.5)
	C	8 (16.3)	5 (17.2)	4 (8.2)	4 (13.8)	7 (14.3)	4 (13.8)	11 (37.9)	19 (38.8)	120.0 (22.2)	69.5 (12.9)	189.5 (35.1)
	D	-	-	1 (2.1)	1 (3.5)	-	-	1 (3.5)	1 (2.1)	9.0 (1.7)	-	9.0 (1.7)
	E	11 (22.5)	4 (13.8)	10(20.41)	8 (57.6)	2 (4.1)	2 (6.9)	13(44.8)	23 (46.9)	205.0 (37.9)	76.5 (14.2)	281.5 (52.1)
	Total	**21 (42.9)**	**11 (37.9)**	**19 (38.8)**	**16 (55.2)**	**9 (18.4)**	**6 (20.7)**	**29**	**49**	**384 (71.1)**	**156 (28.9)**	**540**
LE[3] cv-1	A	1 (2.8)	1 (4.3)	1 (2.8)	1 (4.3)	-	-	2 (8.7)	2 (5.6)	6.5 (2.9)	2.5 (1.1)	9 (4.1)
	B	-	-	3 (8.3)	2 (8.7)	-	-	3 (13.0)	2 (5.6)	43.0 (19.5)	-	43.0 (19.5
	C	8 (22.2)	6 (26.1)	1 (2.8)	1 (4.3)	5 (13.7)		10 (43.5)	14 (38.7)	38.0 (17.2)	43.0 (19.5)	81.0 (36.7
	D	-	-	-	-	-	-	-	-	-	-	-
	E	7 (19.4)	4 (17.4)	5 (13.7)	3 (13.4)	5 (13.7)	5 (21.7)	9 (39.1)	17 (47.2)	56.5 (25.57)	31.5 (14.3	88.0 (39.8)
	Total	**16 (44.4)**	**11 (47.8)**	**10 (27.8)**	**7 (30.4)**	**10 (27.8)**	**9 (39.1)**	**23**	**36**	**144 (65.2)**	**77 (34.8)**	**221.0**
LE cv-2	A	1 (2.9)	1 (4.8)	-	-	1 (2.9)	1 (4.8)	1 (4.8)	2 (5.9)	2.5 (0.94)	14.0 (5.26)	16.5 (6.20)
	B	-	-	2 (5.9)	1 (4.8)	-	-	1 (4.8)	2 (5.9)	38.0 (14.29)	-	38.0 (14.29)
	C	7 (20.6)	5 (23.8)	1 (2.9)	1 (4.8)	2 (5.9)	2 (9.5)	7 (33.3)	10 (29.4)	46.5 (17.48)	30.5 (11.47)	77.0 (28.95)
	D	-	-	-	-	-	-	-	-	-	-	-
	E	8 (23.5)	4 (19.0)	7 (20.6)	6 (28.6)	5 (14.7)	5 (23.8)	12 (57.1)	20 (58.8)	85.0 (31.95)	49.5 (18.61)	134.5 (50.56)
	Total	**16 (47.1.0)**	**10 (47.6)**	**10 (29.4)**	**8 (38.1)**	**8 (23.5)**	**8 (38.1)**	**21**	**34**	**172 (64.7)**	**94 (35.3)**	**266.0**
LE cv-3	A	1 (3.0)	1 (5.3)	1 (3.0)	1 (5.3)	-	-	1 (5.3)	2 (6.1)	17.0 (6.20)	2.0 (2.73)	19.0 (6.93)
	B	-	-	2 (6.1)	1 (5.3)	-	-	1 (5.3)	2 (6.1)	23.0 (8.39)	-	23.0 (8.39)
	C	7 (21.2)	5 (26.3)	-	-	4 (12.1)	3 (15.8)	7 (36.8)	11 (33.3)	67.0 (24.45)	30.5 (10.95)	97.5 (35.58)
	D	-	-	1 (3.0)	1 (5.3)	-	-	1 (5.3)	1 (3.0)	4.5 (1.64)	-	4.5 (1.64)
	E	7 (21.2)	4 (21.0)	6 (18.2)	4 (21.0)	4 (12.1)	4 (21.0)	9 (47.4)	17 (51.5)	84.5 (30.84)	45.5 (16.61)	133.0 (47.44)
	Total	**15 (45.5)**	**10 (52.6)**	**10 (30.3)**	**7 (36.8)**	**8 (24.2)**	**7 (36.8)**	**19**	**33**	**196 (71.5)**	**78 (28.5)**	**274.0**
LE cv-4	A	1 (3.6)	1(5.3)	-	-	-	-	1(5.3)	1 (3.6)	5.0 (2.20)	2.5 (1.10)	7.5 (3.30)
	B	-	-	1 (3.6)	1(5.3)	-	-	1(5.3)	1 (3.6)	8.5 (3.74)	-	8.5 (3.74)
	C	6 (21.4)	4 (21.1)	-	-	4 (14.3)	4 (21.1)	7 (36.8)	10 (35.7)	55.0 (24.23)	33.5 (14.76)	88.5 (38.98)
	D	-	-	-	-	-	-	-	-	-	-	-
	E	6 (21.4)	4 (21.1)	4 (14.3)	3 (15.8)	6 (21.4)	6 (31.6)	10 (52.6)	16 (57.1)	71.5 (31.5)	51.0 (22.5)	122.5 (53.94)
	Total	**13 (46.4)**	**9 (47.4)**	**5 (17.9)**	**4 (21.1)**	**10 (35.7)**	**10 (52.6)**	**19**	**28**	**140 (61.7)**	**87 (38.3)**	**227.0**
LE cv-5	A	1(3.2)	1(5.3)	-	-	-	-	1(5.3)	1(3.2)	8.0 (3.39)	2.0 (0.85)	10.0 (4.24)
	B	-	-	2 (6.5)	2 (10.5)	-	-	2 (10.5)	2 (6.5)	13.0 (5.51)	-	13.0 (5.51)
	C	7 (22.6)	5 (26.3)	1(3.2)	1(5.3)	4 (12.9)	4 (21.1)	9 (47.4)	12 (38.7)	55.0 (23.31)	34.0 (14.41)	89.0 (37.7)
	D	-	-	-	-	-	-	-	-	-	-	-
	E	7 (22.6)	4 (21.1)	6 (19.4)	3 (15.8)	3 (9.7)	3 (15.8)	7 (36.8)	15 (48.4)	93.0 (39.4)	31.0 (13.1)	124.0 (52.54)
	Total	**15 (50.4)**	**10 (52.6)**	**9 (29.0)**	**6 (31.6)**	**7 (22.6)**	**7 (36.8)**	**19**	**31**	**169 (71.6)**	**67 (28.4)**	**236.0**

1. Values in parenthesis indicate percent fungal isolates over total isolates recorded.
2. Values in parenthesis calculated in terms of percent incidence over total incidence
3. LE – *Lycopersicon esculentum* Mill.; cv-1 – Pusa Rubi; cv-2 – Pusa Early Dwarf; cv-3 – Sioux; cv-4 – Co-1; cv.- 5 – ART-1
4. A – Oomycota; B – Zygomycota; C – Ascomycota; D – Basidiomycota; E – Deuteromycota.

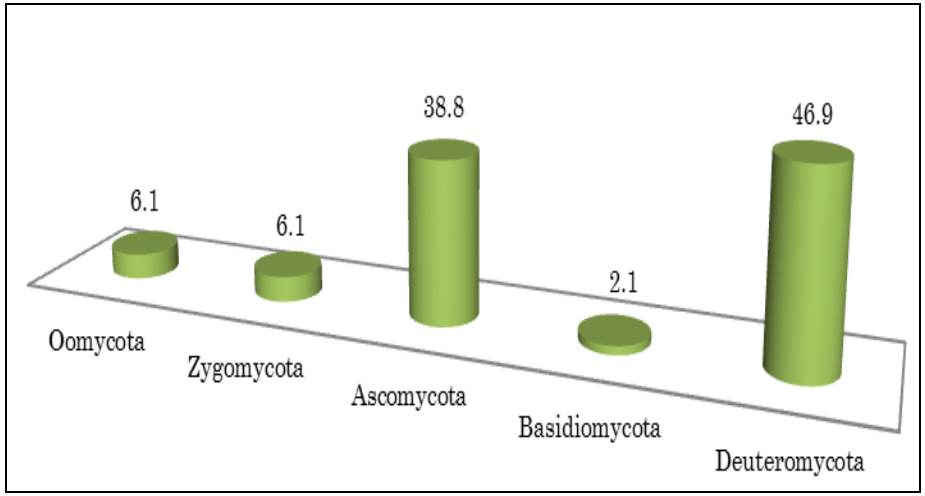

Fig. 1: Distribution of fungal flora of mixed seed samples of diffrents cultivars of *Lycopersicon esculentum* Mill

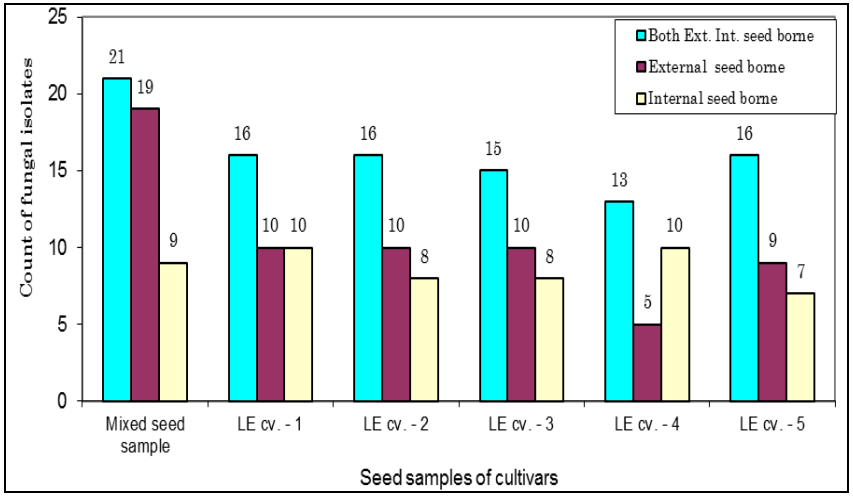

Fig. 2: Distribution of fungal isolates on stored seeds of *Lycopersicon esculentum* Mill

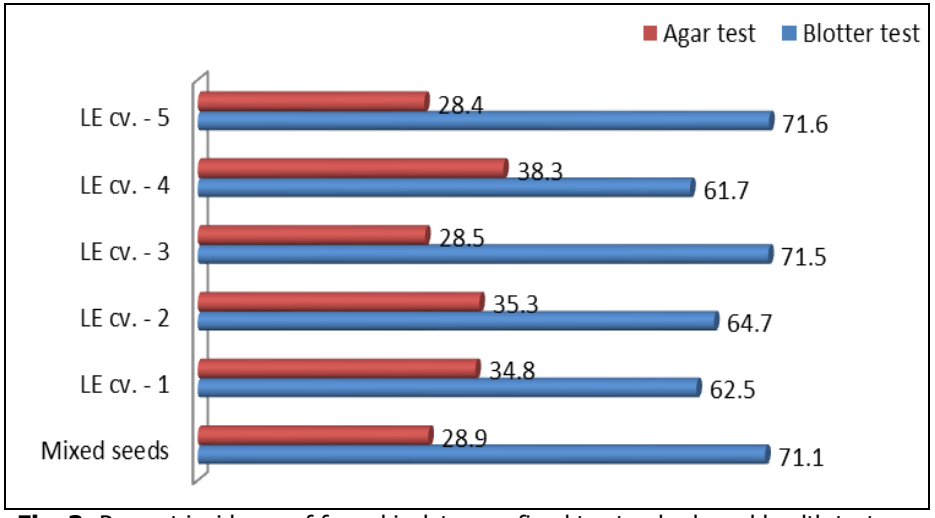

Fig. 3: Percent incidence of fungal isolates confined to standard seed health tests

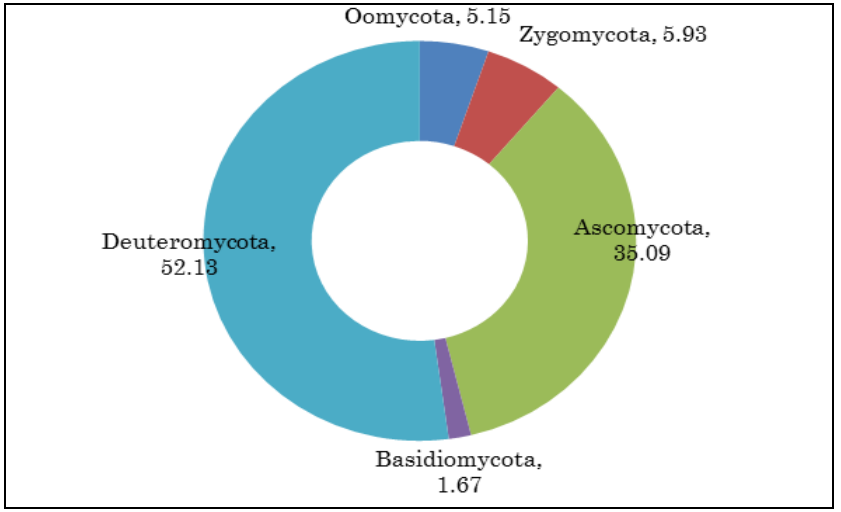

Fig. 4: Percent Incidences of fungal flora in storage of mixed seed samples of *Lycopersicon esculentum* Mill

Of the randomly selected four hundred seeds, 50% seeds without pretreatment were placed in ten sterilized petri plates containing three layered sterile moistened absorbent blotter papers for isolation of external seed borne fungal flora. The remaining 50% 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 plating technique. The pretreated twenty seeds were transferred aseptically to each sterile petri plate containing semi-solid agar nutrient sterile medium composed of peeled potato ($400gm^{-1}$), dextrose ($20gm^{-1}$**)** and agar ($20gm^{-1}$**)** in a liter of distilled water. All petri plates were allowed incubate for seven days in B.O.D incubator at 25±2°C under alternating cycles of 12 hours light and darkness.

e. Identification of fungal isolates: After examination of incubated seeds under stereoscopic microscope the fungal growth appearing on surfaces was identified on the basis of colony colour and sporulation type. Fungal isolate count 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.

RESULTS AND DISCUSSION

Seed is primary mean to transmission and the spread of seed borne disease in seed producing plants. Fungal micro-propagules infect healthy seed mostly when they either 'crawl' all the way to seed on the outside of the plant, or else send out spores that land on the seed. They tend to be restricted on outside, in layers of seed coat or reside inside embryonic cells of seed. Spores on seed coat are more to either dry up or die, or else to get sloughed off with seed coat during seed germination, initiate infection and proceed to spread by attacking cells of outer layers, thereby falling to cause disease on next generation of plants (Lew-Smith, 2013). Seed infection by fungal organisms and prevalence of their propagules in seed lots is vitally important because infected seed(s) may fail to germinate, cause infection to seedlings and make these unhealthy (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 *Lycopersicon esculentum* Mill. seeds (Bhajbhuje, 1989).

Mycological analysis of mixed seed samples of five different commonly grown cultivars of *Lycopersicon esculentum* Mill obtained from farmers of Vidarbha revealed the prevalence of total 49 fungal pathogens belongs to 29 genera on seed surfaces in varying incidence. Of these, isolates belong to *Deuteromycota* are most predominant ones, represented by 13 genera and 23 species followed by *Ascomycota*, contributing 11 genera and 19 species. *Oomycota* and *Zygomycota* were represented by 2 genera and 3 species while *Basidiomycetes* had only one genus and single species. Individual genus, *Fusarium* dominated with 8 species, while *Aspergillus* was represented by 7 species. Two species of *Alternaria, Chaetomium, Curvularia, Penicillium, Phytophthora, Rhizoctonia* and *Rhizopus* have been encountered while others had single species. Of the total isolates, *Aspergillus nidulans, Aspergillus sulphureus, Aspergillus ochracious, Aspergillus amstelodomi, Fusarium moniliformae, F. semitectum, Geotrichum candidus, Paecilomyces varioti* and *Penicillium oxalicum* has been recorded as seed borne fungal pathogens for the first time in India from stored seeds lots of *Lycopersicon esculentum* Mill (Table 1 & 2).

Percent incidence of seed borne fungal pathogens from mixed seed samples of all cultivars was presented in Table 1. A total 21 fungal species of 11 genera including *Alternaria porri, A. solani, A. flavus, A. fumigatus, A. niger, Aureobasidium sp., Botrytis cinera, Colletotrichum capsici,, Curvularia lunata, C. ovoides,, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, F. oxysporum, F. semitectum, F. solani, Fusarium sp., Penicillium oxalicum, P. nigricans*, *Pythium aphanidermatum and Phytophthora infestans* have been encountered in stored seeds as both external and internal seed borne by both seed health tests.

Of the total isolates, *Curvularia lunata* (33%) and *Aspergillus fumigatus* (26%) were appeared to be predominant exhibiting higher incidence followed by *Aspergillus flavus* (24%), *Alternaria porri* (23%), *Fusarium lycopersici* (22.5%), *Curvularia ovoides* (22%) and *Aspergillus niger* (21%). *Alternaria solani, Colletotrichum capsici, Fusarium semitectum, Penicillium oxalicum* and *Phytophthora infestans* were recorded subdominant with fungal incidence varied between 13.0 to 18.5% while others had 6.5 to 9.5% incidence. Low count of fungal incidence was detected for *Aureobasidium sp., Pythium aphanidermatum and Fusarium oxysporum* by both health testing techniques.

Prevalence of 19 fungal species belongs to 16 genera in varying frequency were confined to blotter test only. These isolates included *Chaetomium sp., Cladosporium tenuissium, Corynespora cassicola, Drachslera rostrata, Fusarium culmorum, F, equiseti,, Myrothecium roridum, Paecilomyces variotii, Phoma destructiva, Phytophthora parasitica, Rhizoctonia solani, R. bataticola, Rhizopus stolonifer, Rhizopus sp., Sporotrichum pulverulentum, Stachybotrys atra,, Syncephalastrum racemosus, Trichoderma viride, and Trichothecium roseum.* Among these, *Rhizoctonia solani* was appeared to be most dominant with 18% incidence followed by *Rhizopus stolonifer* and *Rhizoctonia bataticola* (15%).

The frequency of incidence, 14% was detected for *Cladosporium tenuissium, Phoma destructiva and Rhizopus sp.* Moderate 10% fungal incidence was recorded for *Myrothecium roridum, Paecilomyces variotii, and Stachybotrys atra* while six isolates, *Chaetomium* sp., *Drachslera rostrata, Fusarium culmorum, Phytophthora parasitica, Trichoderma viride* and *Trichothecium roseum* has been detected with 4% incidence. *Sporotrichum pulverulentum* and *Fusarium equiseti* had 9% and 6% incidence respectively. Low frequency of incidence was recorded for *Syncephalastrum recemosum* (Table 1). A total of 8 isolates fall under 6 genera were restricted only to agar plate included 8 species fall under six genera. The isolates, *Aspergillus amstelodomi* and *Helminthosporium tetramera* has been isolated with 9% incidence while 5 % incidence was recorded for *Aspergillus ochracious, Aspergillus sulphureus* and *Dendrophoma* sp. The frequency of incidence 7% and 5.5% has been recorded for *Geotrichum candidum* and *Aspergillus nidulans* respectively. Low frequency of incidence was detected for *Chaetomium globosum* and *Ulocladium chartarum* (Table 1).

The data on seed mycoflora of five different cultivars is presented in table 1 & 2.

The seeds LE cv.-1 (*Pusa Rubi*) exhibited association of 36 fungal isolates belongs to 23 genera (table 1). Of these count, 16 species of 11 genera encountered on blotter paper as well as agar plates were *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Aureobasidium sp., Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, Fusarium sp., Penicillium oxalicum and Phytophthora infestans.* The two species of *Aspergillus*, *A. fumigatus* and *A. niger* were appeared to be most predominant with 17% incidence followed by *Alternaria porri* (14.5%). The frequency of fungal incidence varied between 6.0 to13% has been recorded for *Alternaria solani, Aspergillus flavus, Colletotrichum dematium, Curvularia lunata, Penicillium oxalicum* and *Phytophthora infestans* while others had 3 to 5% incidence (Table 1). However, total of 10 fungal species belongs to 7 genera, *Drachslera rostrata, Fusarium culmorum, F. equiseti, Phoma destructiva, Pythium aphanidermatum, Rhizoctonia solani, R. bataticola, Rhizopus stolonifer, Rhizopus sp. and Syncephalastrum recemosum were encountered on blotter paper only.* Both species of *Rhizopus* (18%) and *Rhizoctonia solani* (14%) were seemed to be predominant while other isolates were recorded with 2 to 7% incidence. Of the total count, 10 species of 9 genera, *Aspergillus ochracious, A. sulphureus, Chaetomium glabosum, Curvularia ovoides, Dendrophoma sp., Fusarium solani, Geotrichum candidum, Paecilomyces varioti, Stachybotrys atra and Ulocladium chartarum were restricted to agar plate only* (Table 1). *Paecilomyces varioti* was recorded predominant whereas very low frequency of incidence (0.5%) has been detected for *Curvularia ovoides*. Other isolates were recorded with incidence ranged between 1 to 3% (Table 1).

The seeds of LE cv.-2 (*Pusa Early Dwarf*) exhibited prevalence of altogether 34 fungal species classified under 21 genera. Of the total count, 16 species of 10 genera, including *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger*, *Aureobasidium sp., Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, F. oxalicum, F. semitectum* and *Phytophthora infestans* has been recorded by both seed health tests. *Aspergillus niger* was appeared to be predominant exhibiting higher, 25.5% incidence. The frequency of incidence 12.5% was recorded for *Alternaria solani, Aspergillus flavus* and *A. fumigatus* while other isolates excluding *Aureobasidium* sp. had 2.5 to 10% incidence. However, total 8 species of 8 genera, *Aspergillus sulphureus, Chaetomium glabosum, Curvularia ovoides, Fusarium solani, Geotrichum candidum, Paecilomyces varioti, Phytophthora parasitica and Stachybotrys atra* were restricted to agar plates only. Of the count, *Paecilomyces variotii*, *Phytophthora parasitica* and *Stachybotrys atra* were seemed to be dominant and others were detected with frequency ranged between 2 to 5%. The fungal isolates of 10 species of 8 genera were restricted only to blotter test. These included *Corynespora cassicola*, *Drachslera rostrata Fusarium culmorum, F. equiseti, Penicillium nigricans, Rhizoctonia solani, Rhizopus stolonifer, Rhizopus* sp., *Trichoderma viride* and *Trichothecium roseum.* Both species *Rhizopus* were recorded most predominant with 18-20% incidence. The frequency of incidence, 14% was recorded for *Fusarium culmorum* and *Rhizoctonia solani* followed by *Fusarium equiseti* (12%) whereas other isolates has been detected with incidence ranged between 2.0 to 4.5% (Table 1).

The seeds of LE cv.-3 (Sioux), exhibited association of 33 fungal species of 19 genera (Table1). Of these, 8 species belongs to 7 genera including, included *Aspergillus, amstelodomi, Aureobasidium sp., Chaetomium glabosum, Chaetomium sp, Curvularia ovoides, Fusarium solani, Geotrichum candidus* and *Helminthosporium tetramera* were confined to agar plating only. Excluding *Aspergillus amstelodomi,* the percent incidence recorded for remainings was varied between 2.5 to 5.0%. Prevalence of 10 isolates of 8 genera has been recorded by blotter test only, included *Drachslera rostrata, Fusarium culmorum, F. equiseti, Phytophthora parasiticus, Rhizoctonia solani, R. bataticola, Rhizopus stolonifer, Rhizopus sp., Sporotrichum pulverulentum and Trichothecium roseum*. The higher frequency of incidence, 12% was recorded for *Fusarium culmorum, Phytophthora parasiticus, Rhizoctonia bataticola and*

Rhizopus sp. followed *Rhizoctonia solani and Rhizopus stolonifer* (11%) while others had 3 to 6% frequency . However, of the total count, 15 species belongs to 10 genera have been restricted to both seed health tests, included *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, F. oxysporum, Penicillium oxalicum and Phytophthora infestans.* Among these, *Aspergillus niger* was appeared to be most predominant, detected with 22.5% incidence followed by *Aspergillus fumigatus* (19.5%), *Penicillium oxalicum* (18%). The isolate *Botrytis cinera* had low incidence (4%) while remainings were detected5.0 to 13.5% incidence (Table 1).

From LE cv.-4 (Co-1) seeds altogether 28 species classified under 19 genera have been detected in varying frequencies (Table 1). Of these, 13 species of 9 genera, including *Alternaria porri*, A. solani, *Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici and Phytophthora infestans* were confined to both seed health tests. *Aspergillus fumigatus* and *A. niger* were appeared to be predominant, exhibiting higher, 22.5% incidence followed by *Cladosporium tenuissium* (17.5%) and *Fusarium lycopersici* (16%). Low frequency of incidence was recorded for *Botrytis cinera* (3%) and *Didymella lycopersici* (1%) whereas remaining isolates had 6.5 to 13% incidence. However, prevalence of 5 species of 4 genera has been confined to blotter paper only included *Drachslera rostrata, Fusarium culmorum, F. equiseti, Rhizoctonia solani* and *Rhizopus stolonifer.* The higher incidence was recorded for *Fusarium equiseti* and *Rhizoctonia solani* while others had 2.0 to 8.5% incidence. Of the total count, 10 isolates, *Aspergillus nidulans*, *Chaetomium glabosum, Curvularia ovoides, Fusarium solani, Geotrichum candidum, Helminthosporium tetramera, Paecilomyces varioti, Penicillium oxalicum, Trichothecium roseum* and *Ulocladium chartarum* were restricted only to agar test. All isolates had incidence varying between 2.0 to 5.5% incidence (Table 1).

The seeds of LE cv. - 5 (*ART-1*) exhibited associations of 31 fungal species belong to 19 genera. Of the total count, 15 species of 10 genera has been confined to both the seed health tests, included *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium tenmissimum, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici, F. semitectum., Penicillium oxalicum and Phytophthora infestans.* The higher frequency of incidence, 18.5% was recorded for *Aspergillus fumigatus* and *A. niger* followed by *Fusarium lycopersici* (17.5%). Excluding *Botrytis cinera*, *Didymella lycopersici* and *Fusarium semitectum,* the others had 5 to 12% incidence. However, 7 isolates of 7 genera, *Aspergillus amstelodomi, Aureobasidium sp., Chaetomium glabosum, Curvularia ovoidea, Dendrophoma sp., Fusarium solani,* and *Geotrichum candidum* were restricted to agar plates only. All isolates have been detected in frequency of incidence ranged from 1.5 to 5.0%. Altogether 9 species representing 6 genera including *Drachslera rostrata, Fusarium culmorum, F. equiseti, F. oxysporum, Myrothecium roridum, Rhizoctonia solani, R. bataticola, Rhizopus stolonifer and Syncephalastrum recemosum* have been isolated by blotter test only. Both species of *Rhizoctonia* were appeared to be predominant exhibiting higher incidence, followed by *Rhizopus stolonifer* (10%), *Fusarium oxysporum* (8%). The frequency of incidence 4% was recorded for *Drachslera rostrata, Fusarium culmorum, F. equiseti, Myrothecium roridum.* The isolate *Syncephalastrum recemosum* had 3% incidence (Table 1).

Seed samples of five cultivars of *Lycopersicon esculentum* Mill representing LE cv.-1, 2, 3, 4 and 5 were obtained from various cultivators of Vidarbha region of Maharashtra to ascertain the degree of fungal association with the seeds. Isolation was made by standard blotter and agar plating technique (ISTA, 2012). Altogether 49 isolates classified under 30 genera were encountered in the mixed seed samples. *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium tenuissium, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici Phytophthora infestans* were appeared to be common on the seeds of all cultivars, and detected by both seed health tests (Table 1). *Drachslera rostrata, Fusarium culmorum, F. equiseti, Rhizopus stolonifer Rhizoctonia solani* were confined to blotter paper while *Chaetomium globosum, Curvularia ovoides, Fusarium solani, Geotrichum candidum* were restricted to agar plating only.

The storage environment is one of the factor play important role for proliferation of seed borne pathogens. 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 cultivars as well as individual cultivar, included *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Colletotrichum dematium, Curvularia lunata, Didymella lycopersici, Fusarium moniliformae, F. lycopersici* and *Phytophthora infestans.* The isolate *Cladosporium tenuissium* was confined to seed of all cultivars. Some fungal pathogens remain restricted to specific cultivar in storage environment. *Aureobasidium* sp. from seeds of LE cv.-1,2; *Penicillium oxalicum* from LE cv.-1,3,5; *Fusarium semitectum from* LE cv.-2,5; and *Fusarium sp* from LE cv.-1 have been detected as both external and internal seed borne pathogens. *Curvularia ovoidea, Fusarium oxysporum, F. solani, Penicillium sp., Phytophthora parasitica, Pythium*

aphanidermatum, were appeared frequently on seeds of some cultivars by both seed heath test in one cultivar and either blotter or agar test in others (Table 1).

The fungal pathogens remain restricted to outer seed coat of all cultivars and confined to blotter paper only belongs to species, *Drachslera rostrata, Fusarium culmorum, F. equiseti, Rhizoctonia solani and Rhizopus stolonifer*. The isolates detected as surface contaminants in variable percent incidence in some cultivars were *Phoma destructiva, Pythium aphanidermatum* & *Trichothecium viride* (LE cv.-1); *Corynespora crassicola* & *Penicillium nigricans, Trichoderma viride* (LE cv.-2); *Sporotrichum pulverulentum* (LE cv.-3); *Myrothecium roridum* (LE cv.-5); *Rhizopus* sp. (LE cv.-2,3); *Rhizoctonia bataticola* (LE cv.-1,3,5); *Syncephalastrum racemosus* (LE cv.-1,5).

Altogether four fungal pathogens, *Chaetomium glabosum, Curvularia ovoidea, Fusarium solani, Geotrichum candidus* have been detected only by agar test in all cultivars, whereas the majority were confined in some cultivars, included *Aspergillus amstelodomi* (LE cv.-3, 5); A. nidulans (LE cv.-4); *Aspergillus ochracious* (LE cv.-1); *Aspergillus sulphureus* (LE cv.-1,2); *Chaetomium* sp. (LE cv.-3); *Dendrophoma* sp. (LE cv.-1,5); Ulocladium chartarum (LE cv.-1,4); *Helminthosporium tetramera* (LE cv.-3,4); *Paecilomyces varioti* (LE cv.-1,2,4). *Phytophthora parasitica, Fusarium oxysporum, Trichothecium roridum* were detected as an internal as well as external seed borne in some cultivars and either external or internal seed borne in other cultivars (Table 1). *Alternaria porri, A. solani Aspergillus flavus, A fumigatus, A. niger, Curvularia lunata, Fusarium lycopersici, F. semitectum, Phytophthora infestans, Rhizoctonia solani, Rhizopus stolonifer and Rhizopus sp.* were recorded in higher frequency of incidence over other isolates (Table 1).

The seeds samples of *Lycopersicon esculentum* Mill under storage were highly infested by micro-propagules of fungal pathogens. They tend to be restricted to testa of the nutrient rich viable seeds, includes a very large and diverse group of micro-organisms that occupy position of great economic importance in agriculture in developing countries. The screening of seed samples by dry examination technique revealed prevalence of diverse group fungal micro-propagules such as fungal spores, debries, acervuli etc. distributed in variable count on seed coats, cells of embryo and endosperm. These exhibit an enormous heterogeneity in life-history strategies. The routine seed health tests recommended by International Seed Testing Association comprising blotter and agar plating technique are applied for detection of seed surface adhering fungal pathogens as these two tests are inevitable for getting a complete picture of the fungal infection/association with the seeds (ISTA, 2012). Altogether 49 fungal isolates categorized under 29 genera have been encountered in the seeds of mixed samples of cultivars understudy both as external and internal seed borne. *Deuteromycota* predominated compared to other fungal divisions, contributing highest, 46.9% fungal count over the total isolates. *Ascomycota* ranks second highest, contributed 38.9% fungal population followed by *Zygomycota* and *Oomycota* contributed 6.1% fungal isolates. A least count of isolates was recorded with *Basidiomycota* of the total pathogens recorded (Fig. 1). The count of isolates for external and internal seed borne was appeared to be greater with all the cultivars followed by surface contaminants excepting LE cv.-1 & 4. Equal count of external and internal seed borne pathogens was encountered for LE cv. -4. Least count was observed for internal seed borne pathogens in maximum cultivars (Fig.2).

The prevalence of greater count of species confined to genus, *Fusarium* followed by *Aspergillus*. In contrast, *Aspergillus* contributed greatest percent incidence against *Fusarium* over total incidence. *Curvularia* was recorded subdominant. *Rhizopus stolonifer* retrieved from all samples as seed-surface contaminant (Table 2). These results are in confirmation with earlier findings. Shepherd (2012) has reported greater count of species of *Fusarium* from seeds of sweet potatoes and pigeon peas. Shirpurkar and Wahegaonkar (2012) have isolated comparable higher count of *Aspergillus niger, A. terreus, A. fumigatus, A. flavus, A. parasiticus* from maize seeds. Chukunda et al., (2013) reported prevalence of *Alternaria solani, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramera* and *Trichoderma viride* on maize seeds. *Aspergillus, Alternaria, Penicillium, Cladosporium, Fusarium* and *Stachybotrys atra* were confined in variable incidence in infested seeds of *Solanum melongena L* (Bhajbhuje, 2013).

The count of colonies appeared on blotter papers and agar plates gave estimates of fungal incidence on the seeds. *Deuteromycota* contributed more than half, 52.1% incidence followed by *Ascomycota* that contributed one-third (35.1%) incidence over total incidence of all isolates. *Zygomycota* and *Oomycota* had nearly equal count of colonies, contributing, and 5.9% and 5.2% fungal incidence respectively. *Basidiomycota* contributed little incidence (Table 1). 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). Of this, *Deuteromycota* are dominated with 37.9% and 14.2% followed by *Ascomycota*, contributed 22.2% and 12.9%; Oomycota 3.3% and 1.9% fungal incidence by blotter and agar plate test respectively. Moderate fungal incidence, 5.9% was recorded for *Zygomycota* while little 1.7% incidence was encountered for *Basidiomycota* on blotter paper only (Table 3). The higher incidence of isolates was recorded by blotter paper technique from seeds of mixed lots of all cultivars and individual cultivar over agar plate test

(Table 1). These results are in conformity with earlier reports 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); paddy (Devihalli et al., 2011); Sorgham (Yago et al., 2011); maize (Chukunda et al., 2013), *Solanum melongena* L.(Bhajbhuje, 2013).

The stored seed samples were infested by forty nine fungal pathogens. Of them, *Aspergillus nidulans, Aspergillus sulphureus, Aspergillus ochracious, Aspergillus amstelodomi, Fusarium moniliformae, F. semitectum, Geotrichum candidus, Paecilomyces varioti, Penicillium oxalicum and Sporotrichum pulverulentum* were recorded for first time in seeds of *Lycopersicon esculentum* Mill as new records in India. Prevalence of these isolates on seed coats of other crops confirmed their seed borne nature. Al-Askar et al., (2013) reported *Geotrichum candidus* from alfalfa seed. *Penicillium oxalicum* was confined to maize seeds (Debnath et al., 2012); *Aspergillus nidulans* to *Sorghum* seeds (Yago et al., 2011); *Sporotrichum pulverulentum* to oil seeds (Jain, 2008); *Aspergillus amstelodomi, A. sulphureus, Fusarium semitectum*, *Paecilomyces varioti* were encountered in brinjal seeds (Bhajbhuje, 2013); *Aspergillus ochracious and Fusarium moniliformae* were restricted to seed testa of *Bixa orellana* (Venugopalan and Giridhar, 2012). These reports are in conformity of seeds borne nature for first time reported fungal pathogens in India from mixed seed samples of *Lycopersicon esculentum* Mill.

The efficacy of both standard blotter and agar plating tests varied with nature of fungi. The greater count of isolates was confined to blotter paper over agar plate test in maximum cultivars (Fig. 3). Moreover, *Zygomycota* members proliferated rapidly on blotter paper while *Ascomycota* and *Deuteromycota* developed more profusely on agar plating possibly because they require softer medium rich in moisture for their establishment and growth. The standard blotter paper technique was proved comparative superior over agar plating to the fungal pathogens isolation. Chukunda et al, (2013) pointed out that the quick growing saprophytes adhering to the outer seed coat may be troublesome to detect internal slow growing pathogens. These variations may possibly attribute to the prolonged incubation that might lead to the development of deep seated infection (Jain, 2008; Lew-Smith, 2013). The physiochemical nature of seed as well as agricultural practices and storage environment provided for the different cultivars 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 (Debnath et al., 2012; Al-Askar 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 while absent in disinfected seeds and that fungi were contaminated their surface, did not penetrate the inner tissues (Lew-Smith, 2013). This information, although not very precise, can be a starting point to determine proper strategies of seed treatment.

The results of present survey revealed that fungal isolates belong to genera*, Alternaria, Curvularia, Fusarium, Helminthosporium, Rhizoctonia* of *Deuteromycotina* as well as *Aspergilli, Penicilli* and *Phoma* of *Ascomycotina* 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* had comparatively maximum count of isolates (Fig. 1) and greatest frequency of fungal incidence followed by *Ascomycota* (Fig. 4). It may possibly due to prevalence of maximum count of fungal flora associated with seed coat with significant 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 (Bhajbhuje, 1989). 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). Members of *Basidiomycotina* rarely 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, Colletotrichum, Corynespora, Curvularia, Helminthosporium, Fusarium, Paecilomyces, Rhizoctonia and Stachybotrys* and *Ascomycota* including *Aspergillus, Cladosporium, Penicillium* and *Phoma* appeared to be highly predominant on *Lycopersicon esculentum* Mill seeds. These isolates are among the most abundant and widely distributed organisms on the globe (Venugopalan and Giridhar, 2012; Lew-Smith, 2013). *Fusarium* species exist under very wet storage environment as saprophytes on seeds and plant debris or parasites of many crops causes wilting. *Fusarium*

contributed greatest count of species over other genera isolated with 14.4% incidence. *Aspergilli* exists as obligate saprophytes on nutrient rich stored food material and survive in the environment without causing disease (Jyoti and Malik, 2013). *Aspergillus amstelodomi, A. flavus, A. fumigatus and A. niger* had the highest count of occurrence and greater frequency of incidence (Fig. 5). 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), ESFA (2011) and Shephard (2012). **The** mycotoxins are known to cause chromosomal breakage, create disturbances in normal karyokinesis in 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 decline in the membrane potential of metabolically active meristematic cells of the plant system. Primary metabolites induce growth stimulating response while secondary metabolites appear to create disturbances in normal cell metabolism, growth and karyokinesis in mitotic cell cycle of metabolically active meristematic cells causing cytological abnormalities (Bhajbhuje, 2013). The species of genus *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 culmorum* produces vomitoxin that causes a serious feed refusal and vomiting in animals and *also* associated with allergy; *F. solani* and *F. moniliformae* were reported to cause *keratitis* and also associated with wound and infections of the eyes and fingernails (EFSA, 2011)

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. 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). Most *Alternaria* mycotoxins induced considerable mutagenic and cytotoxic effects. Moreover, the growth of the isolated pathogen 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 lead to spoilage of seeds (Jyoti and Malik, 2013).

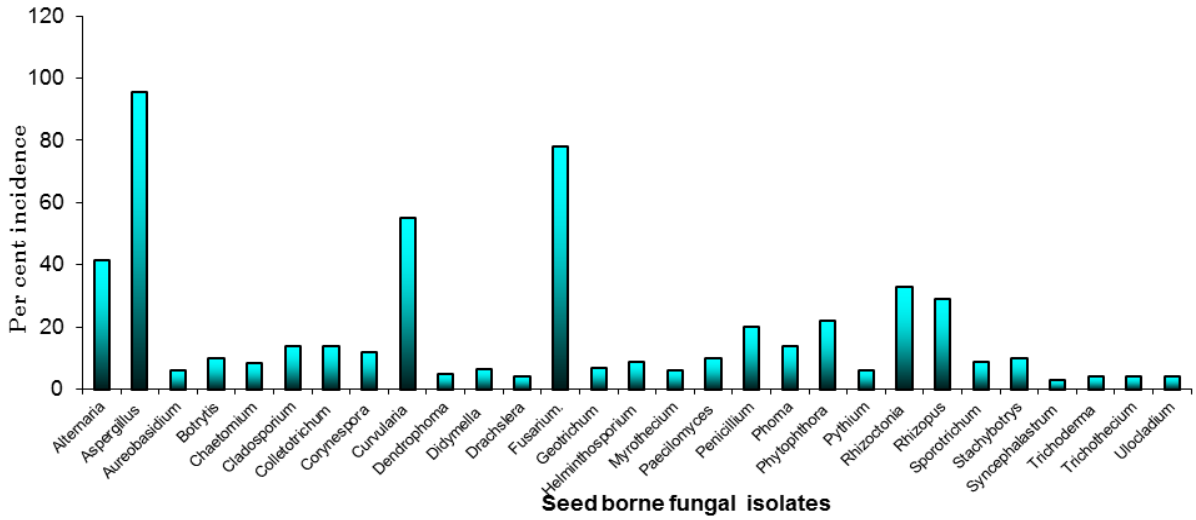

Fig.5. Percent incidence of micro-fungal genera on stored mixed seed samples of *Lycoerpsicon esculentum* Mill

The susceptible cultivars of *Lycopersicon esculentum* Mill are affected with several fungal diseases including early blight (*Alternaria solani*); late blight (*Phytophthora infestans*); septoria leaf spot (*Septoria lycopersici*); buckeye rot (*Phytophthora parasitica*); Fusarium wilt (*Fusarium oxysporum*); Damping off (*Pythium* & *Rhizoctonia* spp.); Southern blight (*Sclerotium rolfsii*). Most of these disease causing pathogens were retrieved as seed surface contaminants, caused physiological damage to the seeds and transmitted from seeds to seedlings during seedling emergence. 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 25-30% (Clemson, 2013).

The data on the diversity and incidence of fungal species on may be a great importance in the region for predicting the extent of pre-and post-infections. Results on present study indicated that *Lycopersicon esculentum* Mill seeds harbor arrays of fungal contamination as a result of improper storage management (Clemson, 2013). Most of the fungal isolates reported in this study, had been encountered in various kinds of stored seeds. 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 proliferation of fungal isolates on inner and outer testa of seeds leads to changes in various cellular and metabolic activities; 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).

Majority of fungal isolates involved in seed deterioration of *Lycopersicon esculentum* Mill are xerophilic moulds such as *Aspergilli* and *Penicilli* of *Ascomycotina* as well as *Alternaria, Curvularia, Fusarium, Helminthosporium* of *Deuteromycotina* (Bhajbhuje, 2013). 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 foundation and vehicles for spread of new life. They constitute basic agricultural productivity. The seeds carrying pathogens may help to spread diseases generation to generation, 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 seed lots of *Lycopersicon esculentum* Mill from Vidarbha cultivators are more prone to fungal attack and carried greater count of fungal propagules on seed surface, leads to deterioration of nutritional components posing damage to the seeds. 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 high quality 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 use improved scientific methods of storage to discourage proliferation of these organisms on seeds.

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