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Dynamics of Seed Borne Micobiota on Genotype of Mungbean *Vigna radiata* (L.) Wilczek at the Different Period of Storage

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Abstract: Good quality seed is the prerequisite to agriculture and though of such vital significance very less attention has been paid to their efficient storage and storage losses. The present study was undertaken to screen for isolation and percent incidence of seed borne mycoflora wherein two Mungbean varieties viz. HUM-4 and HUM-12 were screened by standard blotter paper and agar plate methods at the different period of storage. Seeds of Mungbean varieties were obtained from agriculture farm, Banaras Hindu University, Varanasi. The surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) for 1 min and washed thoroughly with distilled water. Both sterilized and unsterilized seeds were used for detection of seed borne pathogen. A total of 22 different fungi including one bacterial isolate belonging to 12 distinct genera were isolated were from these seed samples. The percentage incidence of different fungi varied with the storage periods. *Aspergillus niger* recorded at maximum level in 22.33, 43.27, 49.95, 19.98, 22.98 and 39.00% in Agar plate method from fresh, 180 and 360 days period of storage in HUM-4 as well as HUM-12 genotype. Highest incidence of seed borne fungi was recorded in genotype HUM-4 in Agar plate followed by HUM-12 through blotter condition. In all storage period unsterilized seed yielded most number of seed borne fungi as compared to sterilized seed. Result revealed that in all storage period *Aspergillus* sp. *Penicillium* sp. *Curvularia lunata* were recorded highest in HUM-4 through Agar plate method, *Fusarium* sp. were recorded highest in HUM-4 blotter method and lowest in HUM-12 agar plate methods, *Rhizopus Cephaliofhora irregularis*, *Chaetomium globosum* and *Cladosporium cladosporoids* in HUM-12 under Agar condition. Result showed that maximum number of fungi belonged to Deuteromycotina (68. 98%) followed by Ascomycotina (13. 89%) and Zygomycotina (9. 09%).

Key words: Mungbean, incidence, seed borne fungi, genotype

INTRODUCTION

Pulses are rich source of vegetative protein and play an important role in nutritional security of majority of vegetarian population in India. The country is the largest producer and consumer of pulses occupying 33% of the world's area and 22% of the production (Srivastava *et al*., 2008). Pulse production in the country has fluctuated widely between 13 and 15 million tonnes (mt) with no significant growth trend between 1991 and 2010. The latest estimate indicates that the present production of pulses has reached 14.7 million tons (mt) with productivity of 637 kg ha^{-1} although the projected pulse requirement by the year 2030 (32 mt) is estimated to be more than double the current production level (Anonymous, 2011). Mungbean (*Vigna radiata* (L.) Wilczek) is a short duration; herbaceous, annual, self-pollinated legume pulse crop under the family. It also has the ability to fix atmospheric nitrogen in soil, which enriches the soil quality (Nadeem *et al*., 2004). It is an excellent source of proteins considered as a "poor men's protein" (Mian, 1976). It contains 26% protein, 51% carbohydrate, 10% moisture, 4% minerals and 3% vitamins (Khan, 1981). Areas for cereals and other pulses have decreased, that for mungbean has doubled in the last two decades with an annual rate of 2.5%. The area under pulses in India is around 24.38 million hectares with a production of 14.52 million tonnes. Nearly 8% of this area is occupied by mungbean which is the third important pulse crop of India in terms of area cultivated and production next to gram and pigeon pea (Sathyamoorthi *et al*., 2008). The estimates for 2010-11 indicate that the total pulse production is 17.29 million tons from 25.51 million ha area which is all times high and is the only exception year. The total area and production under green gram in India (2010-11) was about 3.44 mha and 1.20 mt and productivity was 351 kg ha^{-1} almost 90% of mungbean production on a world scale is produced in Asia, with India, the world's

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largest producer, accounting for more than 50% of world production (Vijayalakshmi *et al*., 2003). The average yield of mungbean is very low (763.50 kg ha^{-1}) as compared to its potential yield of 2-4 ton ha^{-1} (Ramakrishna *et al*., 2000). Rajasthan, Maharashtra andhra Pradesh, Karnataka, Odisha and Bihar are the major mungbean producing states. The average yield of mungbean is very low (763.50 kg ha^{-1}) as compared to its potential yield of 2-4 ton ha^{-1} (Ramakrishna *et al*., 2000). This is due to various factors which are responsible for low yield of mungbean in present country use of poor quality seed and disease infestation in the field are the most important (Bakr and Rahman, 1998) resulting low germination, loss in viability that directly affect the production of mungbean. High moisture content, impure seeds and storage condition are also responsible for low yield of mungbean seed which create many hazards in the field like suboptimal crop population and enhancement of weed infestation. High moisture content and impure seed accelerate number of seed borne fungi. Most of the crops are propagated by seed which carry a many externally or internally harmful seeds borne fungi become active under favourable conditions resulting extensive damage to seeds and diseases on crops raised from them resulting poor germination and poor seedling vigour. Pathogens can spread over a long distance and uninfected field may be infected by the seeds in which different pathogens are present (Fakir, 2001). A large number of mycoflora was reported to be associated with the mungbean seeds. *Alternaria* sp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium equiseti*, *Myrothecium roridum*, *Drechslera* sp., *Aspergillus flavus*, *A. niger* and *Macrophomina phaseolina* were found in mungbean (Bakr and Rahman, 2001).

MATERIALS AND METHODS

Visual examination of seeds: It is very common method to examine seeds in the laboratory by naked eye and also by dissecting microscope. Fresh as well as stored seeds were examined by naked eye and under dissecting microscope periodically for about a year. Three replication of each treatment at different periods of interval were undertaken.

Detection of seed mycoflora The experiment was conducted in the laboratory of department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Seed samples of two Mungbean varieties viz., HUM-4 and HUM-12 were collected from the Department of Genetics and Plant Breeding, BHU, Varanasi for the study on seed mycoflora and fungi were isolated by using different methods viz., Standard blotter paper method and Agar plate method, as recommended by International Seed Testing Association (ISTA, 1966). Seed were surface sterilized using 2.5% bleach-$NaOCl_2$ for one min and washed thoroughly with distilled water. These seeds were dried back again to its original weight and used for further study and three replication of each treatment were prepared. The experimental data were recorded from fresh as well as stored, after every six months (fresh seed, 180 days and 360 days) of storage period. Mycoflora associated with seeds were detected by standard methods and agar plate method (Muskett, 1948).

AGAR PLATE METHOD (Muskett, 1948)

The nutrient medium used for observation and isolation of seed mycoflora was Potato Dextrose Agar (PDA) medium. Sterilized (15 psi for 20 min in an autoclave) melted medium was poured aseptically into sterilized (180°C for 20 min in a hot air oven) Petri dishes were allowed to cool and solidify. Maximum sixteen seeds were placed in each Petri dish containing solidified PDA medium. Both surfaces sterilized and unsterilized seeds were taken for isolation of fungi. Surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) solution (Ramakrishna *et al*., 1991). The seeds were externally sterilized by 0.1% mercuric chloride solution to 1-2 min then washed by sterilized distilled water (Habib *et al*., 2007). All the Petri dishes containing seeds were incubated usually for 7 days at 25±2°C under 12 h alternating cycles of light (Provided by two 40 W fluorescent tube lights, placed horizontally 40 cm above the plates) and darkness. Fungi growing on seeds were isolated and identified.

STANDARD BLOTTER TECHNIQUE (Doyer, 1938)

Seeds were placed on sterilized, moist blotting papers in sterilized Petri dishes with the help of sterilized forceps under aseptic conditions. Both surface sterilized and unsterilized seeds were taken for isolation of fungi. Petri dishes were incubated for two weeks in an incubator at 25±2°C under 12 h alternating cycles of light (Provided by two 40 W fluorescent tube lights, placed horizontally 40 cm above the plates) and darkness. Plated seeds were periodically observed for the presence and growth of fungal species on the seeds. The %incidence of fungi of particular species within a genus of fungi was calculated (Ghiasian *et al*., 2004):

$$\text{Incidence (\%)} = \frac{\text{No. of infected seed}}{\text{Total No. of seed}} \times 100$$

RESULT

Blotter and Agar plate methods were employed for this study and two genotype of mungbean seed HUM-4 and HUM-12, two sets of seeds were analyzed i.e., unsterilized and surface sterilized seeds during 0, 180 and 360 days period of storage. In the present study, it was found that both the agar and blotter paper methods of fungal isolation are effective, routinely and consistently applicable and provide reliable results. The occurrence of fungi most frequently encountered is recorded in terms of mean value with standard error.

Incidence of seed borne fungi on genotype of mungbean seed at the different period of storage: Blotter and Agar plate methods were employed for this study and two genotype of mungbean seeds HUM-4 and HUM-12, two sets of seeds were analyzed i.e., unsterilized and surface sterilized seeds during 0, 180 and 360 days period of storage. All fungi were identified on the basis of their cultural and morphological characteristics. In the present study, it was found that both the agar and blotter paper methods of fungal isolation are effective, routinely and consistently applicable and provide reliable results. The occurrence of fungi most frequently encountered is recorded in terms of mean value with standard error.

Incidence of seed borne fungi on fresh mungbean seed: A total of 15 different fungi belonging to 8 distinct genera were isolated (Table 1). The prominent observation of seed associated mycoflora of mungbean are the *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terrus*, *A. fumigatus*, *A. orchareus*, *A. candidates Penicillium citrinum*, *P. rubrum*, *Curvularia lunata*, *Fusarium moniliformae*, *Fusarium clamydosporum*, *Chaetomium globosum* and *Rhizopus stolonifer*. Highest total number of (15) fungi were isolated by Agar plate method in genotype HUM-4 under unsterilized condition and minimum (6) in genotype HUM-12 by blotter method under sterilized condition (Table 4). Result showed that incidence of *Aspergillus niger* was recorded at maximum level in (22.33%) in genotype HUM-4 through Agar plate methods followed by (19.98%) in genotype HUM-12 through Blotter paper methods then *Penicillium rubrum* (19.00%) in genotype HUM-4 through Agar plate and Fusarium moniliformae and Fusarium clamydosporum (12.83 and 8.67%) in Genotype HUM-4 and HUM-12 through Blotter plate method. Incidence of *Aspergillus flavus*, *Alternaria alternata*, *Aspergillus terrus*, *Aspergillus fumigatus*, *Aspergillus candidates*, *Aspergillus orchareus* and *Curularia lunata* (12.25, 9.68, 8.00, 8.86, 7.50, 7.35 and 7.78%, respectively) were recorded highest in genotype HUM-4 through Agar plate method as comparisons to (9. 08, 7. 51, 5. 58, 3. 86, 3. 33, 0. 00 and 5. 78%) in genotype HUM-12 under Agar plate and lowest in Genotype HUM-12 through blotter paper methods. Some fungi like Chaetomium globosum, Cladosporium cladosporoids, (7.67 and 6.08%) were recorded highest in genotype HUM-12 through Agar plate followed by HUM-4 under Agar plate methods. Seed treated with Potassium nitrate showed complete absence of certain fungi viz., *Aspergillus fumigatus*, *A. candidates*, *A. orchareus*, *A. terrus*, *Penicillium rubrum* and *P. citrum* in HUM-12 blotter condition or low incidence of fungi viz., *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus teruus.*

Incidence of seed borne fungi on mungbean seed varieties at 180 d ays of storage: A total of 20 different fungi

Table 1: Incidence of seed borne fungi on genotype of mungbean seed

	HUM-4				HUM-12			
	Agar plate method		Blotter plate method		Agar plate method		Blotter plate method	
Genotype fungus species	US	S	US	S	US	S	US	S
Alternaria alternata	9.68±2.000	1.98±1.25	7.56±1.50	1.63±0.58	7.51±1.980	1.90±1.53	3.30±1.63	1.75±0.50
Aspergillus flavus	12.25±3.76	5.00±0.50	2.98±0.50	0.00±0.00	9.08±1.500	1.75±0.50	6.08±2.08	0.00±0.00
Aspergillus niger	22.33±4.58	12.25±3.90	18.63±2.58	14.65±1.68	19.98±4.67	5.45±1.73	12.67±1.58	3.85±0.58
Aspergilus fumigates	8.86±1.680	1.78±1.00	6.96±1.68	0.00±0.00	3.86±2.000	2.67±0.25	3.33±1.53	0.00±0.00
Aspergilus candidates	7.50±2.000	2.56±1.00	3.50±2.00	0.00±0.00	3.33±1.980	0.00±0.00	0.00±0.00	0.00±0.00
Aspergillus orchareus	7.35±2.000	3.30±1.67	0.00±0.00	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00
Aspergillus terrus	8.00±3.510	2.36±0.50	4.98±3.51	0.00±0.00	5.58±1.150	0.00±0.00	3.67±1.10	0.00±0.00
Curularia lunataa	7.78±1.580	2.65±1.08	3.50±1.45	2.00±1.00	5.78±1.080	1.65±0.58	0.00±0.00	0.00±0.00
Chaetomium globosum	5.67±1.060	2.00±0.67	2.25±2.00	0.00±0.00	7.67±1.060	1.56±1.08	3.45±2.00	0.00±0.00
Cladosporium clamdosporum	3.00±0.980	0.00±0.00	0.00±0.00	0.00±0.00	6.08±1.780	4.56±2.00	2.08±1.33	0.00±0.00
Penicillium citrum	8.60±1.500	2.52±1.00	4.60±2.00	2.52±2.00	7.60±1.730	2.00±1.00	4.65±1.73	0.00±0.00
Penicillium rubrum	19.00±1.68	9.67±1.71	17.67±1.50	12.67±2.45	17.86±2.00	4.39±2.08	14.66±1.50	2.33±1.08
Fusarium clamydosporum	5.59±1.150	1.67±0.33	8.67±2.08	3.00±1.50	2.50±1.450	2.88±1.00	6.35±2.00	2.00±0.50
Fusarium moniliformae	8.83±2.080	4.30±2.37	12.83±2.00	4.00±2.08	4.98±1.560	0.00±0.00	9.25±2.76	3.67±0.98
Rhizopus stolonifers	4.00±1.080	0.00±0.00	3.70±2.09	0.00±0.00	9.50±2.080	1.19±1.33	6.75±0.50	2.67±1.76

Table 2: Incidence of seed borne fungi on genotype of mungbean seed at the 180 days period of storage

	HUM-4				HUM-12			
	Agar plate method		Blotter plate method		Agar plate method		Blotter plate method	
Genotype fungus species	US	S	US	S	US	S	US	S
Alternaria alternata	16.67±2.67	5.64±1.08	10.33±2.08	2.69±1.33	12.08±2.67	2.69±0.50	5.46±2.26	1.80±0.67
Aspergillus flavus	20.67±1.23	8.80±1.33	10.00±1.50	3.34±1.67	20.65±1.67	10.67±2.83	15.67±0.55	4.67±2.45
Aspergillus niger	40.27±1.08	14.55±2.50	28.25±1.67	9.70±2.25	34.49±2.56	12.85±1.33	22.98±2.25	5.33±2.00
Aspergilus fumigates	18.42±3.33	6.39±1.67	11.42±3.33	2.00±1.55	11.08±1.33	5.86±0.67	12.08±2.56	0.00±0.00
Aspergilus candidates	14.67±1.85	5.60±2.00	8.56±2.08	1.89±1.50	8.33±1.530	4.00±1.30	1.67±0.85	0.00±0.00
Aspergillus orchareus	9.78±1.330	2.67±0.50	8.67±2.00	2.67±1.00	7.38±3.210	1.50±0.67	5.67±1.45	1.58±1.08
Aspergillus terrus	17.46±2.00	9.49±1.85	15.36±3.89	3.83±1.67	11.87±2.67	2.75±1.33	10.65±2.67	2.75±1.50
Curularia lunataa	20.47±1.67	3.85±1.20	14.89±1.98	4.69±2.00	15.54±2.50	7.92±0.98	2.89±3.00	0.00±0.00
Cephaliophora irreguarie	8.56±1.670	1.60±0.65	7.78±2.00	0.00±0.00	10.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00
Chaetomium globosum	12.67±2.34	2.00±1.50	10.33±3.67	1.54±0.25	16.67±2.00	3.85±1.65	5.55±2.65	0.00±0.00
Cladosporium cladospori	14.70±1.85	6.78±2.08	13.33±1.33	6.67±1.33	18.85±2.35	2.67±1.33	10.33±2.00	0.00±0.00
Penicillium citrum	20.45±1.33	10.74±2.08	11.67±2.67	2.74±1.33	11.78±2.35	5.74±2.33	8.83±1.33	1.73±0.55
Penicillium rubrum	26.80±3.50	9.75±2.50	22.65±0.58	5.67±3.67	18.87±1.25	1.67±0.67	13.39±1.33	1.67±0.25
Fusarium cladosporium	10.56±1.67	0.00±0.00	15.00±1.65	1.60±0.55	8.67±3.350	0.00±0.00	13.46±1.00	3.85±2.52
Fusarium moniliformae	19.68±3.36	7.33±2.08	26.08±3.00	15.26±1.50	16.67±3.33	1.90±0.50	22.00±1.67	9.33±2.85
Mucor racemosus	10.00±1.65	1.33±0.13	18.00±3.33	2.95±1.45	12.26±2.89	8.00±2.00	15.0±0.500	4.34±1.50
Trichoderma viridae	6.00±3.510	2.89±1.67	0.00±0.00	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00
Trichoderma harzianum	8.78±2.520	1.67±0.50	0.00±0.00	0.00±0.00	4.98±1.560	0.00±0.00	0.00±0.00	0.00±0.00
Rhizopus stolonifers	17.53±2.00	7.57±0.25	16.67±2.88	1.67±0.98	22.73±1.56	10.00±0.50	18.98±3.00	8.87±2.00
White sterile mycelium	7.00±1.500	0.00±0.00	0.00±0.00	0.00±0.00	2.67±2.150	0.00±0.00	0.00±0.00	0.00±0.00

belonging to 11 distinct genera were isolated (Table 2). The prominent observation of seed associated mycoflora of mungbean are the *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terrus*, *A. fumigatus*, *A. orchareus*, *A. candidates*, *Penicillium citrinum*, *P. rubrum*, *Curvularia lunata*, *Fusarium moniliformae*, *Fusarium clamydosporum*, *Claddosporium cladosporoids*, *Chaetomium globosum*, *Rhizopus stolonifer*, Mucor *Racemosus*, *Chaetomiun globosum*, *Trichoderma viridae*, *Trichoderma harzianum* and white sterile mycelium. Highest (20) fungi were isolated by agar plate method in HUM-4 under unsterilized condition and lowest (11) fungi by blotter plate method under sterilized condition (Table 4). After the storage duration of 180 days some saprophytic storage fungi recorded in higher percentage which was not recorded from fresh seed. Among all these fungi such as *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp., *Chaetomium globosum*, *Cladosporium cladosporoids*, *Penicilium glabuorum* and *Alternaria alternata* boasted up to high level due to increase in storage period. At the 180 days of storage the level of *Aspergillus niger* boasted up to (40.27%) which showed maximum incidence followed by (34.49%) in genotype HUM-12 through Agar condition then *Penicillium rubrum* (26.08%) in genotype HUM-4 under Agar plate and *Fusarium moniliformae* (26.08%). Some fungi like *Curvularia lunata*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terrus*, *Aspergillus candidates* and *Aspergillus orchareus* (20.67, 16.67, 20.67, 18.42, 17.46, 14.67 and 9.78%, respectively) were recorded highest in genotype HUM-4 through Agar plate method followed by (15.54, 12.08, 20.65, 11.08, 11.87, 8.33 and 7.38%) and lowest in genotype HUM-12 through Blotter paper methods and lowest in HUM-12 through blotter method. Highest incidence of *Cephaliofhora irregularis, Chaetomium globosum* and *Cladosporium cladosporoids* (10.00, 16.67 and 18.85%) was recorded highes in genotype HUM-12 under Agar plate followed by (8.56, 12.67 and 14.70%) in Genotype HUM-4 through Agar plate method. Highest incidence of *Fusarium clamydosporum*, *Fusarium moniliformae* and *Mucor racemosus* (15.00, 26.08 and 18.00%, respectively) in genotype HUM-4 under blotter condition as compared to (13.46, 22.00 and 15.00%, respectively) genotype HUM-12 under blotter condition and lowest in HUM-12 under agar condition. Incidence of unknown fungus, white sterile mycelium was recorded (7.00%) only in genotype HUM-4 under agar condition. Result showed that unsterilized seed yielded highest number of fungi as comparison to sterilized seeds. Least incidence was recorded *Curularia lunata*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporoids*, *Trichoderma harzianum* and *T. viridae* in HUM-12 under Blotter methods.

Incidence of seed borne fungi on mungbean seed varieties at 360 days of storage: A total of 22 different fungi including one bacterial isolate belonging to 12 distinct genera including Bacterial isolate were isolated (Table 3). The prominent observation of seed associated mycoflora of mungbean seed are the *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terrus*, *A. fumigatus*,

Table 3: Incidence of seed borne fungi on genotype of mungbean seed at the 360 days period of storage

	HUM-4				HUM-12			
	Agar plate method		Blotter plate method		Agar plate method		Blotter plate method	
Genotype fungus species	US	S	US	S	US	S	US	S
Alternaria alternata	20.37±3.00	8.60±2.08	13.56±2.67	5.00±1.58	18.89±3.65	4.38±2.50	7.43±2.26	2.80±1.34
Aspergillus flavus	24.43±2.56	14.56±2.08	14.00±1.50	4.34±1.67	22.86±2.80	13.67±3.83	17.67±1.55	6.60±4.45
Aspergillus niger	50.56±4.98	23.50±2.50	30.85±4.67	3.85±2.00	40.67±3.98	15.45±2.33	25.56±2.67	14.33±2.00
Aspergilus fumigates	20.67±2.36	9.39±2.67	15.56±2.98	8.50±2.55	16.37±2.67	8.85±1.67	13.08±2.56	2.39±1.67
Aspergilus candidates	16.98±2.80	9.56±2.56	14.00±3.80	3.85±2.50	10.67±2.45	4.85±2.30	3.67±0.85	2.75±1.50
Aspergillus orchareus	11.56±2.35	4.85±2.50	9.80±1.60	3.56±2.60	9.80±2.67	3.50±1.67	7.80±2.40	3.46±2.80
Aspergillus terrus	18.86±3.80	11.56±2.8	17.68±2.69	8.80±2.00	13.77±3.00	4.00±2.45	11.49±2.67	4.56±2.50
Curularia lunataa	17.69±2.00	1.77±0.58	10.56±2.56	2.49±1.56	12.00±1.78	5.65±2.00	1.84±2.80	0.00±0.00
Cephaliophora irreguaries	4.58±1.35	1.90±0.85	6.69±1.56	0.00±0.00	8.50±2.08	2.86±2.00	0.00±0.00	0.00±0.00
Chaetomium globosum	10.39±4.78	1.00±0.50	8.30±3.67	0.00±0.00	13.37±3.46	1.85±0.78	3.52±1.60	0.00±0.00
Cladosporium cladosporium	13.68±2.0	4.85±1.56	10.56±2.67	4.67±2.56	15.75±1.68	1.60±1.33	8.65±2.00	1.67±0.25
Penicillium citrum	26.67±2.58	13.56±3.85	13.33±5.80	2.30±1.50	12.98±3.00	5.50±2.35	10.80±2.85	3.35±1.55
Penicillium rubrum	29.95±2.00	15.70±3.50	22.65±0.58	9.53±2.85	25.56±2.47	11.44±2.08	15.56±2.85	3.35±1.85
Fusarium cladosporium	12.00±2.00	4.67±1.98	18.85±2.00	11.60±3.55	8.67±3.35	0.00±0.00	15.46±2.30	4.36±2.38
Fusarium moniliformae	22.56±2.76	10.38±3.08	28.38±3.56	17.67±2.56	18.80±5.30	2.90±1.50	24.50±2.80	10.33±3.80
Mucor racemosus	12.50±2.50	4.39±1.13	21.50±2.33	3.66±2.00	15.56±4.80	8.53±2.60	17.65±3.50	5.37±2.50
Trichoderma viridae	8.56±1.98	1.56±0.56	4.76±1.33	0.00±0.00	6.30±1.87	2.50±1.33	0.00±0.00	0.00±0.00
Trichoderma harzianum	9.00±3.53	2.98±1.55	5.00±1.30	2.30±0.56	5.00±2.85	1.43±0.00	0.00±0.00	0.00±0.00
Rhizopus stolonifers	19.73±1.58	10.57±0.25	18.67±3.98	3.67±2.00	25.56±2.67	14.30±2.50	20.90±3.50	10.87±2.00
White sterile mycelium	9.95±2.55	3.00±0.50	1.98±2.60	0.00±0.00	4.56±1.59	0.00±0.00	0.00±0.00	0.00±0.00
Dark sterile mycelium	6.43±2.00	3.50±1.56	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bacterial Isolate	6.67±1.85	0.00±0.00	0.00±0.00	0.00±0.00	4.00±1.67	0.00±0.00	0.00±0.00	0.00±0.00

Table 4: Total No. of seed borne fungi of mungbean seed under sterilized and unsterilized condition

	HUM-4				HUM-12			
Days	US	S	US	S	US	S	US	S
0	15	13	13	7	14	11	12	6
180	20	19	18	16	19	14	16	12
360	22	19	17	13	21	13	17	12

A. orchareus, *A. candidates*, *Penicillium citrinum*, *P. rubrum Curvularia lunata*, *Fusarium moniliformae*, *Fusarium cladosporium*, *Claddosporium cladosporoids*, *Cephaliophora irreguaries*, *Chaetomium globosum*, *Rhizopus stolonifer* Mucor, *Chaetomiun globosum*, *Trichoderma viridae*, *Trichoderma harzianum*, white sterile mycelium, dark sterile mycelium and one bacterial isolate. Highest (22) fungi were isolated by agar plate method in genotype HUM-4 under unsterilized condition and lowest in (14) fungi under sterilized condition (Table 4). At the end of storage some new saprophytic fungi like, Dark sterile mycelium and some unknown bacterial isolate were observed (6.43 and 6.67%, respectively) through Agar plate. Due to prolongation of storage period incidence of some storage fungi like *Aspergillus* sp., *Penicillium* sp., *Rhizopus stolonifer*, *Mucor racemosus* boasted up to high level. *Aspergillus niger* was recorded highest incidence (50.56%) was recorded in HUM-4 method followed by (40.67%) in HUM-12 through agar plate then *Penicillium rubrum* (29.95%) in Hum-4 through Agar plate, *Fusarium moniliformae* (28.38%) in HUM-4 under Blotter methods and *Rhizopus stolonifer* (25.56%) in genotype HUM-12 through Agar condition. Some fungi like *Curvularia lunata*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terrus*, *Aspergillus candidates* and *Aspergillus orchareus* (17.69, 20.37, 24.43, 20.67, 18.86, 16.98 and 11.56%, respectively) were recorded highest in genotype HUM-4 through Agar plate method followed by (12.00, 18.89, 22.86, 16.37, 13.77, 10.67 and 13.77%) and lowest in genotype HUM-12 through Blotter paper method. Highest incidence of *Cephaliofhora irregularis*, *Chaetomium globosum* and *Cladosporium cladosporoids* (8.50, 13.37 and 15.75%) was recorded highest in genotype HUM-12 under Agar plate followed by (4.58, 10.39 and 13.68%) in Genotype HUM-4 through Agar plate method. Highest incidence of *Fusarium clamydosporum* and *Mucor racemosus* (18.85 and 21.50%, respectively) in genotype HUM-4 under blotter condition as compared to (15.56 and 17.65%, respectively) genotype HUM-12 under blotter condition and lowest in HUM-12 under Agar condition. Incidence of unknown fungus, white sterile mycelium was recorded (7.00%) only in genotype HUM-4 under agar condition. Sterilized seed reduced the incidence of some seed borne fungi viz., *Curularia lunata*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporoids*, *Trichoderma harzianum* and *T. viridae* but some fungi

Table 5: Percent occurrences of various classes of fungi on mungbean seed

Classes of fungi	No. of sp. isolated	Occurrence (%)
Zygomycotina	2	9.09
Ascomycotina	3	13.89
Deutermycotina	15	68.98
Mycelia sterilla	2	9.09
Total No. of isolated fungi	22	

such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* species and *Rhizopus* sp. and their incidence were recorded in very few amount. Least effect of treatment was recorded on seed borne fungi (Table 5) Result revealed that in all storage period *Aspergillus* sp. *Penicillium* sp. *curvularia lunata* were recorded highest in HUM-4 through Agar plate method *Fusarium* sp. were recorded highest in HUM-4 blotter method and lowest in HUM-12 agar plate methods, *Rhizopus Cephaliofhora irregularis*, *Chaetomium globosum* and *Cladosporium cladosporoids* in HUM-12 under Agar condition. Lowest incidence and highest incidence was recorded in HUM-12 under blotter method and HUM-4 under Agar plate methods during treated with seeds. Table 4 showed that maximum number of fungi belonged to Deuteromycotina (68.98%) followed by Ascomycotina (13.89%) and Zygomycotina (9.00%) (Table 5).

DISCUSSION

The fungi isolated from mungbean seed were main cause of deterioration of seed under the different storage period (Rahman *et al*., 1999). The seeds of green gram are found to be heavily infested with variety of fungi (Ramnath *et al*., 1970). In this study a total of 22 fungal species were isolated by Agar and blotter method from mungbean seed under sterilized and sterilized condition at 0, 180 and 360 days period of storage. Present result showed that saprophytic fungi viz., *A. niger* was predominant among the fungi isolated. Such similar reports have been made by Dawar and Ghaffar (1991) on sunflower seed (Rasheed *et al*., 2004) on groundnut seed. *A. niger* were the predominant storage fungi of groundnut seeds (Mukherjee *et al*., 1992) and soybean seed (Tariq *et al*., 2005). Fungal contamination of stored seed varied with storage duration condition. The dominant fungi and fungal growth depended on period of storage and environmental conditions. Dominant group of fungus *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terrus*, *Penicilium rubrum*, *Penicillium citrum*, *Fusarium clamydosporum*, *Fusarium moniliformae*, *Chatomium globosum* and *Cladosporium cladosporoids* were isolate from mungbean seed through agar and blotter method increased due to storage period. The occurrence of these fungi in mungbean seed has been reported by many other workers (Bakr and Rahman, 2001; Fakir, 2001). Similar result was reported by Al-Yahya (1999) and Krasauskas *et al*. (2005). The result indicated the high incidence of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terrus*, *Alternaria Alternata*, *Penicilium rubrum*, *Penicillium citrum*, *Rhizopus stolonifer* and *Mucor racemosus* and low incidence of field fungi like *Alternaria Alternata*, *Fusarium clamydosporum*, *Fusarium moniliformae*, *Fusarium semitectum*, *Chatomium globosum* and *Cladosporium cladosporoids*. This was due to ability of saprophytes to colonize, rapid germination of spore fast hyphal invasion high competitive nature and their ability to utilize a wide variety of substrate and their nutrient composition (Novak *et al*., 2001). In most of cases agar plate was found to be superior than blotter for the isolation of seed mycoflora (Godbole, 1982). The prevalence of fungal community may be attributed generally to abiotic variables and nature of substrate. Khanna (1964), Williams and Gray (1974), Rai and Srivastava (1982), Thormann *et al*. (2003) reported that appearance of some new fungi, only on agar and which did not found in blotter method indicates that these fungi need some external supply of nutrients. On the contrary, low incedecne of *Chaetomium globosum*, *Syncephalstrum* sp., *Trichothecium roseum* in agar plate might be due to antagonistic effect of *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans*, *Cladosporium herbarum*, *Drechslera tetramera*, *Curvularia lunata* which were dominant in agar plate. Similar type of observations reported by Aulakh *et al*. (1976) that in agar method *Aspergillus niger*, *Penicillium* sp., *Rhizopus arrhizus* suppressed the growth of other fungi of maize seeds. Result showed that treated seeds yielded less population of seed-borne fungi than the untreated seeds that is close conformity with those of Limonard (1968), Bhutta *et al*. (1998) and Sharfun-Nahar *et al*. (2005). Similar results have also been reported from seeds other than sunflower e.g., groundnut seeds by Rasheed *et al*. (2004) and legume seeds by Embaby and Abdel-Galil (2006). These results are in agreement with the findings of Sundaresh and Hiremath (1978) in soybean seed mycoflora. Result showed that incidence of storage fungi increased due to prolongation of storage period storage fungi. Similar result was reported by Macedo *et al*. (2002). The variation in fungal frequency mainly due to isolate technique used in this study. The increasing trend regarding fungal detection was observed the blotter paper method as it already proved that blotter found to be the most efficient economical and reliable method by different researcher (Bhutta *et al*., 1999; Fakhrunnisa and Hashmi, 1992). According to another point of view, there are

several biotic and abiotic factor to increase the incidence of seed borne fungi as seed sources, moisture level, storage condition and duration. At the initial stage of storage, incidence of different group of storage fungi boasts from the beginning of storage to the end of 180 and 360 days of storage. In maximum occurrences of fungus in over time was observed by Katta and Bullerman (1995).

CONCLUSION

The fungi associated with seed samples were *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terrus*, *A. fumigatus*, *A. orchareus*, *A. candidates*, *Penicillium citrinum*, *P. rubrum*, *Curvularia lunata*, *Fusarium moniliformae*, *Fusarium cladosporium*, *Claddosporium cladosporoids*, *Cephaliophora irreguaries*, *Chaetomium globosum, Rhizopus stolonifer*. Mucor, *Chaetomiun globosum*, *Trichoderma viridae*, *Trichoderma harzianum*, white sterile mycelium, Dark sterile mycelium and one bacterial isolate. Among all seed borne fungi *Aspergillus niger* showed maximum incidence in genotype HUM-4 as well as HUM-12 under Agar plate and blotter paper method and white sterile mycelium, dark sterile mycelium and *Trichoderma* sp. showed lowest incidence.

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