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Seed Health Evaluation of Various Pulses by Incubation Methods

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A B S T R A C T

The present investigation was carried out in the Dept. of Plant Pathology, IGKV, Raipur (C.G) during 2015-16. Basic seed pathological work were conducted with five pulse crops seeds namely green gram, black gram, chickpea, pea and pigeonpea periodically from 1-6 months at monthly interval. The seed health evaluation of various pulses by physical and incubation method was investigated. In all, eleven seed borne mycoflora viz. *Aspergillus niger*, *Tricoderma* sp., *A. flavus*, *Fusarium* sp., *Rhizopus* sp., *Alternaria* sp., *Mucor* sp., *A. fumigatus*, *Aspergillus* sp., *Penicillium* sp. and others (unidentified fungi) isolated by incubation methods viz. standard blotter and agar plate method. Seed lots were subjected to incubation methods i.e. standard blotter and agar plate shows increasing trend in frequencies of seeds borne mycoflora and decreasing trend in seed germination as the storage period increases. The reduction in seed germination due to association of various seed borne mycoflora clearly suggesting of role of seed borne mycoflora in reducing seed germination in all the pulses under study.

Introduction

Pulses may be defined as the dried edible seeds of cultivated legumes. They belong to the family Fabiaceae. Pulses for being environment friendly, major source of protein and complementing cereals both in production and consumption will have a vital role to play under the present circumstances. Seed is dormant phase of future crop and reviewing constant global attention due to the demand for high yields and increasing agricultural co-operations. Good and healthy seeds are not only essential requirement but also an important component for any successful production programme. Seeds are regarded as means of transporting plant pathogen (Agarwal and Sinclair, 1996). Seed born pathogen may cause seed abortion, seed rot/ necrosis, reduction or elimination of germination capacity as well as seedling damage (Khanzada *et al.,* 2002). Leguminous crops commonly carry seed born mycoflora is a major factor affecting seed health (Neergaard, 1977).

The seed mycoflora of pigeonpea *viz. Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A.* sp., *Penicillium* sp., *Mucor* sp., mycelia sterilia and *Fusarium udum* reduce the seed germination of different pigeonpea varieties (Pradhan *et al.,* 2015). Seed-borne mycoflora have been isolated in agar plate and blotter test were belonging to six genera namely

Aspergillus niger, *Penicillium* sp., *Fusarium moniliforme*, *Fusarium oxysporum* f.sp. *ciceri*, *Rhizopus* sp. and *Trichoderma harzanium*. Since seed borne infections attack in the initial stage of seed germination (Trivedi and Rathi, 2015). Seed health refers primarily to the presence or absences of the microorganisms of various kinds (ISTA, 1985). It has been realized time and again that seed health, a paramount importance for crop stand and yield returns. Hence, aptly quoted "A healthy seed leads to a healthy harvest''. In this content, present investigation was carried out to know the seed health evaluation of various pulses by incubation methods.

Materials and Methods

Seed sampling

The five crops and its varietiy green gram (HUM-16), black gram (Indira urd-1), chickpea (Vaibhav), pea (Arkel) and pigeonpea (Asha) were taken for detection of seed mycoflora and dry seed examination and procure from various projects of IGKV, Raipur and kept in cloth bags, labeled and stored at room temperature during entire course of investigation. The following standard methods recommended were used in the present study of seed health evaluation.

Standard blotter method (ISTA, 1976)
Agar plate method (Muskett and Malone, 1941)

Incubation methods

Standard blotter method

This method was used to detect the presence of fungi on or in the seeds after incubation. By this test, the fast growing fungi are better detected than the slow growing ones. In each petriplate of 9.0 cm diameter, two good quality blotter papers of the same diameter were kept and moistened with sterilized distilled water. In each plate, 10 seeds were placed on the moistened blotters in such a manner that 9 formed the outer circle and one at the center. For each variety, 40 replicated plates were maintained (total of 400 seeds tested for each variety). Incubate the plate at 22±1ºC for 7 days in alternating cycles of 12 hours darkness and 12 hours light. Observations were recorded as described earlier. All seeds of the outer ring were examined first and finally the seed in the centre of the dish and expressed in per cent seed mycoflora associated.

Agar plate method

Potato dextrose agar medium (15-20 ml) was poured in each sterilized petriplate. To avoid bacterial contamination, a little amount of Streptomycin sulphate was added in the medium at the time of pouring. Seeds of each variety were surface sterilized with 1.0% NaOCl solution for 30 seconds and immediately washed twice with sterile distilled water thoroughly to remove NaOCl solution that adhered if any. Seeds were placed on the previously poured medium in petriplate in such a way that 9 in the outer circle and one at the centre. For each variety, 40 replicated plates were maintained and incubated at 22±1ºC under alternate cycles of 12 hrs dark and 12 hrs light in NUV. Observations were recorded as described earlier.

Results and Discussion

Seed health evaluation of various pulses by incubation method

Black gram

Seeds of black gram variety Indira urd-1 were examined for associated seed borne mycoflora by two incubation methods i.e. standard

blotter and agar plate as per ISTA and data presented in table 1. Germination of seeds was maximum in first month of storage and then decreasing trend was observed upto sixth month of storage period in both the incubation methods.

It was also observed that total mycoflora was in increasing trend as the storage period increases with the range from 40.3 to 80.00 in stander blotter method and from 58.1 to 159.2 in agar plate method.

A variety of mycoflora was observed with varying frequencies in both the methods. In standard blotter method, increasing trend in the frequency of *A. niger*, *A. flavus*, *Fusarium* sp., *Alternaria* sp., *Mucor* sp., *A. fumigatus*, *A.* sp., *Penicillium* sp., and unidentified mycoflora except in *Tricoderma* sp., and *Rhizopus* sp. In these last two mycoflora, frequency of occurrence was erratic.

Among the mycoflora detected over the study period of sixth month, overall occurrence was maximum of *Rhizopus* sp. (9.73%) followed by *Fusarium* sp. (9.38%) and *Tricoderma* sp. (9.11%). Frequency was least in occurrence of *Penicillium* sp. (1.28%).

In agar plate method, increasing trend in frequencies of associated mycoflora were observed.

Among the mycoflora detected, frequency was maximum in *Alternaria* sp. (25.04%) followed by *Fusarium* sp. (22.67%) and *A. niger* (13.67%) over the storage period taken in the study. *A. fumigatus*, *Aspergillus* sp. and *Penicillium* sp. were not detected in agar plate method in any of the month of storage.

Majority of seed borne mycoflora and less seed germination found in the present study were also reported by Rahman *et al.*, (1999) are in conformity with the result of this study.

Green gram

It was depicted from the data of table 2 that decreasing trend in seed germination was observed in both the incubation methods as the storage period increases.

On the other side increasing trend was observed in total seed borne mycoflora associated with seeds ranging from 71.64 to 108.8 and 55.4 to 187.0 in standard blotter and agar plate method, respectively.

Data presented in table 2 also reveal that various types of mycoflora were found associated with green gram seeds in varying frequencies over the storage period of six months. In general, increasing trend in the frequencies of occurrence of detected mycoflora was observed in standard blotter method. Among the detected mycoflora, maximum average frequency was observed in *Aspergillus niger* (23.28%) which was followed by *Fusarium* sp. (15.04%), *Rhizopus* sp. (12.14%) and least in *A. fumigatus* (1.93). *Penicillium* sp. was not detected in green gram variety HUM 16.

In the same table, data on mycoflora detected is presented and observed that increasing trend in the frequency of mycoflora irrespective of genus of mycoflora. Among them, highest frequency was observed in *Mucor* sp. (20.95%) followed by *Aspergillus niger* (13.64) and *Rhizopus* sp. (12.74%). *Aspergillus flavus* was not detected in any of the month of storage.

Commonly occurring seed borne mycoflora found associated with seeds of green gram were reported by Sharma and Singh (2001), Barua *et al.*, (2007), Ashwini and Giri (2014) and Sarita *et al.*, (2014) which were in confirmity with the findings of present study in which most common seed borne fungi in varying frequencies were recorded.

Pea

Seeds of pea variety Arkel were subjected to standard blotter and agar plate method for seed health evaluation and data obtained were presented in table 3. Germination percentage of seeds was gradually fall down from first month to sixth month of storage whereas total seed mycoflora was increases with the increase in storage months. It ranges from 50.63 to 125.4 in standard blotter and 30.0 to 172.4 in agar plate method.

In standard blotter method, increasing trend was observed in frequencies of associated seed borne mycoflora. Frequency was maximum in *Alternaria* sp. (30.70%) followed by *Fusarium* sp. (21.98%) and *Rhizopus* sp. (12.46%) though it was not observed in first month.

Frequency was least in *Tricoderma* sp. (4.86%). Commonly detected mycoflora like *A. fumigatus, A.* sp. and *Penicillium* sp. was not detected as in other pulses under study.

Similarly, increasing trend in frequencies of detected mycoflora was recorded in agar plate method. Among the mycoflora recorded, frequency was highest in *Alternaria* sp. (20.44%) followed by *Fusarium* sp. (18.6%) and unidentified mycoflora (16.06%).

Frequency was least in *Aspergillus flavus* and *Rhizopus* sp. (8.57%). *Tricoderma* sp., *A.fumigatus* and *A.* sp. was not at all recorded in any of the months of study.

Wilman *et al.,* (2014) identified various mycoflora from pea seeds and observed seed borne fungi were responsible for less seed germination. These findings are in agreement with the findings of present study in which decreasing trend in seed germination and increasing trend in seed mycoflora were recorded.

Chickpea

Like other pulses under study, decreasing trend in germination percent and increasing trend in associated mycoflora with seed was observed in chickpea variety Vaibhav in both incubation methods (table 4).

Total mycoflora recorded in standard blotter method was ranging from 67.96 to 154.3 and 58.7 to 172.1 in agar plate method.

In standard blotter method, detected mycoflora show increasing trend in frequencies of different mycoflora.

Among them, frequency was highest in *Tricoderma* sp. (29.99%) which was closely followed by *Aspergillus flavus* (29.67%).

Frequency was lowest in *Aspergillus niger* (5.91%). *Rhizopus* sp., *Mucor* sp. and *Penicillium* sp. were not detected in any of the months of study.

In agar plate method, frequency of mycoflora increases with the increase in storage months.

Among the mycoflora recorded, highest frequency was found in *A. flavus* (23.95%) followed by *Mucor* sp. (17.64%) and *Tricoderma* sp. (7.97%). *Penicillium* sp. and *A.* sp. not recorded over the study period.

Various genera of seed borne fungi were found associated with chickpea seeds in varying frequencies with less germination and poor seedling vigour was reported by several earlier worker (Prasad *et al.,* 1998; Rauf, 2000; Shahnaz *et al.,* 2007; Razia and Pathak, 2013 and Trivedi and Rathi, 2015) corroborating with the findings of present study in which decreasing trend in seed germination and increasing trend in different seed borne mycoflora with the increase in storage period were recorded.

Table.1 Detection of mycoflora associated with seeds of black gram (Indira urd-1) by incubation methods

S.N.	Mycoflora Associated	Standard Blotter Method (frequency of mycoflora %)							Agar Plate Method (frequency of mycoflora %)						
		Month						Average	Month						Average
		L	I.	L	II.	L	III.		L	I.	L	II.	L	III.	
(a)	**Germination (%)**	88.00	76.80	73.60	70.80	68.8	68.00	74.34	76.00	74.40	72.00	68.00	66.50	60.40	69.55
								7.95							
(b)	**Mycoflora**							9.11							
1	*Aspergillus niger*	5.33	6.00	8.00	8.80	9.60	10.00		2.10	4.30	12.40	16.30	20.50	26.40	13.67
2	*Tricoderma* sp.	10.66	11.2	7.20	7.60	8.40	9.60	8.95	2.40	2.30	4.20	6.40	10.40	12.50	6.37
3	*A. flavus*	7.33	8.00	8.40	9.20	10.40	10.40	9.37	4.10	6.50	8.30	10.30	14.30	18.30	10.30
4	*Fusarium* sp.	6.66	8.40	9.20	10.00	10.40	11.60	9.73	16.30	18.30	22.30	24.50	26.30	28.40	22.67
5	*Rhizopus* sp.	10.33	7.20	9.60	10.00	10.40	10.80	6.4	4.40	6.50	8.50	12.40	14.30	16.30	10.40
6	*Alternaria* sp.	-	3.20	5.60	6.80	7.60	8.80	1.52	20.20	20.30	24.50	26.40	28.30	30.50	25.04
7	*Mucor* sp.	-	0.40	0.80	1.60	2.00	2.80	3.84	6.30	6.20	8.00	10.40	14.40	14.40	9.95
8	*A. fumigatus*	-	1.60	3.60	4.40	4.80	4.80	3.28	-	-	-	-	-	-	-
9	*Aspergillus* sp.	-	2.00	2.40	3.20	4.00	4.80	1.28	-	-	-	-	-	-	-
10	*Penicillium* sp.	-	0.40	0.80	1.20	1.20	2.80	2.76	2.30	4.40	8.30	10.50	10.40	12.40	-
11	Others	-	1.60	2.00	3.20	3.40	3.60								8.05
11	(Unidentified fungi)														
	Total	**40.31**	**50.00**	**57.6**	**66.00**	**72.20**	**80.00**		**58.1**	**68.80**	**96.5**	**117.2**	**138.9**	**159.2**	

Table.2 Detection of mycoflora associated with seeds of green gram (HUM-16) by incubation methods

S.N.	Mycoflora Associated	Standard Blotter Method (frequency of mycoflora %)							Agar Plate Method (frequency of mycoflora %)						
		Month						Average	Month						Average
		L	I.	L	II.	L	III.		L	I.	L	II.	L	III.	
(a)	**Germination (%)**	79.33	72.0	70.4	68.00	67.00	66.00	70.46	86.20	78.00	74.60	70.10	68.20	64.20	73.55
(b)	**Mycoflora**														
1	*Aspergillus niger*	27.66	19.60	21.60	22.80	23.60	24.40	23.28	4.30	8.30	12.20	16.30	18.40	22.30	13.64
2	*Tricoderma* sp.	7.66	9.20	10.80	11.20	12.00	13.60	10.75	4.40	6.50	10.20	12.30	14.50	18.30	11.04
3	*A. flavus*	6.33	7.20	9.60	10.40	10.80	11.20	9.26	-	-	-	-	-	-	-
4	*Fusarium* sp.	15.00	16.00	14.00	14.40	15.20	15.60	15.04	4.10	6.40	6.30	8.30	12.40	14.40	8.65
5	*Rhizopus* sp.	10.00	11.20	14.00	12.00	12.40	13.20	12.14	6.60	6.80	10.20	14.20	18.60	20.00	12.74
6	*Alternaria* sp.	3.00	4.00	6.00	7.20	7.60	8.40	6.04	4.50	4.70	8.40	10.60	12.50	16.50	9.54
7	*Mucor* sp.	-	1.60	2.40	3.20	3.60	4.00	2.96	14.30	16.60	18.20	22.60	26.00	28.00	20.95
8	*A. fumigatus*	0.33	0.80	1.60	2.00	2.80	4.00	1.93	4.30	6.60	10.30	12.60	16.40	18.50	11.45
9	*Aspergillus* sp.	1.00	3.20	6.00	6.80	7.60	8.40	5.5	2.30	4.60	6.30	8.60	10.00	16.50	8.05
10	*Penicillium* sp.	-	-	-	-	-	-	-	6.30	6.50	12.30	14.20	16.40	18.50	12.37
11	others	0.66	1.60	4.00	4.80	5.20	6.00	3.71	4.30	4.60	6.60	10.10	12.00	14.00	8.6
1	(Unidentified fungi)														
	Total	**71.64**	**74.40**	**90.00**	**94.80**	**100.8**	**108.0**		**55.4**	**71.6**	**101.0**	**129.8**	**157.2**	**187.0**	

Table.3 Detection of mycoflora associated with seed of pea (Arkel) by incubation methods

S.N.	Mycoflora Associated	Standard Blotter Method (frequency of mycoflora %)							Agar Plate Method (frequency of mycoflora %)						
		Month						Average	Month						Average
		L	I.	L	II.	L	III.		L	I.	L	II.	L	III.	
(a)	**Germination (%)**	74.66	69.40	67.40	66.50	65.44	63.50	67.83	90.20	84.30	78.40	72.30	66.30	62.00	75.59
(b)	**Mycoflora**														
1	*Aspergillus niger*	2.66	3.40	4.60	5.30	6.30	7.50	4.96	4.40	6.50	10.40	14.50	18.00	22.50	12.72
2	*Tricoderma* sp.	1.33	2.40	4.70	5.80	6.50	8.40	4.86	-	-	-	-	-	-	-
3	*A. flavus*	2.00	3.30	6.70	9.60	9.60	11.60	7.14	2.20	4.30	6.00	10.00	12.40	16.50	8.57
4	*Fusarium* sp.	16.66	20.40	21.70	23.30	24.30	25.50	21.98	12.30	14.30	16.00	18.50	22.00	28.50	18.6
5	*Rhizopus* sp.	-	8.40	10.30	13.30	14.30	16.00	12.46	2.20	4.30	8.40	10.00	12.55	14.00	8.57
6	*Alternaria* sp.	26.66	28.60	30.30	31.30	33.30	34.00	30.70	12.70	12.40	18.00	22.50	26.50	30.50	20.44
7	*Mucor* sp.	0.66	2.60	4.40	5.10	8.60	8.40	4.96	4.20	4.30	6.40	10.00	12.00	18.50	9.23
8	*A. fumigatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	
9	*Aspergillus* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	
10	*Penicillium* sp.	-	-	-	-	-	-	-	-	-	8.50	10.50	14.50	15.40	12.23
11	Others	0.66	4.40	8.60	9.10	12.30	14.00	8.18	-	10.30	12.40	14.50	16.40	26.50	16.02
1	(Unidentified fungi)														
	Total	**50.63**	**73.50**	**91.30**	**102.8**	**115.2**	**125.4**		**30.0**	**56.4**	**86.1**	**110.5**	**134.35**	**172.4**	

Table.4 Detection of mycoflora associated with seeds of chickpea (Vaibhav) by incubation methods

S.N.	Mycoflora Associated	Standard Blotter Method (frequency of mycoflora %)							Agar Plate Method (frequency of mycoflora %)						
		Month						Average	Month						Average
		L	I.	L	II.	L	III.		L	I.	L	II.	L	III.	
(a)	**Germination (%)**	84.06	75.00	72.00	70.30	68.60	65.10	72.51	90.10	80.00	74.40	70.40	68.30	66.10	74.89
	Mycoflora														
(b)	*Aspergillus niger*														
1	*Tricoderma* sp.	1.33	4.00	5.00	7.30	8.40	9.40	5.91	2.30	4.00	6.40	8.40	12.40	14.30	7.97
2	*A. flavus*	30	26.30	28.40	30.30	31.50	33.40	29.99	8.30	10.00	14.50	16.40	20.50	24.30	15.67
3	*Fusarium* sp.	25.33	26.00	29.00	30.40	32.00	35.40	29.67	20.30	20.00	20.40	24.50	28.30	30.20	23.95
4	*Rhizopus* sp.	5.33	7.30	11.40	13.00	15.50	17.50	11.67	2.30	6.00	8.50	12.30	16.00	18.30	10.57
5	*Alternaria* sp.	-	-	-	-	-	-	-	6.30	8.00	12.40	14.30	14.30	16.30	11.93
6	*Mucor* sp.	2.66	9.10	10.00	12.40	13.00	14.70	10.31	4.40	6.00	8.50	12.30	14.30	14.20	9.95
7	*A. fumigatus*	-	-	-	-	-	-	-	10.80	12.00	14.60	18.30	28.00	22.10	17.64
8	*Aspergillus* sp.	-	4.20	10.20	11.50	13.00	15.60	10.9	4.00	4.60	8.70	10.30	10.00	14.10	8.62
9	*Penicillium* sp.	-	4.20	8.00	10.40	15.50	17.60	11.14	-	-	-	-	-	-	-
10	Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	(Unidentified fungi)	3.33	2.00	6.20	7.40	9.50	10.70	6.53	-	4.00	8.00	12.30	14.20	18.30	11.36
11															
	Total	**67.96**	**83.1**	**108.2**	**122.7**	**138.4**	**154.3**		**58.7**	**74.6**	**102.0**	**129.1**	**158.1**	**172.1**	

Table.5 Detection of mycoflora associated with seeds of pigeonpea (Asha) by incubation methods

S.N.	Mycoflora Associated	Standard Blotter Method (frequency of mycoflora %)							Agar Plate Method (frequency of mycoflora %)						
		Month						Average	Month						Average
		L	I.	L	II.	L	III.		L	I.	L	II.	L	III.	
(a)	**Germination (%)**	86.66	76.44	73.40	71.50	69.50	65.50	73.84	76.50	74.40	70.60	68.60	64.40	60.40	69.15
	Mycoflora														
(b)	*Aspergillus niger*														
1	*Tricoderma* sp.	1.33	3.33	7.50	8.60	10.50	18.00	8.21	4.50	6.50	8.60	12.50	16.50	18.50	11.18
2	*A. flavus*	13.33	14.45	15.60	16.70	17.40	20.50	16.33	-	4.50	18.60	10.50	12.00	14.60	12.05
3	*Fusarium* sp.	23.33	22.50	24.60	26.50	28.50	29.50	25.83	12.60	12.50	14.00	18.60	22.40	26.00	17.69
4	*Rhizopus* sp.	9.33	11.60	13.50	15.50	17.50	19.00	14.41	16.00	18.50	20.40	24.40	28.00	32.50	23.3
5	*Alternaria* sp.	-	2.55	3.55	6.50	9.40	11.50	6.7	18.50	20.70	20.50	24.30	27.40	32.00	23.9
6	*Mucor* sp.	10.00	11.60	13.50	15.50	17.30	19.40	14.55	4.50	4.50	6.40	8.40	12.00	14.50	8.39
7	*A. fumigatus*	-	-	-	-	-	-	-	12.50	14.50	16.00	20.30	22.40	24.60	18.39
8	*Aspergillus* sp.	-	2.50	3.50	6.60	8.40	10.50	6.3	4.50	6.50	10.30	12.50	14.50	16.50	10.8
9	*Penicillium* sp.	-	2.50	4.40	6.50	7.50	9.00	5.99	-	-	-	-	-	-	-
10	Others	-	-	-	-	-	-	-	-	-	8.50	12.50	16.00	18.60	13.9
11	(Unidentified fungi)	-	-	-	-	-	7.50	7.5	4.40	6.60	8.60	12.30	14.50	16.60	10.5
	total	**57.32**	**71.03**	**86.15**	**102.4**	**116.5**	**144.9**		**77.5**	**94.8**	**131.9**	**156.3**	**186.7**	**214.4**	

Pigeonpea

Seeds of pigeonpea variety Asha were evaluated for associated mycoflora by two incubation methods and data presented in the table 5. It is clear from the table that as in other pulses under study, decreasing trend in germination percentage and increasing trend in total mycoflora with the range from 57.32 to 144.9 and 77.5 to 214.4 in standard blotter and agar plate method, respectively.

As far as frequencies of various mycoflora were concern, trend was increasing with the increase in storage month in standard blotter method. *A. flavus* frequency was maximum (25.83%) followed by *Tricoderma* sp. (16.33%) and *Alternaria* sp. (14.55%) among the mycoflora recorded. Least frequency was observed in *Aspergillus* sp., (5.99%) *Mucor* sp. and *Penicillium* sp. were not recorded and unidentified mycoflora recorded in only sixth month of storage.

In agar plate method, all the mycoflora found associated with the seeds shows increasing trends as the month of storage increased from one to six month. Maximum frequency was recorded in *Rhizopus* sp. (23.9%) which was closely followed by *Fusarium* sp. (23.3%) and *Mucor* sp. (18.39%). *Mucor* sp. was not detected in standard blotter method. Frequency was minimum in *Alternaria* sp. (8.39%). *Penicillium* sp. was not recorded in first two month and later shows increasing trend upto sixth month. *A.* sp. was not detected in any of the months of study.

The mycoflora found associated with pigeonpea seeds in the present study were also reported by several workers in varying frequencies (Chakravarthy *et al.,* 2002; Reddy *et al.,* 2006; Pandey *et al.,* 2007; Jalander and Gachande, 2011; Singh *et al.,* 2011; Patil *et al.,* 2012; Rathod *et al.,* 2012; Narayan and Ayodhya, 2013; Shivani and Sreelaksmi, 2013 and Pradhan, 2014) confer the findings of this study.

Seeds of black gram, green gram, pea, chickpea and pigeonpea were evaluate for associated seed borne mycoflora periodically at one month interval upto six months of storage by incubation methods i.e. standard blotter and agar plate method.

In general, seed germination shows decreasing trend and mycoflora shows increasing trend during six months of storage. The seed borne mycoflora found associated with various pulses were *A. niger*, *A. flavus*, *A. fumigatus*, *Fusarium* sp., *Tricoderma* sp., *Muco*r sp., *Rhizopu*s sp., *Alternaria* sp., *Aspergillus* sp., *Penicillium* sp. and some unidentified mycoflora in varying frequencies in both the methods of incubation in the storage period under study.

Morphological features of mycoflora found associated with the seeds of various pulses

Aspergillus flavus

The fungus produces compact globose conidial heads (vesicle) in yellow– green shades. Mycelium was white to gray. Conidiophores simple, unbranched, transparent and stand erect on the seed, terminates into vesicle, deep yellow green. Conidia hyaline, single celled, globose and produced in chains.

Aspergillus fumigatus

The fungus produces conidial heads in dark blue- green shades with powdery texture. Conidiophores were short, smooth walled and terminates into clavate vesicle. Single row of phialides was present on the upper two third portion of vesicle. Conidia green, globose, produced in chains, single celled and rough walled.

Aspergillus niger

The fungus produces abundant conidial heads which were in black shades. The mycelium was white to light yellow. Conidiophores arise directly from the seed coat, long, light brown, unbranched, erect, terminates into conidial heads or vesicle which appear globose, black, radiating in conidial chains.Conidia single celled, dark brown, globose and echinulate.

Fusarium sp.

The fungal growth on seeds consists of white to pink light mycelium and branched monophialides. On branched monophialides, dry, white, powdery (false) heads produced. Microconidia single-bicelled, hyaline, ovoid to fusoid, mostly curved. Macroconidia hyaline, falcate, apical cell narrowed towards the tip, hooked apices, frequently 3 septate.

*Mucor s*p.

The fungal growth on seeds was grey- brown and fluffy. Sporangiophores erect, short. Sporangia globose, black in colour. Sporangiospores are erect and slightly elongated.

Penicillium sp.

The fungal growth on seeds was dark blue shade. Conidiophores simple, erect, branched about 2/3 of the way to the tip, bears phialides on which conidia produced. Conidia ovoid, unicellular, hyaline to light green, smooth walled.

Alternaria sp.

The fungus produces woolly or powdery chain of dark brown conidia of variable lengths and shapes. The mycelium may be either sparse or abundant and very variable in color, usually light olive green to brown. conidiophores are simple, erect and often clustered. Conidia have transverse, oblique septa, ovoid to obovoid, obclavate, obpyriform, ellipsoidal, muriform, with an elongated terminal cell.

Rhizopus sp.

Rhizopus sp. grow as filamentous, branching hyphae that generally lack cross wall (i.e., they are coenocytic). They reproduce by forming asexual and sexual spores.

In asexual reproduction, sporangiospores are produce inside a spherical structure, the sporangium. Sporangia are supported by a large apophysate columella atop a long stalk, the sporangiophore. Sporangiophores arise among distinctive, root like rhizoids.

In sexual reproduction, a dark zygospore is produced at the point where two compatible mycelia fuse.

Tricoderma sp.

Conidiophores hyaline, upright, much branched, not verticillate; phialides single or in groups; conidia hyaline, single-celled, ovoid, borne in small terminal clusters; usually easily recognized by the rapid growth and green patches or cushions of conidia; saprophytic on seed or in soil, very common, some species reported as parasite on other fungi.

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