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SEED HEALTH OF STORED LENTIL COLLECTED FROM MAJOR LENTIL GROWING AREAS OF BANGLADESH

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Abstract

An experiment was conducted in the Plant Pathology Laboratory, Regional Agricultural Research Station, BARI, Jashore using 126 lentil seed samples collected from farmer's store of different lentil growing areas of Bangladesh to determine physical status and fungal association with lentil seeds. The collected seeds contained different varieties and locations. The seeds were grouped into four grades according to physical status. Among the varieties the maximum apparently healthy seed i. e. grade-1 was found in BARI Masur-7 (90.92 %) and the minimum was in local (59.34 %) varieties. In respect of location the maximum grade-1 seed was recorded from Madaripur (90.75 %) and minimum in Kushtia (47.67 %) district. Germination percentage was the highest in BARI Masur-7 (94.00 %) and the lowest in BARI Masur-3 (75.42 %) variety. Among districts, the highest germination was found in seeds of Meherpur (89.83 %) and the lowest in Narail (62.97 %). A linear positive relation was found between percent germination and apparently healthy seed in case of both varieties and locations. Six fungal genera were associated with lentil seed samples *viz*. *Fusarium* sp., *Alternaria* sp., *Stemphylium* sp., *Curvularia* sp., *Penicillium* sp. and *Aspergillus* spp. *Aspergillus niger* caused highest infection (17.58 %) followed by *Fusarium oxysporum, Alternaria tennuis, Stemphylium botryosum, Curvularia lunata, Penecillium* sp., *Aspergillus flavus, Aspergillus paraciticus* and *Aspergillus candidus* and the lowest (4.67 %) by *Aspergillus ocraceous*. Average association of fungi was the highest in local variety (3.27 %) and it was the lowest in BARI Masur-7 (0.50 %).

Keywords: Lentil seed, fungal association, germination, Bangladesh.

Introduction

Lentil is the oldest and most popular pulse crop in the world as it is grown since 8000 years ago. It has high protein content and special capacity for fixing atmospheric nitrogen. In Bangladesh pulses constitute an integral part of the daily diet as a direct source of protein for human beings (Sattar *et al.*, 1996). It is cultivated allover Bangladesh but intensively in Mid-Western region namely Jashore, Jhenidah, Magura, Faridpur, Rajbari, Kushtia, Chuadanga, Meherpur and Pabna district (Afzal *et al.*, 1999). The yield of lentil in Bangladesh is low compared to other countries like Syria, Turkey, Canada, USA and Ethiopia. In 2016-2017 the crop covered an area of 2,49,000 hector with a total production of

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2,69,000 metric tons in Bangladesh (Azad *et al,.* 2017). There are various factors responsible for low yield of lentil and low quality seed. Pulse seeds are rapidly deteriorated by high temperature and relative humidity during storage. In most of the cases farmers' stored seeds are badly infested with stored grain pests and moulds those resulted very poor germination. Although seeds can be affected by or can harbour seed-borne pathogens and can be contaminated with foreign materials such as crop debris, soil particles, weed seeds, seeds with other crops and of other varieties (Khokon *et al.,* 2005). Out of 16% annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne diseases (Fakir, 2004). Lentil suffer from the attack of a number of seed-borne diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew and downy mildew which are caused by *Fusarium oxysporum f.sp. lentis, Sclerotium rolfsii, Rhizoctonia solani, Botrytis cinerea, Uromycis fabae, Erysiphe polygoni* and *Peronospora lentis,* respectively (Singh and Dwivedi, 1980 and Khare, 1981).

The supply of quality seeds of pulses is very limited in Bangladesh where Bangladesh Agricultural Development Corporation supplies only 1.15% seeds of total requirement. Remaining 98.85% seeds of pulses are produced by the farmers. In 2011-12 BADC supplied only 1092 metric ton pulse seeds all over the country. As such, the quality of farmers' stored seed is very poor (Fakir *et al.,* 2007). Pulses have inherently low yield potential and susceptibility to diseases and insect pests and sensitivity to microclimate changes (Fakir and Rahman, 1989). More than 60 pathogenic fungi including 20 major ones can infect seeds and transmit different diseases in the field causing considerable yield loss (Bakr and Rashid, 2007).

The aim of the research work was to determine health status and fungal incidence in farmers' stored lentil seed from major lentil growing regions of Bangladesh.

Materials and Methods

The experiment was carried out in the Plant Pathology laboratory, Regional Agricultural Research Station, BARI, Jashore.

Collection of seed samples

Seed samples were collected from the farmers of Mid-Western region covering intensively lentil growing districts of Bangladesh. Collection of seed samples was done from 1 March to 30 April 2012 directly from the store of the farmer. Lentil seeds were collected from 63 upazillas under 12 districts of Bangladesh such as Jashore, Jhenidah, Magura, Narail, Rajbari, Faridpur, Gopalgonj, Madaripur, Shariatpur, Chuadanga, Meherpur and Kushtia. Two samples from each upazila i.e., 126 working seed sample were collected from the farmers who had about one acre of lentil field and used own stored seed for sowing. The samples were collected directly from the storage of the farmers according to ISTA (1999) rules. Each sample was 250-300 g and the samples were enclosed in polythene bags with proper labeling and kept in the refrigerator at 5 ± 1^{0}C.

Dry inspection test

Dry inspection test was carried out according to ISTA (2001). A working seed sample of 400 seeds was drawn from each of the 126 samples. The seed samples were inspected visually under magnified hand lens and under steriobinocular microscope. The seeds were checked and grouped into four different grades following a standard grading scale such as Grade-1= Apparently healthy seed, Grade-2= Shriveled seed, Grade-3= Discolored seed and Grade-4= Broken seed. The graded seeds were separated using hand lens and stereo-binocular microscope and the data were expressed in percentage.

Germination test and blotter incubation test

The germination test was conducted during 11 August to 22 December 1013. The germination of the seeds was determined following blotter incubation test. Four hundred seeds were taken randomly from all the seed samples. The seeds were placed on three-layered moistened blotting paper in sterilized glass petridish (9 cm) using sterilized forceps and each petridish contained 25 seeds. The petridish were kept at room temperature under natural light and darkness for seven days. The germination were counted after seven days of incubation.

The associations of different fungi in lentil seed were also detected by blotter incubation method (ISTA, 2001). At that time, petridishes were observed under stereo-binocular microscope for observing the seed-borne mycoflora and also observed under compound microscope for confirmation. For the confirmation of identification, pure culture were prepared and studied under compound microscope. Pathogenicity test was also done of major fungi found from blotter incubation method (Ramanath *et. al.,* 1970, Neergaard, 1974). The association of fungi were counted and expressed in percentage.

Results and Discussion

Prevalence of seeds under different grades

Among 126 seed samples, the prevalence of grade-1, grade-2, grade-3 and grade-4 seeds ranged 59.34-90.92, 2.42-22.89, 1.67-20.45 and 3.01-4.83% with means 74.85, 13.27, 8.23 and 4.36%, respectively. The highest prevalence of grade-1, grade-2, grade-3 and grade-4 seeds were found in varieties BARI Masur-3, BARI Masur-5, Local and BARI Masur-3 and the lowest in Local variety, BARI Masur-7, BARI Masur-3 and BARI Masur-3, respectively (Table 1).

The prevalence of grade-1, grade-2, grade-3 and grade-4 seeds collected from different districts ranged 47.63-90.75, 6.63-20.52, 1.60-42.03 and 0.22-10.00% with mean 69.12, 4.34, 15.49 and 3.82%, respectively. The maximum prevalence of grade-1 seeds was recorded from samples collected from the district of Madaripur and the lowest samples from Kushtia. The highest prevalence of grade-2 seeds were found in samples from Jhenidah and the lowest in samples from Madaripur. The lowest prevalence of grade-3 seeds were found in samples

from Gopalganj and the highest in Kushtia. Under grade-4, the lowest and highest prevalence of seeds were observed in samples from Madaripur and Shariatpur (Table 2).

Table 1. Prevalence (%) of lentil seeds of different varieties and grades into four grades

Varieties	Grade-1 (%)	Grade-2 (%)	Grade-3 (%)	Grade-4 (%)	Germination (%)
BARI Masur-3	78.83	14.67	1.67	4.83	75.42
BARI Masur -5	63.06	22.89	9.50	4.65	76.98
BARI Masur -6	82.10	11.02	4.34	3.01	89.59
BARI Masur -7	90.92	2.42	5.17	4.50	94.00
Local variety	59.34	15.13	20.45	4.81	75.65
Range	59.34-90.92	2.42-22.89	1.67-20.45	3.01-4.83	75.42-94.00
Mean	74.85	13.27	8.23	4.36	82.33
SD	13.29	7.43	7.39	0.77	8.80

N.B. Grade-1: Apparently healthy seed, Grade-2: Shriveled seed, Grade-3: Discolored seed, Grade-4: broken seed,

Germination= Averages of four grades

Table 2. Germination of lentil seeds collected from different districts under four grades

District	Grade-1 (%)	Grade-2 (%)	Grade-3 (%)	Grade-4 (%)	Germination (%)
Jashore	71.55	13.27	11.20	3.98	82.03
Jhenidah	70.58	20.52	2.94	4.29	80.83
Magura	71.84	15.69	8.25	4.22	84.16
Narail	53.88	8.10	35.63	2.41	62.97
Rajbari	49.66	19.47	37.92	2.44	71.28
Faridpur	59.61	17.38	20.14	2.81	79.77
Gopalganj	77.08	18.15	1.60	3.20	86.93
Madaripur	90.75	6.63	2.38	0.22	85.00
Shariatpur	73.28	11.25	5.84	10.00	84.18
Chuadanga	82.19	13.19	1.88	5.09	87.66
Meherpur	81.33	14.04	3.42	1.21	89.83
Kushtia	47.67	11.58	42.03	5.67	73.94
Range	47.67-90.75	6.63-20.52	1.60-42.03	0.22-10.00	71.28-89.83
Mean	69.12	14.11	14.44	3.80	80.72
St. Dev.	13.64	4.34	15.49	2.50	7.77

N.B. Grade-1: Apparently healthy seed, Grade-2: Shriveled seed, Grade-3: Discolored seed, Grade-4: broken seed.

Germination= Averages of four grades.

Germination

The maximum average germination of seeds was found in BARI Masur-7 (94.00%) followed by BARI Masur-6 (89.59%). The lowest germination was recorded in samples of variety BARI Masur-3 followed by BARI Masur-3, Local and BARI Masur-5. These three varieties showed 75.42, 75.65 and 76.95% germination, respectively (Table 1).

The germination of seeds collected from the districts of Jashore, Jhenidah, Magura, Narail, Faridpur, Magura, Gopalganj, Madaripur, Shariatpur, Chuadanga and Kushtia were respectively 82.03, 80.83, 84.16, 62.97, 79.77, 86.93, 85.00, 84.18, 87.66, 89.83 and 73.94%. The maximum germination of 89.83% was recorded in seed samples collected from Meherpur and the lowest of 71.28% in the seeds from Rajbari (Table 2).

The percentage of germination was positively and linearly correlated with percentage of apparently healthy seeds collected from different districts. Their relationship could be explained by the regression equation Y=42.12 + 0.537X, where Y= estimated germination and X= regression coefficient. The correlation coefficient was r=0.811 (Fig. 1).

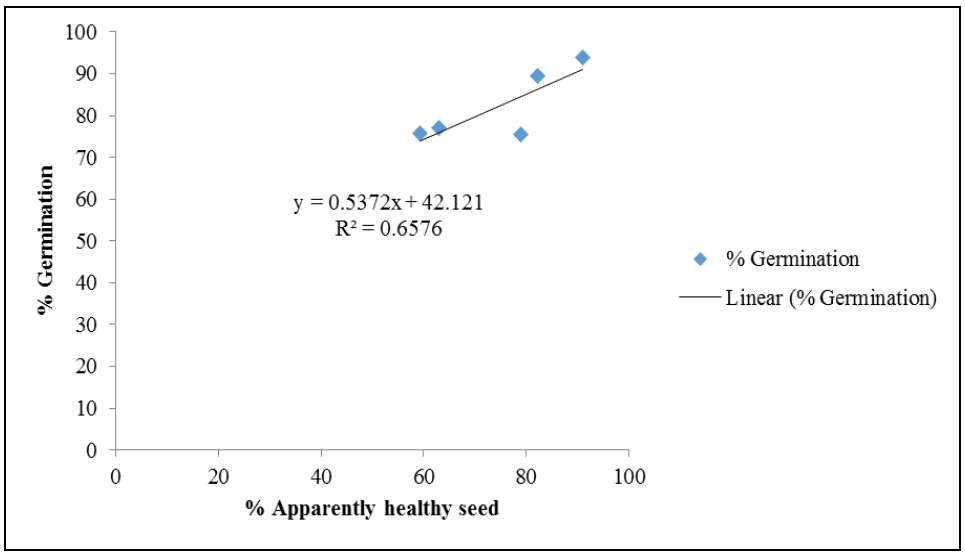

Fig. 1. Relationship between percent of germination and apparently healthy seed.

Association of fungi with seeds samples of five varieties

Six fungal genera were found to be associated seeds of five masur varieties. Those were alphabetically *Alternaria*, *Aspergillus*, *Curvularia*, *Fusarium*, *Penicillium* and *Stemphylium* (Table 3). Five species of *Aspergillus* (*A. candidus*, *A. flavus*, *A. niger*, *A. ocraceous*, *A. paraciticus*), one species of *Alternaria* (*A. tennuis*), one species of *Curvularia* (*C. lunata*), one species of *Fusarium* (*F.*

oxysporum) and one species of *Stemphylium* (*S. botryosum*) were identified. Prevalence of different species of fungi in BARI Masur-3, BARI Masur-5, BARI Masur-6, BARI Masur-7 and Local variety ranged respectively 0.50-7.17, 1.44-5.71, 0.74-3.09, 0.00-1.08 and 1.65-6.10% with mean 3.03, 2.98, 1.54, 0.05, 3.27 and 2.17%. The average prevalence of *A. tennuis, A. candidus, A. flavus, A. niger, A. ocraceous, A. paraciticus, C. lunata, F. oxysporum, Penicillium* sp. and *S. botryosum* under five lentil varieties were 0.58-3.24, 0.00-5.17, 0.17-4.25, 0.58-7.17, 0.00-1.81, 0.50-2.83, 0.67-2.36, 1.00-4.00, 0.75-5.05 and 0.58-3.13 % with mean 1.78, 1.99, 2.38, 4.49, 0.94, 1.49, 1.55, 2.40, 3.49 and 2.10%, respectively. The maximum prevalence of different varieties was found in *A. niger* and the lowest in *A. ocraceous.* The mean was 2.17% (Table 3).

Table 3. Prevalence (%) of fungi associated with lentil seeds of five varieties collected from different districts

Fungi (Alphabetically listed)	Prevalence (%) of fungi on five varieties					
	BARI Masur-3	BARI Masur-5	BARI Masur-6	BARI Masur-7	Local variety	Mean
Alternaria tennuis	1.17	2.46	1.44	0.58	3.24	1.78
Aspergillus candidus	5.17	1.44	1.06	0.00	2.31	1.99
Aspergillus flavus	4.25	3.58	0.84	0.17	3.08	2.38
Aspergillus niger	7.17	5.71	2.89	0.58	6.10	4.49
Aspergillus ocraceous	0.50	1.81	0.74	0.00	1.65	0.94
Aspergillus paraciticus	2.83	1.78	0.84	0.25	1.75	1.49
Curvularia lunata	0.67	2.35	1.30	1.08	2.36	1.55
Fusarium oxysporum	2.00	3.00	2.00	1.00	4.00	2.40
Penecillium sp.	3.83	4.73	3.09	0.75	5.05	3.49
Stemphylium botryosum	2.67	2.94	1.18	0.58	3.13	2.10
Mean	3.03	2.98	1.54	.050	3.27	2.17
SD	2.12	1.36	0.85	0.39	1.43	1.23

Association of fungi with seeds samples of twelve districts

Among 12 districts, the highest seed infection was caused by *F. oxysporum* recorded from the seeds of Narail (6.81%) followed by Kushtia (6.17%) and Rajbari (4.50%) district while the lowest seed infection was found in the seeds of Meherpur (1.71%) district (Table 4). The highest seed infection caused by *Alternaria tennuis* was recorded from local varieties (3.24%) followed by BARI Masur-5 (2.46%), BARI Masur-6 (1.44%) and BARI Masur-3 (1.17%). Only 0.58% seed infection was recorded in BARI Masur-7 (Table 3). Similarly, the highest infection of *Alternaria tennuis* was recorded from the seeds of Kushtia (5.05%) followed by Narail (3.94%) and Rajbari (3.10%) district and the lowest

seed infection was found in the seeds of Chuadanga (1.44%) district (Table 4). In case of lentil varieties the highest seed infection caused by *Stemphylium botryosum* was recorded from local varieties (3.13%) followed by BARI Masur-5 (2.94%), BARI Masur-3 (2.67%), BARI Masur-6 (1.18%) and BARI Masur-7 (0.58%) variety (Table 3). Highest seed infection due to *S. botryosum* was recorded from the seeds of Kushtia (7.30%) followed by Gopalganj (4.30%) and Narail (3.10%) district and the lowest infection was found in Meherpur (1.00%) district (Table 4). Highest seed infection caused by *C. lunata* was recorded from local variety (2.36%) followed by BARI Masur-5 (2.35%), BARI Masur-6 (1.30%), BARI Masur-7 (1.08%) and BARI Masur-3 (0.67%) variety (Table 3). Infection of *Curvularia lunata* was higher in Madaripur (8.00%) followed by Meherpur (7.00%) and Kushtia (4.80%) district and the lowest seed infection of *C. lunata* was found in Jashore (1.10%) district (Table 4). In case of *Penicillium* sp. the highest infection was recorded from local varieties (5.05%) followed by BARI Masur-5 (4.73%), BARI Masur-3 (3.83%), BARI Masur-6 (3.09%) and BARI Masur-7 (0.75%) variety (Table 3). Among 12 districts the highest infection of *Penicillium* sp. was recorded from the seeds of Kushtia (22.75%) followed by Faridpur (17.00%), Shariatpur (15.75%) and lowest in both Narail (6.50%) and Gopalganj (6.50%) district (Table 4). Higher infection of *Aspergillus niger* was recorded from BARI Masur-3 (7.17%) followed by local varieties (6.10%), BARI Masur-5 (5.71%), BARI Masur-6 (2.89%) and BARI Masur-7 (0.58%) variety (Table 3). *Aspergillus niger* was higher in Kushtia (19.50%) followed by Faridpur (18.75%) and Shariatpur (17.50%) and lower in Meherpur (5.00%) district (Table 4). *Aspergillus flavus* was maximum in BARI Masur-3 (4.25%) followed by BARI Masur-5 (3.58%), local varieties (3.08%), BARI Masur-6 (0.84%) and BARI Masur-7 (0.17%) (Table 3). Similarly, *A. flavus* was more in Faridpur (12.50%) followed by Jhenidah (9.50%) and Kushtia (9.50%) district. It was less in Madaripur (1.00%) and Meherpur (1.00%) district (Table 4). On the other hand, the highest *Aspergillus ochraceus* was recorded from BARI Masur-5 (1.81%) followed by local varieties (1.65%), BARI Masur-6 (0.74%) and BARI Masur-3 (0.50%) and no infection of this fungus was found in BARI Masur-7 (Table 3). The species *A. ochraceus* was highest in the seeds of Faridpur (7.25%) followed by Kushtia (6.50%) and Jhenidah (5.00%) and lowest in Gopalganj (1.25%) district (Table 4). The seed infection caused *A. paraciticus* was the highest in BARI Masur-3 (2.83%) followed by BARI Masur-5 (1.78%), local variety (1.65%), BARI Masur-6 (0.84%) and BARI Masur-7 (0.25%) (Table 3). Among 12 districts, highest seed of *A. paraciticus* was recorded from Chuadanga (7.50%) followed by Kushtia (7.00%) and Faridpur (5.00%), and the lowest was found in Shariatpur (0.50%) district (Table 4). *A. candidus* was highest in BARI Masur-3 (5.17%) followed by local variety (2.31%), BARI Masur-5 (1.44%) and BARI Masur-6 (1.06%), while no infection was found in BARI Masur-7 (Table 3). Among 12 districts, the highest seed infection by *A. candidus* was recorded from Kushtia (10.25%) followed by Jhenidah (7.00%), Faridpur (6.00%) and the lowest was in Chuadanga (1.50%) (Table 4).

Table 4. Prevalence (%) of fungi associated with lentil seeds collected from different districts

District	Prevalence (%) of fungi									
	Alternaria sp.	*A. candidus*	*A. flavus*	*A. niger*	*A. ocraceous*	*A. paraciticus*	*Curvularia* sp.	*Fusarium* sp.	*Penecillium* spp.	*Stemphylium botryosum*
Jessore	1.80	5.25	7.25	16.00	3.50	4.50	1.10	2.78	10.25	2.30
Jhenidah	1.69	7.00	9.50	16.75	5.00	5.00	1.20	2.63	12.50	2.50
Magura	2.10	2.50	3.50	9.50	3.25	1.75	1.80	2.91	10.00	1.40
Narail	3.94	5.25	5.75	11.50	2.25	3.75	1.50	6.81	6.50	3.10
Rajbari	3.10	6.50	7.75	15.50	3.50	4.00	1.90	4.50	10.50	2.40
Faridpur	2.30	6.50	12.40	18.75	7.25	6.25	3.80	2.98	17.00	2.40
Gopalganj	1.95	4.75	3.75	7.00	1.24	4.25	1.90	2.28	6.50	4.70
Madaripur	1.72	3.00	1.00	5.00	1.49	2.50	8.00	2.33	7.00	1.80
Shariatpur	2.63	2.50	9.25	17.50	4.00	0.50	3.10	2.55	15.75	1.30
Chuadanga	1.44	1.50	4.25	5.25	1.25	7.50	2.10	2.53	8.50	2.50
Meherpur	1.83	3.25	1.00	6.50	2.25	1.75	7.00	1.71	7.00	1.00
Kushtia	5.05	10.25	9.50	19.50	6.50	7.00	4.80	6.27	22.75	7.30
Total	29.55	58.25	74.9	148.75	41.48	48.75	38.2	40.28	134.25	32.7
Mean	2.46	4.85	6.24	12.40	3.46	4.06	3.18	3.36	11.19	2.73
St. dev.	1.08	2.48	3.62	5.54	1.98	2.18	2.30	1.63	5.04	1.73

The results of the present investigation reveal that variable associations of different fungi exist in various varieties of lentil seeds which is preserved by the farmers for next year sowing. BARI Masur-7 shows lower fungal association than other varieties and more associations were found in local and old varieties. It indicated that the seeds of latest varieties were more tolerant against fungal infection. In blotter incubation test six fungal genera were detected in lentil seeds such as *Fusarium* sp., *Alternaria* sp., *Stemphylium* sp., *Curvularia* sp., *Penicillium* sp. and *Aspergillus* sp. Five species of *Aspergillus* viz. *A. niger, A. flavus, A. ocraceous, A. paraciticus* and *A. candids* were detected in seeds of lentil. Six fungal pathogens from 16 lentil seed samples collected from farmer's store of Meherpur by other authors and found lower germination percentage in case of lower percentage of apparently healthy seeds (Hasan *et al.,* 2010). Sarkar *et al.* (2010) recorded eight fungal species under six genera in lentil seeds. Similarly, Dev *et al.* (2002), El-Nagerabi and Elshafie (2001) and Javed *et al.* (2000) also identified 6, 7 and 9 fungal genera from lentil seed. Based on findings of the present study, it may be concluded that prevalence of higher apparently healthy seed may give higher germination percentage and lower fungal infection.

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