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PREVALENCE OF FUNGI IN DIFFERENT VARIETIES OF CHICKPEA (*CICER ARIETINUM* L.) SEEDS IN STORAGE

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ABSTRACT

A total of nine species of fungi were found to be associated with seeds of nine varieties of chickpea. The isolated fungi were *Alternaria alternata, Aspergillus flavus, A. niger, A. fumigatus, A. nidulans, Curvularia lunata, Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride*. Association of fungi with chickpea seeds was recorded three times, within a week after harvest, after two and five months of storage. The fungal association varied with duration of storage period. Species of *Aspergillus, Penicillium* and *Rhizopus* become predominating fungi with increase of the storage period. The fungal association with seeds of chickpea also affects germination, seedling mortality and seedling height. Germination of BARI chola 6 and 7 were completely inhibited due to prevalence of *Rhizopus stolonifer* in the seeds examined within seven days of harvest. The total fungal infection in chickpea seeds was highest in BARI chola 8 (168) and lowest in BARI chola 1 (65).

Key words*:* Chickpea seeds, Fungi, Prevalence, Seedling height, Seedling mortality

INTRODUCTION

Chickpea, *Cicer arietinum* L. belongs to Fabaceae and it is one of the important pulse crops grown in Bangladesh. It is an important protein rich crop and occupies third position both in production and in acreage in pulse production of Bangladesh (Anon. 1995). Chickpeas serve as an energy and protein source not only in human nutrition but also as animal feed which lead to an increase in egg and milk production.

In the last several years, chickpeas cultivation area and production has sharply declined because of some major constraints (Bakr *et al.* 2002). Chickpea is invaded by more than 50 diseases reported from different parts of the world (Nene 1980, Fakir 1983). Most of the diseases (more than 30) are caused by fungi. In Bangladesh so far 17 chickpea diseases have been recorded, 12 of which are caused by fungi (Bakr *et al.* 2007). Out of 12 fungal diseases of chickpea, 4 diseases, namely Botrytis grey mould (BGM), wilt, root rot, blight and collar rot are the major ones (Bakr 1994).

Chickpea seeds in storage, carry a mycoflora of 'field' and 'storage' fungi. Field fungi gradually disappear and storage fungi then predominate. Most of the storage pathogen

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species are *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. The storage fungi may cause discoloration of the seeds and germination failure (BARI 1986). These fungi grow vigorously and initiate grain spoilage. These also bring about several undesirable changes making them unfit for consumption and sowing.

Lot of research has been done at home and abroad on chickpea diseases and its control but information on storage mycoflora of chickpea seeds is inadequate (Dwivedi 1989, Lal and Singh 1997, Salam 2004). Considering the importance of this popular pulse crop the present research work was undertaken to search the prevalence of fungi in different chickpea seeds in storage conditions.

MATERIALS AND METHODS

The present study was carried on storage seeds of chickpea. Seed samples of Barichola 1-9 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. Samples were collected after harvesting and placed in clean brown paper bags, labeled properly and preserved at room temperature for subsequent use.

The fungi were isolated from the samples following the Tissue Planting method on PDA medium (CAB 1968) and Blotter method of ISTA (1996). Two hundred seeds of each sample were placed on three layers of moist blotting paper (Whatman No. 1) in Petri plates. The seeds were surface sterilized by dipping in 10% Chlorox solution for 5 minutes and then washed three times with sterilized water. Seeds were placed in each plate and incubated at $25 \pm 2°C$ for 5 - 7 days.

Fungi isolated from seed inocula were transferred to separate PDA plates and PDA slants for further studies and preservation. Identification of the isolates were determined based on morphological characteristics observed under a compound microscope following the standard literatures (Barnett and Hunter 2000, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976). Per cent frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Wetly 1972).

$$\% \text{ frequency} = \frac{\text{No. of inocula from which fungal isolates were raised}}{\text{No. of inocula cultured}} \times 100$$

For the determination of germination, 300 seeds of each sample were taken and placed in 30 PDA plates. Each plate contained 10 seeds. Plates were then incubated at room temperature for 7 days. Seeds producing both plumule and radicle were considered as germinated seeds. Germination was recorded after 7 days. Data were expressed as percentage.

Seedling mortality was determined after 10 days of incubation following the formula (Anon. 2014).

$$\text{Mortality percentage of seeds} = \frac{\text{Number of dead seed}}{\text{Number of total germinated seed}} \times 100$$

Seedling height of different varieties of chickpea seeds at 7^{th} day was also recorded.

RESULTS AND DISCUSSION

Nine species of fungi belonging to six genera were found to be associated with seeds of nine varieties (BARI chola 1 - BARI chola 9) of chickpea. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. niger* Van Tiegh, *A. fumigatus* Fresenius, *A. nidulans* Eidam, *Curvularia lunata* (Wakker) Boedijn, a species of *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers, (Figs 1, 2).

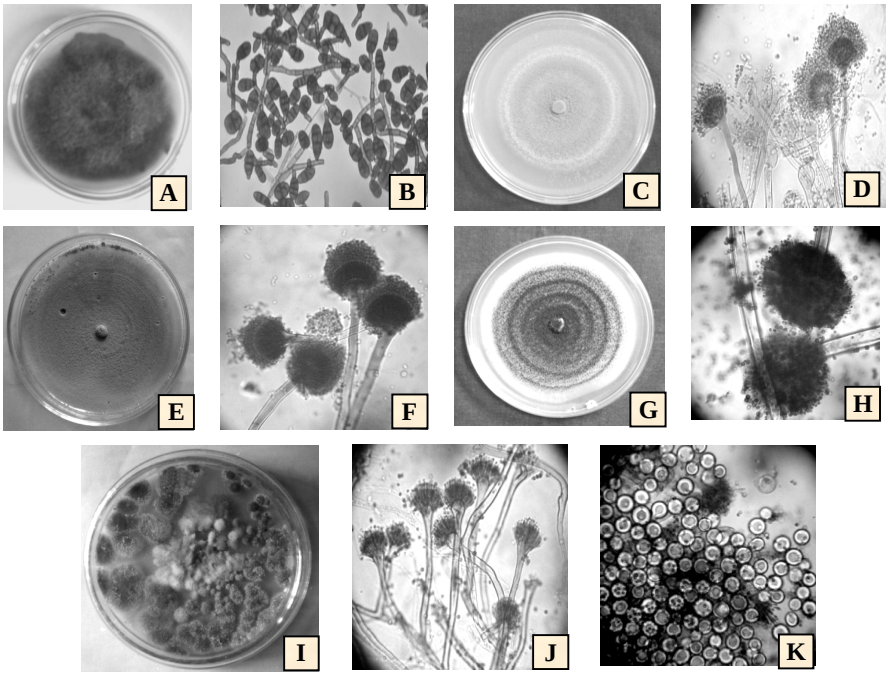

Fig. 1. A: Culture plate and B: Conidiophores and conidia of *Alterneria alternata;* C: Culture plate and D: Conidiophores and conidia of *Aspergillus flavus*; E: Culture plate and F: Conidiophores and conidia of *A. fumigatus*; G: Culture plate and H: Conidiophores and conidia of *A. niger*; I: Culture plate, J: Conidiophores, conidia and K: Hulle cell of *A. nidulans* (Bar = 50 μm).

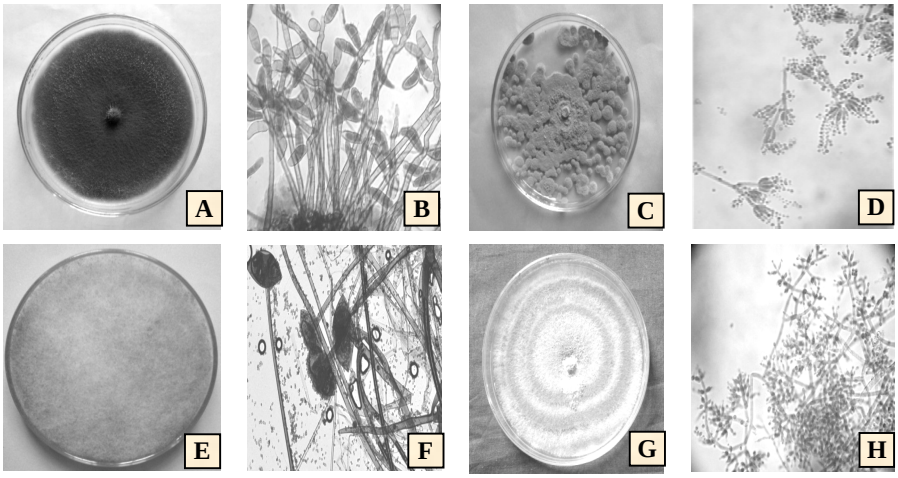

Fig. 2. A: Culture plate, B: Conidiophores and conidia of *Curvularia lunata*; C: Culture plate, D: Conidiophores and conidia of *Penicillium* sp.; E: Culture plate, F: Conidiophores and conidia of *R. stolonifer*; G: Culture plate, H: Conidiophores and conidia of *Trichoderma viride* (Bar = 50 µm).

Association of fungi with chickpea seeds was recorded three times, within a week after harvest of seeds, after 2 and 5 months of storage. The association of fungi varied with duration of the storage period.

Eight species of fungi belonging to five genera of Deuteromycetes were found to be associated with nine varieties of chickpea seeds. Prevalence of *R. stolonifer* was highest (100%) in BARI chola 6 and BARI chola 7, whereas prevalence of *A. flavus*, *A. nidulans* and *Curvularia lunata* were lowest (2%) (Table 1).

Table 1. Frequency percentage of association of fungi with different varieties of chickpea seeds.

Name of fungi	Frequency percentage of fungi with chickpea seeds								
	BRC 1	BRC 2	BRC 3	BRC 4	BRC 5	BRC 6	BRC 7	BRC 8	BRC 9
Alternaria alternata	-	4	3	4	-	-	-	-	-
Aspergillus flavus		2	-	4	-	-	-	-	-
A. fumigatus	20	15	28	17	7	-	-		10
A. niger	17	7	43	13	30	-	-	11	4
A. nidulans	-	-	2	-	-	-	-	-	-
Curvularia lunata	-	2	-	-	-	-	-	-	-
Penicillium sp.	14	8	10	14	3	-	-	-	-
Rhizopus stolonifer	-	16	-	20	58	100	100	88	57

'-' = No isolate, BRC = BARI chola.

Table 2 shows the association of fungi with chickpea seeds after two months of storage. The frequency percentage of *A. flavus* was highest (56%) in BARI chola 7 and lowest (2%) with *C. lunata*, *Penicillium* sp. and *R. stolonifer* (Table 2).

Table 3 shows the prevalence of fungi with seeds of chickpea after five months of storage. The frequency percentage of *Aspergillus* spp., *R. stolonifer* and *Penicillium* sp. gradually increased with increase of the storage period. Prevalence of *A. flavus* was highest (57%) in BARI chola 7. Lowest 2% *Penicillium* sp. and *R. stolonifer* was recorded in BARI chola 3 and 5 (Table 3).

Table 2. Frequency percentage of association of fungi with different varieties of chickpea seeds after two months of storage.

Name of fungi	Frequency percentage of fungi with chickpea seeds								
	BRC 1	BRC 2	BRC 3	BRC 4	BRC 5	BRC 6	BRC 7	BRC 8	BRC 9
Alternaria alternata	-	18	3	8	-	-	-	-	-
Aspergillus flavus	4	-	-	-	-	-	56	54	15
A. fumigatus	42	52	22	18	21	19	40	46	24
A. niger	8	4	28	14	3	19	30	32	26
Curvularia lunata	-	2	-	-	-	3	-	-	-
Penicillium sp.	4	4	2	2	4	5	-	-	-
Rhizopus stolonifer	14	-	14	14	2	-	-	38	13
Trichoderma viride	8	-	-	8	-	-	8	10	-

'-' = No isolate, BRC = BARI chola.

Present result supports the observation of Rodrigues (1984), who isolated 23 fungi from the seeds of chickpea.

Table 3. Frequency percentage of association of fungi with different varieties of chickpea seeds after five months of storage.

Name of fungi	Frequency percentage of fungi with chickpea seeds								
	BRC 1	BRC 2	BRC 3	BRC 4	BRC 5	BRC 6	BRC 7	BRC 8	BRC 9
Alternaria alternata	-	19	3	7	-	-	-	-	-
A. flavus	7	4	-	-	-	-	57	55	16
A. fumigatus	43	52	23	19	29	20	41	46	25
Aspergillus niger	9	5	28	14	7	19	30	33	26
Penicillium sp.	4	6	2	5	4	6	-	-	-
Rhizopus stolonifer	19	-	15	16	2	-	-	39	12

'-' = No isolate, BRC = BARI chola.

Table 4 shows that nine species of fungi were found to be associated with nine varieties of chickpea seeds. Prevalence of *A. fumigatus, A. niger, Penicillium* sp. and *R. stolonifer* were highest in all varieties examined and frequency percentage of association of these fungi were also higher, whereas *A. nidulans* was exclusively isolated from BARI chola 3. The total infection of fungi in chickpea seeds was highest in BARI chola 8 and lowest in BARI chola 1. The frequency percentage of *C. lunata* and *A. nidulans* were lowest.

Table 4. Per cent incidence of fungal infection with nine varieties of chickpea seeds.

Name of Varieties	Per cent incidence of fungal infection, (Average of three isolations)									
	Alternaria alternata	*Aspergillus niger*	*A. flavus*	*A. fumigatus*	*A. nidulans*	*Curvularia lunata*	*Penicillium* sp.	*Rhizopus stolonifer*	*Trichoderma* sp.	Total infections
BRC 1	-	9	3	34	-	-	7	9	3	65
BRC 2	9	4	4	56	-	2	4	11	-	90
BRC 3	2	28	-	35	1	-	6	5	-	77
BRC 4	4	11	7	14	-	-	10	32	3	81
BRC 5	-	15	3	29	-	-	7	30	-	84
BRC 6	-	9	3	13	-	1	7	54	-	87
BRC 7	-	14	21	28	-	-	6	41	3	113
BRC 8	-	26	30	26	-	-	18	64	4	168
BRC 9	-	20	14	16	-	-	26	34	-	110

'-' = No isolate, BRC = BARI chola.

The fungal association with seeds of chickpea also effects on germination, seedling mortality as well as seedling height. Germination of BARI chola 6 and 7 was completely inhibited due to prevalence of *R. stolonifer* after 7 days of harvest. The storage mycoflora also affect the seedling mortality and seedling height. Germination percentage of seeds was highest in BARI chola 1 and lowest in BARI chola 9. The percentage of seedling mortality was highest in BARI chola 8 and lowest in BARI chola 1. The length of shoot was highest in BARI chola 1 and lowest in BARI chola 3, whereas root length was highest in BARI chola 1 and lowest in BARI chola 6 (Table 5).

Table 5. Effect of storage mycoflora of chickpea seeds on germination, seedling mortality and seedling height.

Name of varieties	Germination (%) (7^{th} day)	Mortality (%) (after 7 days)	Seedling height (after 7 days)	
			Root (mm)	Shoot (mm)
BRC 1	55	22	18	12
BRC 2	36	61	17	10
BRC 3	37	34	13	5
BRC 4	40	39	12	11
BRC 5	25	30	10	4
BRC 6	29	27	4	10
BRC 7	28	35	13	7
BRC 8	45	79	5	10
BRC 9	19	75	9	6

BRC = BARI chola.

One author (Anon. 1986) reported the association of 5 fungi with chickpea seeds. *Aspergillus* spp. were the most prevalent fungi. *A. flavus* and other *Aspergillus* spp. were commonly associated with stored seed and responsible for reducing germination.

Dwivedi (1989) recorded *A. flavus*, *A. niger*, *Fusarium moniliforme* and *Penicillium oxalicum* from chickpea seeds. Lal and Singh (1997) reported that seed mycoflora superficially associated with the freshly collected seeds. Dominant fungal spp. noticed were *Alternaria, Aspergillus, Cladosporium, Curvularia* and *Penicillium* in stored seeds. Moreover *Aspergillus flavus, A. fumigatus, A. niger* and *A. luchuensis* were dominant in seeds stored for 1 year.

Storage fungi deteriorate the quality and quantity of seeds in storage. Fungal population of nine varieties of chickpea seeds have been extensively studied up to 5 months of storage. Associated fungi were isolated and identified. Association of *A. nidulans* with chickpea seed is new addition to the field of Mycology and Plant Pathology. Present findings will be helpful for designing the management of mycoflora of chickpea in storage.

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