

## ESTIMATION OF INTERRELATIONSHIP AMONG SEED GERMINATION, PURITY, SEEDLING MORTALITY AND ASSOCIATION OF FUNGI WITH SEEDS OF CHICKPEA

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### Abstract

A total of 9 species of fungi belonging to 6 genera were found to be associated with seeds of 9 varieties (BARI chola 1-9) of chickpea. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link., *A. fumigatus* Fresenius, *A. niger* Van Tiegh., *A. nidulans* Eidam, *Curvularia lunata* (Wakker) Boedijn, *Penicillium* Link., *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers. Seed quality analysis showed that percentage of pure seed varies from 96 - 99%. The highest percentage of purity was found in BARI chola 4 (99) and lowest purity percentage (96) was found in BARI chola 6 and 8. BARI chola 7 showed highest seed germination percentage (66) while BARI chola 8 showed lowest seed germination percentage (17). The highest mortality percentage was found in BARI chola 8 (82) while lowest percentage was found in BARI chola 1 (22). The frequency percentage of association of fungi was found highest in BARI chola 8 (94) and lowest in BARI chola 1 (63). Present study deals with estimation of some interrelationship among the mentioned parameters through correlation and regression analysis. There were negative correlation between physical purity and occurrence of fungi and between germination rate and occurrence of fungus. On the other hand, positive correlation were found between germination rate and purity percentage and between seedling mortality and occurrence of fungi.

### Introduction

Chickpea is a dicot, sub-shrub, edible, leguminous annual plant under the family Fabaceae. It is invaded by more than 50 diseases reported from different parts of the world (Nene 1980, Ahmed 1985 and Fakir 1983). In Bangladesh so far 17 chickpea diseases are recorded, 12 of which are caused by fungi (Bakr *et al.* 2007). Out of 12 fungal diseases Botrytis grey mould (BGM), wilt, root rot, blight and collar rot are the major ones (Bakr 1994).

A large number of pathogenic fungi are transmitted through seeds. Chickpea seeds in storage, carry a mycoflora of storage fungi. Most of the storage pathogen species are *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. The storage fungi may cause discoloration of the seeds and germination failure (BARI 1986 and Dawar 2007). These fungi especially grow vigorously and initiate grain spoilage. They also bring about several undesirable changes making them unfit for consumption and sowing.

In Bangladesh the quality of the freshly harvested seeds at farm level is very unsatisfactory, resulting crop failure. If seed quality is maintained by testing before sowing, then possible disease could be controlled during seedling and flowering stages. So it is expected that yield improvement could be achieved at higher rate by controlling the quality of seed in Bangladesh (Khandakar 1983).

The study of seed health condition is of vital significance in all consideration. The associated fungi with chickpea seeds is particularly important because they reduce seed germination, affect seedling and flowering stage and also reduce the grain quality.

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However, this work relates the seed health condition of chickpea and estimate interrelationship among some quality factors through correlation and regression analysis which is very much important in controlling seed quality.

### Materials and Methods

The present study was carried on storage seeds of chickpea. Seed samples of BARI chola 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 4°C in refrigerator for subsequent use.

The fungi were isolated from the samples following the Tissue Planting method on PDA medium (Anon.1968) and Blotter method as recommended by ISTA (Anon. 1976 and 2014). Two hundred seeds of each samples were placed on three layers of moist blotting paper (Whatman No. 1) in Petri plates. The seeds were washed with sterile water and then surface sterilized by dipping in 10% Chlorox solution for 5 min. Seeds were placed in each plate and incubated at  $25 \pm 2^{\circ}\text{C}$  for 5 - 7 days.

Fungi grown in the seeds 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 1972, Benoit and Mathur 1970, Ellis 1971, 1976). Per cent occurrence of the fungal isolates was calculated by adopting a formula (Spurr and Wetly 1972).

To calculate the physical purity of seeds 100 gm seeds per sample were examined. To analysis the seed quality, the ratio of pure seeds, inert matter and weed seeds of different samples were separated and recorded. Purity percentage of seeds was determined with the following formula:

$$\text{Purity percentage of seeds} = \frac{\text{Weight of pure seed}}{\text{Total weight of seed}} \times 100$$

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

Interrelationships among storage mycoflora, seed germination, purity and seedling mortality of different varieties of chickpea seeds were measured through correlation and regression analysis (Steel and Torrie 1960).

### Results and Discussion

During the tenure of this investigation a total of 9 species of fungi belonging to 6 genera and a sterile fungus were found to be associated with seeds of 9 varieties (BARI chola 1 - 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, *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers. (Table 1). Among these fungi *Aspergillus* spp., *Penicillium* sp. and *Rhizopus stolonifer* were predominant. *Aspergillus nidulans*, *Curvularia lunata* and *Trichoderma viride* were recorded only with a few varieties of chickpea seeds. Maximum of seven species of fungi were found to be

associated with BARI chola 2. Per cent frequency of association of *Rhizopus stolonifer* was the highest (68) and the lowest was in *Curvularia lunata* (1) (Table 1).

The per cent of seeds associated fungi were ranged from 63 - 94 (Table 2). Maximum fungal association (94%) was recorded in BARI chola 8 and minimum (63%) in BARI chola 1. Mortality of seedling was recorded the highest in BARI chola 8 (82) and the lowest in BARI chola 1 (22%) (Table 2). The highest purity percentage were in BARI chola 4 (99) and lowest in BARI chola 6 (96). BARI chola 7 showed the highest seed germination (66%) while BARI chola 8 showed the lowest (17%) (Table 2). *Aspergillus flavus* and other *Aspergillus* sp. were predominant and commonly associated with stored seed of chickpea and responsible for reducing germination (Dawar *et al.* 2007). However, the differences in germination status might be due to differences in storage and handling. The prevalence of seed-borne infection is also responsible for lower germination (Fakir 1983)

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

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
<i>Alternaria alternata</i>	-	4	3	4	-	-	-	-	-
<i>Aspergillus flavus</i>	3	4	-	4	3	3	21	-	14
<i>A. fumigatus</i>	28	55	28	17	27	-	28	10	14
<i>A. niger</i>	17	7	43	13	30	9	14	21	19
<i>A. nidulans</i>	-	-	2	-	-	-	-	-	-
<i>Curvularia lunata</i>	-	2	-	-	1	-	-	-	-
<i>Penicillium</i> sp.	14	8	10	14	3	7	8	15	25
<i>Rhizopus stolonifer</i>	9	16	5	29	30	54	41	68	57
<i>Trichoderma viride</i>	8	-	9	16	-	-	8	10	-

- = No isolate, BRC = BARI chola.

**Table 2. Effect of storage mycoflora on purity, germination and seedling mortality of different varieties of chickpea seeds.**

Name of varieties	Purity (%)	Germination (%) (7th day)	Mortality (%) (after 7 days)	% occurrence of fungi
BARI chola 1	98	60	22	63
BARI chola 2	98	36	67	88
BARI chola 3	98	59	34	75
BARI chola 4	99	46	40	77
BARI chola 5	97	40	60	85
BARI chola 6	96	27	62	88
BARI chola 7	97	66	78	90
BARI chola 8	96	17	82	94
BARI chola 9	97	55	57	84

In this study it has been estimated some interrelationships between the quality factor through correlation and regression analysis which is very much important in controlling seed quality. Significant relationship has been estimated in all the cases (Fig. 1A-D).

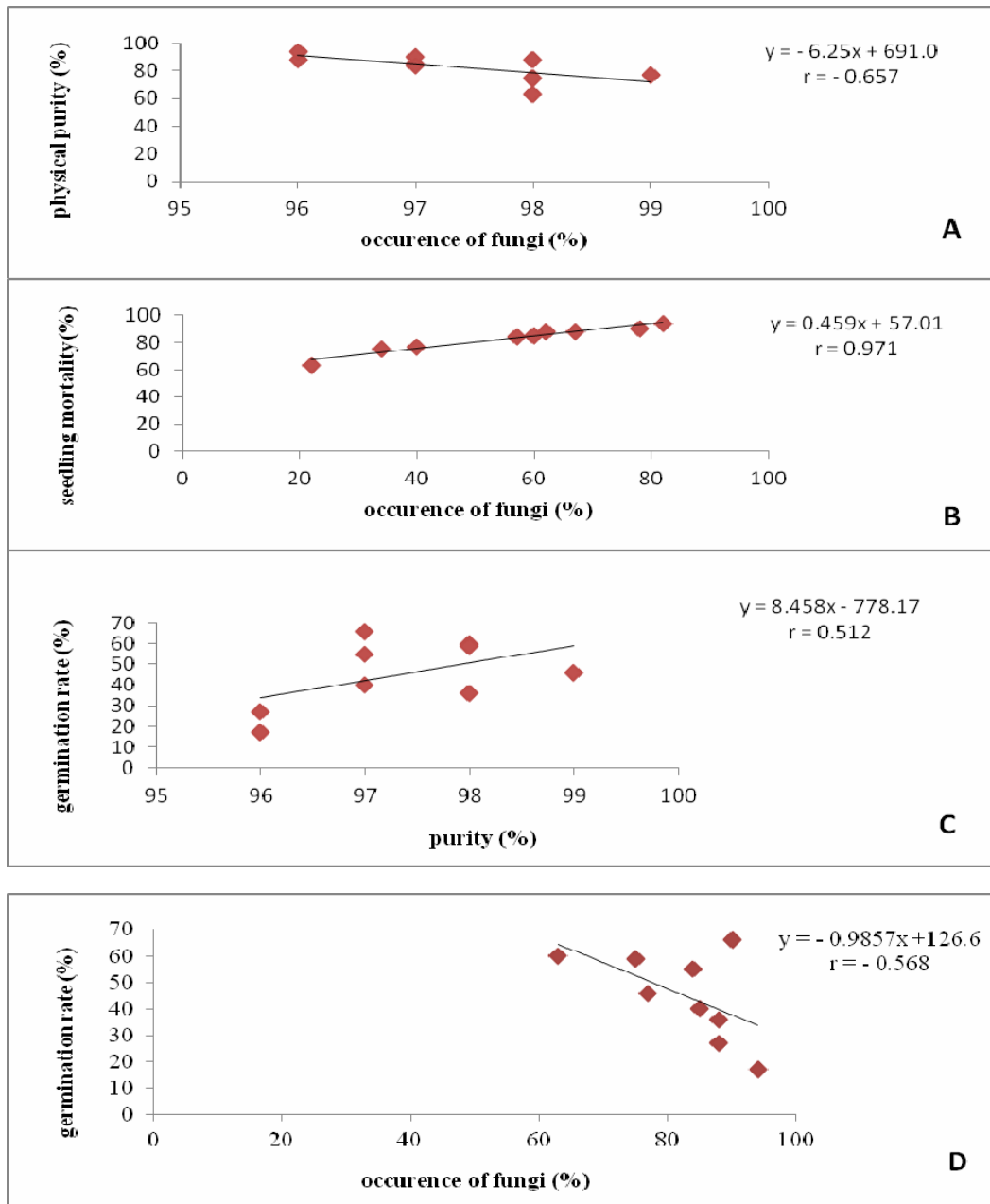


Fig. 1. Correlation co-efficient and regression equation between A = Physical purity (%) and occurrence of fungi (%). B = Seedling mortality (%) and occurrence of fungi (%). C = germination rate (%) and purity (%). D = Germination rate (%) and occurrence of fungi (%).

Fig.1A shows the relationship between percentage of physical purity and percentage of occurrence of fungi and negative correlation between the two variables. From Fig. 1A it is evident that the regression line gives a downward sloping curve, which means that the percentage of fungi decreases when purity of seed increases and vice versa. The correlation co-efficient value between occurrence of fungi and physical purity of seeds was  $-0.657$ .

Fig. 1B shows the relationship between occurrence of fungi and seedling mortality and positive correlation between the two variables. Here regression line gives an upward sloping curve which means that both the variable change in the same direction i.e. the mortality of seed increases when the percentage of fungi increases. The correlation co-efficient value between percentage of fungi and seedling mortality was  $+0.971$ .

Fig. 1C shows the relationship between percentage of germination rate and physical purity of seeds and found positive correlation between the two variables. In this case the regression line gives an upward sloping curve which indicates that both the variables change in the same direction i.e. the germination increases when the purity of seed increases and the germination decreases when the purity of seed decreases. The correlation co-efficient value between purity and germination of seed was  $+0.512$ .

Fig. 1D shows the relationship between germination rate and occurrence of fungi and negative correlation between the two variables. Here regression line gives a downward sloping curve which means that germination of seeds decrease when the percentage of fungi increases or the germination of seed increases when the percentage of fungi decreases. In the present study, the correlation co-efficient value between percentage of fungi and percentage of germination was  $-0.568$ .

The saprophytic fungi though non-pathogenic, have the ability to secrete toxic metabolites which damages the quality of seed in storage (Christensen 1972). Out of saprophytes *Aspergillus flavus*, *A. Fumigatus*, *A. Niger* and *Curvularia lunata* were found to effect germination (Fazli and Ahmed 1959).

The average physical purity of chickpea seeds was recorded to be 97.33% in the present investigation. The result agrees with seed law (National seed board standard) that chickpea seed should not contain more than 2% impurities. The minimum purity percentage according to seed standard is 97 - 98%. The average seed germination of seeds in this study is 41.5%. The result does not agree with the Bangladesh Seed Board recommendation. The standard germination percentage is minimum 75%.

Fig. 1D indicates that increased of one variable (i.e. germination or occurrence of fungi) decreased the other variable (i.e. germination or occurrence of fungi). This result was dissimilar with the result obtained by Khandakar (1987 on jute seeds. But it was similar with the result obtained by Sontakke and Hedawoo (2014).

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