Evaluation of seed infection of fungi in Chickpea

Razia. K.Zaidi* and NehaPathak

Section of Plant Pathology and Nematology,
Department of Botany,
Aligarh Muslim University, Aligarh, India
Email id- razia.kzaidi@gmail.com

Abstract
The study aims at identifying pathogenic fungi associated with gram different categories (*Cicer arietinum* L.) seeds. Seed health testing is a pre requisite for seed improvement, seed production, seed certification and trade in seed. Using blotter, and agar plate methods as recommended by ISTA, the seed mycoflora of different gram seed samples was examined. About thirty fungal species were isolated from these seeds most abundant- among these were *Alternaria alternata, Chaetomium spp., Penicillium citrinum, Aspergillus niger, A. flavus, Rhizopus nigricans, Fusarium oxysporum*. The no of fungal species were reduced in surface sterilized seeds which indicate of that many of the fungi were located on seed coat. Blotter method showed greater incidence of fungi on different parts of seeds followed by agar plate method. The seed mycoflora devalue the seed quality, reduce its nutritional value and cause a germination failure of the seedlings and of the crop raised from such infected seeds.

Key words – Chickpea, Seed fungi, *Aspergillus spp, Alternaria spp, Chaetomium spp., Penicillium spp, Fusarium spp.*

Introduction

Plants are extremely important in the lives of people throughout the world. Gram (*Cicer arietinum* L.), also known as 'chickpea', is the most important legume grown in India and grown over 6.66 m ha of land.
Mainly two types of chickpea are grown, brown seeded types called "Desi" and white seeded called "Kabuli". Chickpea (*Cicer arientium* L.) an important legume crop is, cultivated over an area of 963.0 hectares with a production of about 675.2 tons in Pakistan (Anon., 2004). Chickpea after dehulling is valued for its nutritive seeds with high protein content (12.3-31.5%).
Chickpea seed has 58.9% carbohydrate, 3% fiber, 5.2% oil, 3% ash, 0.2% calcium, and 0.3% phosphorus. Digestibility of protein varies from 76-78% and its carbohydrate from 57- 60%.
Gram husks, green or dried stems and leaves are used for stock feed; whole seeds may be milled directly for feed. Gram is one of the best legumes for human consumption as the seeds are very nutritive. It furnishes an important food for lower classes and the flour is quite nutritious. Among the food legumes, chickpea is the most nutritive pulse extensively used as protein adjunct to starchy diet (Sastri, 1950). Many fungal species viz., *Alternaria porri, A. alternata, Aspergillus amstelodami, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sydowi, A.wentii, Botrytis cinerea, Cladosporium macrocarpum, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina, Myrothecium roridum, Penicillium notatum, Rhizoctonia* sp., and *Rhizopus arrhizus* been reported from chickpea (Ahmad *et al*., 1993).

Significant decrease in protein content due to attack of seed-borne fungi like *Aspergillus flarus* and *Fusarium semitectum* has been observed in seeds of Black gram and Green gram (Bilgrami *et. al. 1976*). Prasad and Pathak (1987) reported loss in protein content of cereals like Wheat, Maize and Barley seeds affected by *Fusarium oxysporum* and *Fusarium semitectum* under different storage condition. Of the different diseases *Ascochyta* blight caused by *Ascochyta rabiei* (Nene, 1980), *Botrytis* grey mould caused by *Botrytis cinerea* appear in the form of grey to brown lesion (Joshi & Singh, 1969). Many workers have detected different mold fungi and their toxin production ability in stored grains which deteriorate the stored products (Afzal *et al*., 1979). Among these fungal diseases are more prevalent. Presence or absence of mycoflora on seed surface is one of the important aspect that determines the quality of seed. Large number of fungi and other organisms are associated with all types of seeds. The fact that the seed is contaminated with a number of organisms does not mean that the resultant crop will be diseased. Seed-borne inoculation can severely endanger seedling and plant vigaur but whether, how much and when they are going to attack depends on their nature and an ecological balance of all the micro-organism in and around the seed. Seed-borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure and /or causing disease to the newly emerged seedlings or growing plants but also contaminate the soil by establishing its inocula permanently (Hasan et al.,2005)
The present study aims to study the seed borne fungal pathogens of important pulse crop namely gram [*Cicer arietinum* (L.)].

Materials and methods
External and internal mycoflora of abnormal seeds of gram was studied. International rules for seed testing (Annonymous, 1966) were followed in the present investigation. Seed samples of gram of different commercial varieties were procured from. Indian Agricultural Research Institute, New Delhi, for isolation of seed mycoflora. Isolations were made from 400 infected seeds of the gram under aseptic conditions. Seeds were tested by ISTA techniques using blotter and PDA methods for the external and internal mycoflora of gram. For the study, seeds were grouped in to different categories as shown in the relevant tables.

Blotter method: The blotter method (Limonard, 1966; Lantos et al., 2002) is one of the important incubation methods. It is useful incubation method to detect the deep seated pathogens where untreated seeds and seeds after sterilized by 0.1% aqueous mercuric chloride solution for 2-3 minutes then washed by sterilized distilled water (Habib et al., 2007) for 10 minutes were plated on water soaked filter papers, and incubated usually for 8 days under 12^{th} alternating cycles of light and darkness. After incubation, fungi developed on seeds are examined under different magnification of a stereomicroscope and identified. The identification of the fungi is based on the way they grow on the seeds on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope. Petriplates with moisten blotting papers and seeds were incubated at 20±2 ºC for 8 days with cycles of 12 hours light and 12 hours darkness. After 8 days of incubation fungi which developed on the seeds were identified. Similarly fungi growing out from the seeds on the **Potato dextros agar medium** (**PDA**) were examined.

CALCULATION AND ANALYSIS
Frequency of the fungus and relative abundance was calculated and percentage

germination of seeds was also recorded.
The frequency of the fungus was calculated by the following formula:

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

Relative abundance of the fungi was calculated by the formula:

$$\frac{\text{Total No. of colonies of a fungus on seed}}{\text{Total No. of colonies of all fungus}} \times 100$$

Results - Different categories of chickpea seeds revealed the presence of a large number of fungal members which can be described as follows along with the percentage germination of seeds.

(A) Wrinkled big seeds

External seed mycoflora- Table (1) shows that fifteen fungi viz., *C.indicum. A. niger, A.flavus., A.terreus, P.vermiculatum, A.alternata, A.dianthi, C.cladosporioides, C.lunata, C.clavata, C.pallescence, D.halodes, H.fuscoatra, F.equiseti,* and *Fusariella Spp,* were recorded from external surface of wrinkled big seeds of gram. Highest frequency value (1.00) and relative abundance (1.00) for *P.vermiculatum, A.alternata , A. dianthi,* and *D.halodes*. The percentage germination of seeds was 30.

Internal seed mycoflora- Table (2) revels that eleven fungi viz., *R.arrhizus, A. niger, A.flavus, A.fumigatus, A.candidus, A.fusispora. A.sonchi, C.lunata, C.clavata, F.oxysporum* and *Fusariella Spp* were recorded from the internal seed surface of wrinkled big seeds of gram. Highest frequency value (9.75) and relative abundance (17.65) were recorded for *C.lunata* and lowest frequency (1.00) and relative abumdance (3.00) for *A.sonchi* and *Fusariella spp*. The percentage germination of seeds was 30.

(B) **Wrinkled small seeds**

External seed mycoflora- It is evident from table (1) that eighteen fungi viz., *M.sphaerosporus, R.stolonifer, C.cucurbitarum. C. flavus, A. terreus, A.fumigatus, A.fusispora, A.alternata, A.sonchi, , A.dianthi, A.clamydospora, C. herbarum, C.lunata, D.australiensis, D.halodes, H.fuscoatra, F.oxysporum* and *Fusariella Spp* were detected from the external surface of wrinkled small seeds of gram. . Highest frequency value (20.75) were recorded for, *F.oxysporum* while lowest frequency (1.00) and relative abundance (1.00) for *M.sphaerosporus, R.stolonifer, , A.clamydospora, C. herbarum, C.lunata,* and *D.halodes.* The percentage germination of seeds was 28.

Internal seed mycoflora – A perusal of the table (2) indicates that thirteen fungi namely *C.magrum, C.spirale, A. fumigatus, A.alternata, A.sonchi, A.dianthi, A.prassicicola, C. oxysporum, C.lunata, D.australiensis, D.halodes, F.orthoceras* and *Fusariella spp.* were isolated from the internal surface of wrinkled small seeds of gram. Highest frequency value (9.75) and relative abundance (20.25) were recorded for *F.orthoceras* while lowest frequency (1.00) and relative abundance (2.00) for

C.spirale, C.oxysporum and *Fusariella spp*. The percentage germination of seeds was 29.50.

(C) Damaged (Injured) Seeds –

External seed mycoflora- It is clear from Table (1) that twenty one fungal species viz., *M.sphaerosporus, R.arrhizus, C.cucurbitarum, A.niger, A.flavus, A.terreus, Afumigatus, P.vermiculatum, A.alternata, A.sonchi, A.clamydospora, C.cladosporioides, C.herbarum, C.clavata, D. australiensis, D.hawaiiensis, D. halodes, H.fuscoatra, F.equiseti, F.oxysporum* and *Fusariella spp.* were recorded from the external seed surface of damaged seeds of gram. Highest frequency value (12.50) and relative abundance (9.50) were recorded for *A. niger* and lowest frequency (1.00) and relative abundance (2.00) were recorded for *M.sphaerosporus, A. fumigatus, C. clavata,* and *F.equiseti,*. The percentage germination of seeds was 15.

Internal seed mycoflora – Table (2) shows that sixteen fungi viz., *R.arrhizus, C.magrum, A.niger, A.flavus, A.candidus, A.alternata, A.sonchi, A.brassicicola, C. oxysporum, C.clavata, D. australiensis, D.halodes, F.equiseti, F.oxysporum, Fusariella spp.* were detected from the internal seed surface of damaged seeds of gram. Highest frequency value (9.75) and relative abundance (11.10) were noticed for *A. niger* and lowest frequency (1.00) and relative abundance (1.00) were recorded for *A.candidus, C.clavata, F.equiseti,*. The percentage germination of seeds was 23.24.

Discussion

All available resources are being mobilized to set up our food production and the farmers are being advised to take up to scientific farming. Increased crop productivity can be achieved by using cultivars of high yielding varieties and avoiding crop failures. This involves the demand of better quality seed in terms of germination, purity and health by the farmers. Seeds carry several destructive pathogens that often take a heavy toll by causing severe diseases on crops raised from them. Gram (*Cicer arietinum* L.) is an important potential legume crop of India. The crop suffers due to a number of diseases several of which are seed-borne (Chupp and Sherf, 1960, Walker, 1956). It's cultivation is hampered due to seed-borne diseases. Keeping the importance of all pulses and cereals to developing country like India and also in view of the fact that quite little work has been carried out on the seed pathology of all crops in India. It was considered desirable to study certain aspects of seed mycoflora so that the losses due to minimized.

In case of gram ,15,18,21 fungi have been recorded from external surface of wrinkled big, wrinkled small, and damaged seeds and 11,13, and 16 from internal surface. Highest frequency value and relative abundance in case of wrinkled big seeds have been recorded for *A. niger*, in external mycoflora and *C.lunata* in internal mycoflora. In case of wrinkled small seeds, highest frequency value and relative abundance have been observed for *F.oxysporum*, in external flora and *F.orthoceras* in internal flora. Surface sterilization also has the advantage of minimizing competition among fungi on the seed (Kaur, 2010)

However in case of damaged seeds, highest frequency value and relative abundance have been noticed for *A.niger*, in both external and internal mycoflora (1.11) Table 12 and fig 2.). Role of germination in abnormal seeds has been quite low in comparison to normal seeds.

By and large, the number of number of fungi detected from damaged seeds is more than normal seeds. For the entry of pathogens damaged seeds act as avenues. Walldon

(1916), Machacek and Greaney (1933) and Koehlar (1957) observed that damaged seeds are much more susceptible to mould, saprophytes and pathogens, than are normal seeds both during storage and under field conditions. The fungi isolated from stored wheat seeds were the main cause of deterioration of seeds during storage (Worange et al., 2008). The variation in the type of fungi in different varieties may be in part due to variation in the agricultural operations. *Aspergillus*, in general, out-numbered all the other fungal species and were widely distributed in the seed samples of different categories of gram. From the above discussion it is clear that the studies on seed mycoflora of food crops like chickpea is an important aspect of the plant protection because without seed health tests we cannot touch the target of food security as the healthy seeds are the prerequared of the healthy agriculture.

Literature

1. Afzal, M.R., A. Cheema and R.A. Chaudhary. 1979. Incidence of aflatoxin producing fungi in animal feed stuff. Mycopathologia, 69(3): 149-51.
2. Ahmad, I., S. Iftikhar and A.R. Bhutta. 1993. Seed borne microorganism in Pakistan. A checklist 1991. Pakistan Agricultural Research Council, Islamabad, Pakistan. 32pp.
3. Annonymous, 1996. International seed testing association. International rules for seed testing. Proc. Int. Seed Test Assoc. 31:752.
4. Anonymous. 2004 Agriculture statistics of Pakistan, 2002-2003. Ministry of food, Agriculture and Co-operatives Food and Agriculture Division (Economic wing), Govt. of Pakistan, Islamabad, 316. pp.
5. Bilgrami, K.S., Jamaluddin and Rizvi, M.A. (1979). Fungi of India, Today and Tomorrow's printers and Publishers, New Dehli.
6. Chupp. C. and Sherf, A.P. (1960) Vegetable diseases and their control. The Ronald Press Company, New York, U.S.A. pp. 104-165.
7. Habib, A., S.T.Sahi, M.U. Ghazanfar and S. Ali. (2007). Location of seed-borne mycoflora of eggplant (*Solanum melongena* L.) in different seed components and impact on seed germinability. Int. J.Agric. Biol.,9:514-516.
8. Hasan, M.M, S.P. Chowdhary, S. Alam, B. Hossain and M.S. Alam, (2005). Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, Seedling health and vigaur index. Pak.J. Biol. Sci., 8:1284-1289.
9. Joshi, M.M and R.S Singh. 1969. A *Botrytis* grey mould of gram. Indian Phytopath., 22: 125-128.
10. Kaur, B. (2010).Development and evalution of methods for the detection of seed borne fungi in rice. Intr. J. Edu. Admin. Vol.No 2,(2):123-130.
11. Kochler, Benjamin (1957). Pericarp injuries in seed corn. Prevalence in dent corn and relation to seedling blights. Univ, Illinois, Agr, Exp. Sta, Bull., 617:72 p.
12. Lantos, F., J. Lantos and E.Lantos. (2002). An unusual twist to the blotter test. The Lantos method Practicing oil analysis, pp:7.
13. Limonard, T. (1966). A modified blotter test for seed health. Neth. J. Pl. Path. **72**: 319-321.
14. Machacek, J.E. and Greaney, F.J. (1933). The effect of mechanical seed injury on the development of root rot in cereals. Can.J.Res.8:276-281.
15. Nene, Y.L. 1980. A Review of *Ascochyta* blight of chickpea. Tropical Pest Management, 28: 61-70.

16. Prasad, T. and Pathak S. S. 1987. Impact of various storage systems on bio-deterioration of cereals. Indian Phytopath. 40(1): 39-46.
17. Sastri, B.N. 1950. The wealth of India. A dictionary of Indian raw materials and industrial
18. Products. Vol. II. Council of Scientific & Industrial Research, New Delhi, 427 pp.
19. Walker, J.C (1952). Diseases of vegetable crops. Mc Graw Hill Book Company Inc., New York, U.S.A. pp. 529.
20. Wallden, J.N. (1916). Troskskada a lvete och rag dess inflytande pa kansligheter for betning och lagring servings. Utsadesforcnngs. Tidskrift. 26, 24-41.
21. Worang, R.L., O.S. Dharmaputra, R.Syarief and Miftahudin, 2008. The quality of physic nut (*Jatropha curcas*) seeds packed in plastic material during storage. Biotropia, 15: 25-36.

Table (1). External Mycoflora of Abnormal Seeds of Gram

S.No.	Fungi Isolated	Wrinkled big seeds		Wrinkled small seeds		Damaged seeds	
		F	R.A.	F	R.A.	F	R.A.
1.	*Mucor sphaerosporus* Hagem	-	-	1.00	1.00	1.00	1.00
2.	*M. globosus* Fischer	-	-	-	-	-	-
3.	*Rhizopus stolonifer* Enrenberg	-	-	1.00	1.00	2.50	3.00
4.	*R. arrhizus* Fischer	-	-	-	-	2.50	3.00
5.	*Cheanephora cucurbitarum* (Berkeley & Ravenel) Thaxter	-	-	4.00	5.54	2.50	3.00
6.	*Chaetomium indicum* Corda	5.50	7.64	-	-	-	-
7.	*C. flavum* Omvik	-	-	2.50	3.34	-	-
8.	*Aspergillus niger* van Tieghem	14.50	15.0	-	-	12.50	9.50
9.	*A. flavus* Link	10.75	13.87	-	-	11.50	9.00
10.	*A. terreus* Thom	6.25	10.64	3.00	5.20	8.75	7.70
11.	*A. fumigates* Fresenius	-	-	9.75	12.90	1.00	2.00
12.	*Penicillium vermiculatum* Dangeard	1.00	1.00	-	-	2.50	3.00
13.	*Acrophialophora fusispora* (Saksena) M.B. Ellis	-	-	5.75	8.00	-	-
14.	*Alternaria alternate* (Fr.) Keissler	1.00	1.00	7.75	10.49	7.50	7.21
15.	*A. sonchi* J.J. Davis	-	-	4.75	5.17	2.50	5.00

16.	*A. dianthi* Stevens & Hall	1.00	1.00	2.50	3.54	-	-
17.	*A. chlamydospora* Mouchacca	-	-	1.00	1.00	4.50	5.00
18.	*Cladosporium cladosporioides* (Fresen) de Vries	5.50	7.60	-	-	2.50	3.00
19.	*C. herbarum* (Pers.) Link	-	-	1.00	1.00	5.00	5.58
20.	*Curvularia lunata* (Wakker) B Boedijin	6.25	10.64	1.00	1.00	-	-
21.	*C. clavata* Jain	2.50	3.60	-	-	1.00	2.00
22.	*C. pallescence* Boedijn	3.75	4.78	-	-	-	-
23.	*D. australiensis* (Bugnicourt) Subram. & Jain	-	-	2.50	3.34	5.00	5.90
24.	*D. hawaiiensis* (Bugnicourt) Subram. & Jain	-	-	-	-	2.50	3.00
25.	*D. halodes (*Drechsler) Subram. & Jain	1.00	1.00	1.00	1.00	5.00	5.42
26.	*D. rostrata* (Drechsler) Richardson & Fraser	-	-	-	-	-	-
27.	*Humicola fuscatra* Traaen	3.50	4.14	4.75	5.77	5.00	5.50
28.	*Fusarium equiseti* (Corda) Saccardo	8.75	12.09	-	-	1.00	2.00
29.	*F. oxysporum* Schlechtendahl	-	-	20.75	25.93	8.75	7.43
30.	*Fusariella* sp.	4.00	6.00	3.75	4.78	5.80	5.36
	Percentage Germination	30.00		28.00		15.00	

F denotes percent frequency, **R.A.** denotes relative abundance and denotes absence of a fungal species.

Table (2). Internal Mycoflora of Abnormal Seeds of Gram

S.No.	Fungi Isolated	Wrinkled big		Wrinkled small		Damaged seeds	
		F	R.A.	F	R.A.	F	R.A.
1.	*Rhizopus arrhizus* Fischer	2.50	4.80	-	-	2.00	3.00
2.	*Chaetomium magnum* Bainier	-	-	3.50	3.50	5.21	3.00
3.	*C. spirale* Zopf	-	-	1.00	2.00	-	-
4.	*Aspergillus niger* van Tieghem	7.75	15.65	-	-	9.75	11.10
5.	*A. flavus* Link	4.75	9.60	-	-	7.25	10.00
6.	*A. fumigates* Fresenius	5.75	14.75	4.75	10.46	-	-
7.	*A. candidus* Link	5.00	10.00	-	-	1.00	1.00
8.	*Acrophialophora fusispora* (Saksena) M.B. Ellis	2.50	4.41	-	-	-	-
9.	*Alternaria alternata* (Fr.) Keissler	-	-	7.00	17.20	625	9.66
10.	*A. sonchi* J.J Davis	1.00	3.00	2.50	4.40	6.75	9.76
11.	*A.dianthi* Stevens & all	-	-	7.75	16.60	-	-
12.	*A. brassicicola* (Schw.) Wiltshire	-	-	2.50	4.40	1.25	3.00

13.	*Cladosporium oxysporum* Berk. & Curt.	-	-	1.00	2.00	4.00	8.42
14.	*Curvularia lunata* (Wakker) Boedijn	3.50	7.86	3.50	5.66	-	-
15.	*C. clavata* Jain	9.75	17.65	-	-	1.00	1.00

16.	*Drechslera australiensis* (Bugnicourt) Subram. & Jain	-	-	3.25	5.00	3.00	5.38
17.	*D. halodes* (Drechsler) Subram. & Jain	-	-	2.75	4.44	4.00	8.81
18.	*Fusarium oxysporum* Schlechtendahe	4.50	9.25	-	-	7.5	10.00
19.	*F. equieti* (Corda) Saccardo	-	-	-	-	6.50	9.55
20.	*F. orthoceras* Appel & Wollenweber	-	-	9.75	20.55	1.00	1.00
21.	*Fusariella* sp.	1.00	3.00	1.00	2.00	3.00	5.32
	Percentage Germination	30.00		29.50		23.25	

F denotes percent frequency, **R.A**. denotes relative abundance and denotes absence of a fungal species.