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SEED BORNE FUNGI ASSOCIATED WITH CHICKPEA IN PAKISTAN

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Abstract

Using blotter, agar plate and deep freezing methods as recommended by ISTA, the seed-borne mycoflora of 14 chickpea seed samples collected from different areas of Pakistan was examined. A total number of 21 species belonging to 13 genera of fungi were isolated. Of these 4 species viz., *Absidia glauca, Rhizoctonia solani, Syncephalastrum* sp., and *Trichoderma harzianum* are new reports from Pakistan. Pathogenic fungi viz., *Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani,* and saprophytic fungi like *Aspergillus niger* and *A. flavus* were pre-dominant. Component plating of chickpea seeds showed that seed coat and cotyledons were infected by greater number of fungi followed by axis (radicle+plumule). *M. phaseolina* and *R. solani* were also isolated from seed coat, cotyledons and axis of seed. The fungal species were reduced in surface sterilized seeds which indicate that most of fungi were located on seed coat. Blotter method showed greater incidence of fungi on different parts of seeds followed by agar plate and deep-freezing method.

Introduction

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). Mainly two types of chickpea are grown, brown seeded types called "Desi" and white seeded called "Kabuli". Chickpea after dehulling is valued for its nutritive seeds with high protein content (12.3-31.5%). Chickpea seeds are eaten fresh as green vegetables, parched, fried, roasted and boiled as snack food, sweet and condiments. Seeds are ground and the flour can be used as soup, dhal and to make bread, prepared with pepper, salt and lemon it is served as a side dish. Young plants and green pods are eaten like spinach. A small proportion of canned chickpea is also used in Turkey and Latin America to produce fermented food. Animal feed is another use of chickpea in many developing countries. Chickpea husks, green or dried stems and leaves are used for stock feed; whole seeds may be milled directly for feed. Leaves are said to yield an indigo like dye. 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%. 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). 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).

Colletotrichum blight caused by *Collectotrichum dematium*, *Alternaria* blight is caused by *Alternaria alternata* (Vishwakarima & Chaudhary, 1974). Mould fungi are also known to produce mycotoxin (Rodricks, 1976). Many workers have detected different mold fungi and their toxin production ability in stored grains which deteriorate the stored products (Afzal *et al*., 1979). Present paper describes the association of fungi with chick pea seed collected from different parts of Pakistan.

Materials and Methods

Fourteen chickpea seed samples were collected from different localities of Paksitan viz., Karachi (3), Lahore (1), Rawalpindi (1), Islamabad (1), Haripur (1), Abbottabad (1), Sahiwal (1), Hyderabad (1), Kotmithan (1), Rajan-pur (1), Sukkur (1) and Chakwaal (1). From each sample 400 seeds were tested. For the standard blotter method, untreated seeds and seeds after treatment with 1% Ca $(OCI)_2$ for 10 minutes were placed on three layers of moistened blotter, 10 seeds per Petri dish. For agar plate method, the untreated seeds and seeds after surface sterilization with 1% $Ca(OCI)_2$ for 10 minutes were placed on potato dextrose agar (PDA), pH 5.5. Ten seeds were placed in each Petri dish and the dishes were incubated at 24± 1°C under 24h of alternating cycle of light and darkness for 7days. In deep freezing method, the untreated seeds and seeds after sterilization with 1% $Ca(OCl)_2$ for 10 minutes were placed on blotter and incubated for 1 day each at 20ºC ± 2ºC followed by 5 days incubation at 24±1ºC under 12 hours of alternating cycle of light and darkness (Anon., 1976).Fungi growing on seeds were identified after references to Barnett (1960), Ellis (1971), Domesch *et al*., (1980), Nelson *et al*., (1983) and Raper *et al*., (1965). For location of fungi five seed samples collected from different localities of Pakistan viz., Karachi (2) Rawalpindi (1) Islamabad (1) and Abottabad (1) were used. The method suggested by Mathur *et al*., (1975) was used to detect the location of seed-borne fungi with slight modification. Seed soaked for 4 hrs in sterilized distilled water in test tubes were dissected aseptically into testa (brown covering), and embryo. Embryos were further dissected into cotyledons, axis (radicle & plumule). ISTA techniques (Anon, 1976) were used to detect fungal infection of different parts of seed where 20 untreated and 20 seeds treated with 1% $Ca(OCI)_2$ were used for blotter, agar plate and deep freezing method. Using blotter method, the treated and untreated seeds components were plated on Petri dish on three layers of sterilized moistened blotter. For Agar plate method, the treated and untreated seed components were plated on PDA, pH 5.5. The dishes were incubated at 24ºC for 7 days. For deep freezing method, the treated and untreated seed parts were placed on blotters and incubated for 1 day each at 20ºC and -2ºC in deep freezer followed by 5 days incubation at 24 ± 1ºC under 12h alternating cycle of ADL and darkness. Fungi growing on different parts of seeds were identified. Data were subjected to Analysis of Variance (ANOVA) or Factorial Analysis of Variance (FANOVA) depending upon the experimental design following the procedure as given by Gomez & Gomez (1984).

Results and Discussion

A total of 13 genera and 22 species of fungi viz., *Absidia glauca* Hagem., *Alternaria porri* (Ellis) Cif., *A. alternata* Nees., *Aspergillus amsteldomi* (Mangin)Thom & Chruch., *A. flavus* Link ex Gray., *A. fumigatus* Fres., *A. niger* Van Tieghem., *A. sydowi* (Brain &

Sart)., *A. wentii* Wehmer., *Botrytis cinerea* Pers. ex Nocca & Baib., *Cladosporium* sp., *Curvularia lunata* Boedijn., *Fusarium equiseti* (Corda) Sacc., *F. moniliforme* Sheld., *F. oxysporum* Schlecht., *F. semitectum* Berk & Rev., *Macrophomina phaseolina* (Tassi) Goid., *Myrothecium roridum* (Tode ex Fr)., *Paecilomyces variotii* Bain., *Rhizoctonia solani* Kuhn., *Syncephalastrum* sp., and *Trichoderma harzianum* Rifai were isolated from chickpea seed (Table 1). Of these 4 species viz., *Absidia glauca*, *Rhizoctonia solani*, *Syncephalastrum* sp., and *Trichoderma harzianum* marked with asterisk were found to be new report from Pakistan (Ahmed *et al*., 1993). About 50% samples of seed were infected by *F. equiseti* by deep freezing method with an infection range of 0.3–0.5% in surface sterilized seeds and 0.8–0.9% in non-sterilized seeds (Table 1). *M. phaseolina* infection was observed on blotter and agar plate method with an infection range of 0.08–0.9% in surface sterilized and non-sterilized seeds ($p<0.001$). *R. solani* showed 0.4% infection in sterilized seeds. About 50% sample of seeds were found to be infected by *P. variotii* with an infection range of 0.07–0.3% in surface sterilized seeds and 0.03–0.18 in non-surface sterilized seeds. Of the 14 samples used for the detection of seed-borne fungi, 100% samples were found to be infected by *A. flavus* and *A. niger*. Surface sterilization with 1% $Ca(OCl)_2$ significantly reduced the infection % of *A. flavus* ($p< 0.5$). Of the blotter, agar plate and deep-freezing methods used for the isolation of seed-borne fungi, the blotter technique yielded significantly higher number of fungi ($p<0.5$) as compared to agar plate and deep freezing methods. Similar results have been observed by Khan *et al*, (1988); Tariq *et al*., (2005) on soybean and Dawar & Ghaffar (1991) on sunflower who found that blotter and agar plate methods were more suitable for the detection of seed-borne fungi. Mathur *et al*., (1975) found that the deep-freezing method was more suitable for the detection of *Fusarium* spp., in sorghum seed. The samples collected from Rawalpindi and Abbottabad showed the highest incidence of pathogenic fungi viz., *F. moniliforme*, *M. phaseolina* and *R. solani* (Table 1).

Component-plating of chickpea seeds showed that higher number of fungi were isolated by blotter technique as compared to agar plate and deep-freezing methods. Most of the fungi were found to be located on seed coat followed by cotyledons and axis of chickpea seed. Of the five samples tested, samples collected from Rawalpindi and Abbottabad showed the highest frequency of fungi viz., *R. solani*, *M. phaseolina*, *F. moniliforme*, *F. equiseti* and *A. alternata* whereas 3 samples from Karachi were contaminated with storage fungi especially *A. flavus* and *A. niger*. Infection of *A. flavus* and *A. niger* were observed on seed coat, cotyledons and axis in sample collected from Karachi whereas infection of *A. flavus* was observed from surface sterilized axis in sample collected from Islamabad. Surface sterilization of seeds with 1% $Ca(OCl)_2$ reduced the incidence of *Aspergillus* spp., Limonard (1968) also reported that microbial contamination was eliminated by chlorine disinfection. Infection of *M. phaseolina* was recorded in all parts of seed viz., seed coat, cotyledons and axis ($p<0.001$). These results are contrary to the reports of Sadashivaiah *et al.*, (1986) who found *M. phaseolina* infection only in pericarp and seed coat. Infection of *R. solani* was recorded in all parts of seed viz., seed coat, cotyledons and axis. Similar results have been reported by Dawar & Ghaffar (1990) on sunflower; Rasheed *et al.*, (2004b) on groundnut. *F. moniliforme* was detected in inner tissues such as seed coat but not in axis. Mathur *et al.*, (1975) and Sultana *et al.*, (1988) found that infection of *F. moniliform* was more conveniently detected in the endosperms than seed coat and embryo of sorghum seed. Infection of *A. alternata* was observed from seed coat and cotyledons but not from axis (Table 2).

Table 1. Seed borne mycoflora of chickpea.

Name of fungi	Sterilized						Non sterilized					
	Agar plate		Blotter method		Deep freezing method		Agar plate		Blotter method		Deep freezing method	
	NSI	1% ± SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD	NSI	1%±SD
**Absidia glauca*	1	0.2 ± 0.6	0	0	0	0	0	0	0	0	0	0
Alternaria alternata	0	0	6	1.57±2.35	0	0	6	0.92±1.32	6	1.71±2.05	4	0.42±0.75
A. porri	5	0.71±1.20	10	2.2±1.92	2	0.14±0.36	6	1.28±1.63	0	0.78±2.40	0	0
Aspergillus flavus	14	3.8±2.40	14	8.5±4.29	14	3.35±1.77	14	6.5±4.09	8	4.64±3.79	14	1.57±2.10
A. fumigatus	8	1.71±2.05	10	4.5±4.29	4	1.21±2.22	0	0	14	2.57±2.87	14	4.35±3.11
A. niger	14	7.7±5.07	14	10.1±4.37	14	4.57±2.73	14	7.57±4.40	9	6.21±3.72	6	0.92±1.30
A. sydowi	7	0.89±1.93	9	2.57±2.4	3	0.64±1.37	2	0.14±0.72	14	0	7	0.21±1.30
A. wentii	3	0.39±1.67	7	1.4±6.38	4	0.57±0.59	8	1.21±1.31	0	1.07±1.93	0	0
Botrytis cinerea	6	0.57±1.30	4	0.5±2.25	0	0	5	0.78±1.25	4	0.78±1.76	0	0
Cladosporium sp.	0	0	0	0	0	0	2	0.21±0.57	3	0	0	0
Curvularia lunata	0	0	0	0	0	0	0	0	0	0.21±0.57	0	0
Fusarium equiseti	8	1.28±1.58	10	2.5±2.17	4	0.35±0.63	9	1.78±1.93	2	2.71±2.99	7	0.85±1.63
F. moniliforme	0	0	5	0.85±1.35	0	0	0	0	8	1.28±2.04	4	0.42±0.75
F. oxysporum	3	0.28±0.61	11	1.91±1.92	0	0	0	0	6	0	0	0
F. semitectum	0	0	0	0	3	0.20±0.61	2	0	0	2.5±2.73	0	0
Macrophomina phaseolina	2	0.21±0.57	7	0.92±1.07	0	0	1	0.07±0.26	10	0.85±1.23	0	0
Myrothecium roridum	5	0.64±1.0	4	0.35±0.93	0	0	0	0	6	0	0	0
Paecilomyces variotii.	7	1.21±1.38	7	0.85±1.32	1	0.07±0.51	6	0.57±0.75	0	1.64±2.3	1	2.0±0.53
**Rhizoctonia solani*	1	0.14±0.53	6	0.85±1.18	0	0	0	0	7	0.35±0.63		
**Sycephalastrum* sp	2	0.14±1.2	3	0.57±1.67	0	0	5	0.6±1.2	4	0.28±0.57	0	0
**Trichoderma harzianum.*	3	0.5±1.2	4	0.7±2.2	0	0	4	1.0±1.9	2	0.6±1.0	0	0

NSI = Number of samples infected out of 14 samples tested
SD = ± Standard deviation
I% = Percentage of infected seed
* = New reports on chickpea

Table 2. Component plating of chickpea seed.

Name of fungi	Seed coat						Cotyledon						Axis (radical + plumule)					
	Agar plate		Blotter method		Deep freezing		Agar plate		Blotter method		Deep freezing		Agar plate		Blotter method		Deep freezing	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	30	34	16	22	4	4	-	-	-	10	-	-	-	-	-	2	-	-
A. porri	20	18	20	32	-	5	11	12	28	37	_	7	5	5	3	5	-	_
Aspergillus flavus	58	62	36	38	2	3	38	47	26	19	2	5	16	8	9	2	6	10
A. fumigatus	20	40	30	38	6	13	38	42	26	35	10	14	24	37	14	28	-	13
A.niger	56	69	50	30	3	6	30	34	2	10	1	3	20	28	6	12	5	7
A. sydowi	6	13	9	14	27	-	-	-	4	8	13	_	_	2	3	_	-	10
A. wentii	7	8	6	18	-	3	2	6	5	17	2	10	4	6	1	6	-	-
Fusarium equiseti	8	8	42	46	-	-	2	3	4	17	_	_	_	5	3	7	-	-
F.moniliforme	9	10	2	8	-	_	_	2	_	_	_	_	_	1	_	_	-	-
F.oxysporum	10	13	16	27	-	2	13	19	18	23	_	3	2	6	4	5	-	1
Macrophomina phaseolina	8	9	10	16	-	-	7	12	7	14	_	2	6	11	2	7	-	2
Myrothecium roridum.	2	6	4	12	-	-	_	7	6	6	_	_	_	5	6	7	-	-
Rhizoctonia solani	6	8	5	10	-	-	-	6	7	10	-	2	-	-	-	4	-	-

A = Surface sterilized seed

B = Non-sterilized seed

Present result showed that saprophytic fungi viz., *A. flavus* and *A. niger* were predominant among the fungi isolated. Such similar reports have been made by Dawar & Ghaffar (1991) on sunflower seed, Rasheed *et al*., (2004a) on groundnut seed. *A. flavus* and *A. niger* were the predominant storage fungi of groundnut seeds (Mukherjee *et al*., 1992) and soybean seed (Tariq *et al*., 2005). These species have been reported to reduce the germination of seed and damage the seeds in storage (Christensen, 1973). Similarly Clinton (1960) reported that *A. flavus* is one of the fungus attacking germinating groundnut seed. Of the three methods used for the detection of seed-borne fungi blotter and agar plate methods were found more suitable for the detection of fungi as compared to deep freezing method. Total number of 17 fungal species was isolated by blotter method, 16 fungal species were isolated by agar plate and 9 fungal species by deep freezing method. Jovicevic (1980) reported that the filter paper method was most practical method for routine analysis of seed health. Khan *et al*., (1988) found blotter and agar plate methods were more suitable for detection of *Fusarium* spp., and *Chaetomium globosum* from rice seed. Such similar results were observed by Dawar & Ghaffar (1991) on sunflower seed and Tariq *et al*., (2005) on soybean seed. Limonard (1968) reported that intrafungal antagonism becomes a problem in agar plate method. Using agar plate method, the quick growing saprophytes like *Apergillus* spp., and *Cladosporium* spp., adhering to seed surface may be troublesome particularly in the detection of slow growing parasite present internally (Tempo, 1970).

Present result showed that surface sterilization of seed reduced the infection of *A. flavus* and *A niger* and increased the incidence of pathogenic fungi viz., *M. phaseolina*, *R. solani* and *F. moniliforme* on chickpea seed. Such similar report have been made by Dawar & Ghaffar (1991) on sunflower and Tariq *et al*., (2005) on soybean seed. Carranza (1965) observed that chickpea wilt which is caused by *Fusarium* spp., occur in the field of chickpea produced root rot and wilt disease. Present result showed that *A. flavus* and *A. niger* were the predominant fungi of chickpea seed. *A. flavus* was the important mycotoxin producer and produce aflatoxin B1, B2, G1 and G2 which are hepatocarcinogenic (Goldblatt, 1969). Mycotoxins can cause severe damage to the liver, kidneys and nervous system of man even in low dosages (Rodricks, 1976). There is therefore need for reducing the pathogenic fungi by treatment of seed for obtaining the good quality of seed and also reduce the mould fungi and mycotoxin production by improving the storage conditions.

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References

Afzal, M.R., A. Cheema and R.A. Chaudhary. 1979. Incidence of aflatoxin producing fungi in animal feed stuff. *Mycopathologia*, 69(3): 149-51.

Ahmad, I., S. Iftikhar and A.R. Bhutta. 1993. *Seed borne microorganism in Pakistan. A checklist* 1991. Pakistan Agricultural Research Council, Islamabad, Pakistan. 32pp.

Anonymous. 1976. International rules of seed testing. *Proc. Int. Seed Test. Assoc.*, 4:3-49.

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.

Barnett, H.L. 1960. *Illustrated genera of imperfect fungi* (second edition). Burgess Pub. Co., 225 pp.

Carranza, J.M. 1965. Wilt of chickpea (*Cicer arietinum*) caused by *Botrytis cinerea* (in Spanish) Revista de la Faculted de agronoma , *Universidad nacional de la Plata*, 41: 135-138.

Christensen, CM.1973. Loss of viability in storage microflora. *Seed Sci. & Technol.,* 1: 547-562.

Clinton, R.S.S. 1960. Seed bed pathogen of groundnut in Sudan, and an attempt to control with an artificial testa. *Empire J. Exptl. Agr*., 28: 211-222.

Dawar,S. and A. Ghaffar .1990. Location of fungi in sunflower seed. *Pak. J.Bot*., 23(2): 117-120.

Dawar, S. and A. Ghaffar. 1991. Detection of the seed borne mycoflora of sunflower. *Pak. J. Bot*., 23(2): 173-178.

Domsch, K.W., W. Gams and T. Anderson. 1980. *Compendium of soil fungi.* Academic Press, London. 859 pp.

Ellis, M.B. 1971. *Dematiaceous hyphomycetes.* CMI, Kew, Surrey, England. 608pp.

Goldblatt, L.A. 1969. *Alfatoxin. Scientific background, control and implications. Academic Press,* New York. 472 pp.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for agricultural research.*2nd Ed. Wiley, New York. 680 pp.

Joshi, M.M and R.S Singh. 1969. A *Botrytis* grey mould of gram. *Indian Phytopath.*, 22: 125-128.

Jovicevic, B. 1980. Contribution to the knowledge of harmful mycoflora on seeds of wheat, maize and sunflower. *Zastita Bilja*, 31: 101-119.

Khan, S.A.J., A.K. Khanzada, N. Sultana and M. Aslam. 1988. Evaluation of seed health testing techniques for assessment of seed-borne mycoflora of rice. *Pak. J. Agric. Res.*, 9: 502-505.

Limonard, T. 1968. *Ecological aspects of seed health testing.* International seed testing Association, Wageningen, Netherlands. 167 pp.

Mathur, K., S.B. Mathur and P. Neergaard. 1975. Detection of seed-borne fungi in sorghum and location of *Fusarium moniliforme* in the seed. *Seed Sci. & Technol.,* 3: 683-690.

Mukherjee, P.S., S.K Nandi and B. Nandi. Deterioratiue. 1992. Changes in groundnut seeds in storage. *Journal of Mycopathological Research*, 30(2): 113-119.

Nelson P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species, An illustration manual for identification.* The Pennsylvania State University Press, USA.

Nene, Y.L. 1980. A Review of *Ascochyta* blight of chickpea. *Tropical Pest Management*, 28: 61-70.

Raper. K.B., D.I. Fennell and P.K.C Austwick. 1965. *The Genus Aspergillus*. The Williams & Wilkins Company, Baltimore.

Rasheed, S., S. Dawar and A.Ghaffar. 2004b. Location of fungi in groundnut seed. *Pak. J. Bot*., 36(3): 663-668.

Rasheed. S., S. Dawar, A. Ghaffar and S.S. Shaukat. 2004a. Seed borne mycoflora of groundnut. *Pak. J. Bot.*, 36(1): 199-202.

Rodricks, J.V. 1976. *Mycotoxins and other fungal related food problems.* Advances in Chemistry series 149. American Chemicals Society, Washington.

Sadashivaiah, A.S., K.G. Ranganathaiah and D.N. Gowda. 1986. Seed health testing of *Helianthus annuus* with special reference to *Macrophomina phaseolina. Indian Phytopathol.*, 39: 445-447.

Sastri, B.N. 1950. *The wealth of India.* A *dictionary of Indian raw materials and industrial Products. Vol. II.* Council of Scientific & Industrial Research, New Delhi, 427 pp.

Sultana, N., S. A. J. Khan and A. K. Khanzada. 1988. Detection and location of seed-borne fungi in soyabean seed. *Pak. J. Bot.*, 20: 239-244.

Tariq, M., S. Dawar, M. Abid and S.S. Shaukat. 2005. Seed-borne mycoflora of soyabean. *Int J. Biol. Biotech.*, 2(3): 711-7113.

Tempo, J. 1970. Routine method for determining the health condition of seeds in the seeds testing station. *Proc. Inst. Seed Test. Assoc.*, 35: 250-296.

Vishwakarma, S.N. and K.C.B. Chaudhary. 1974. Floral blight of gram incited by *Alternaria alternata. Netherlands Journal of Plant Pathology*, 80: 110-112.

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