

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 arietinum* 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 *Collectorotrichum dematum*, *Alternaria* blight is caused by *Alternaria alternata* (Vishwakarma & 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 Pakistan viz., Karachi (3), Lahore (1), Rawalpindi (1), Islamabad (1), Haripur (1), Abbottabad (1), Sahiwal (1), Hyderabad (1), Kotmian (1), Rajan-pur (1), Sukkur (1) and Chakwal (1). From each sample 400 seeds were tested. For the standard blotter method, untreated seeds and seeds after treatment with 1% Ca(OCl)_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(OCl)_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 $24 \pm 1^\circ\text{C}$ 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 $20^\circ\text{C} \pm 2^\circ\text{C}$ followed by 5 days incubation at $24 \pm 1^\circ\text{C}$ 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 Abbottabad (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(OCl)_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 24°C for 7 days. For deep freezing method, the treated and untreated seed parts were placed on blotters and incubated for 1 day each at 20°C and -2°C in deep freezer followed by 5 days incubation at $24 \pm 1^\circ\text{C}$ 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 amstelodami* (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% $\text{Ca}(\text{OCl})_2$ 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% $\text{Ca}(\text{OCl})_2$ 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. moniliforme* 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).

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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