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SEED-BORNE DISEASE PROBLEMS OF LEGUME CROPS IN PAKISTAN

A Rauf Bhutta

Federal Seed Certification and Registration Department, G-9/4, Islamabad, Pakistan

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Twenty four seed-borne fungi belonging to different genera, were detected using blotter paper method, from 145 seed samples of major legume crops in Pakistan. Of these fungi, *Alternaria alternata, Ascochyta* spp., *Colletotrichum* spp., *Fusarium* spp. and *Macrophomina phaseolina* were the most frequent and known as common pathogenic fungi in these crops. Highest number of various types of mycoflora was detected in soybean (14), chickpea (13) followed by mungbean, pea and lentil seeds. Range of seed infection percentage varied in individual samples and crops. Seven fungi, *Aspergillus, Chaetomium, Cladosporium, Mucor, Penicillium, Trichoderma* spp. generally not considered field fungi in legume crops and are known to be involved in deterioration of seeds and production of *mycotoxins*, were also observed. It is needed that the test legume crop seeds may be tested for their health status before sowing and storage so as to produce healthy crops.

Key words: Seed-borne fungi, Legume crops, Pakistan.

Introduction

Grain legumes commonly known as pulses, represent a subject of enormous significance to the common man's diet in Pakistan. They occupy an area of about 1537.70 million hectares which represents about 8 percent of the total area under other crops (Anon 1995 a). The major grain legumes grown in Pakistan are chickpea (*Cicer arietimum*), lentil (*Lens culinaris Med.*), mungbean (*Vigna ungulata,* L), mashbean (*Vigna munga,* L), arhar (*Cajanus cajan* L), common bean (*Phaseolus vulgaris* L), faba bean (*Vicia faba* L) and mothbean (*Vigna aconitifolia* L). Soybean and pea are also leguminous crops but these are considered in vegetable group. All these crops are generally grown throughout the country but on marginal lands under low fertility and low moisture conditions.

In Pakistan, total production of 695500 metric tonnes of major grain legumes is much below the current requirement of 876585.71 metric tonnes for local consumption (Anon 1995 b). The yield of legume crops is very low rather static for long. The major constraints in yield are, low yield potential of varieties, stresses of rainfed conditions, poor agronomic practices, damages from pest and diseases, absence of sound seed certification programme and socioeconomic conditions (Hussain *et al* 1994). Of these constraints, biotic diseases are the major factor (Table 1) contributing to low production in legume crops in Pakistan (Bhutta 1990; Ilyas 1990; Bashir and Malik 1995; El-Ahmed and Bhutta 1995). *Asocochyta* blight, *Fausarium* wilt, root-rot and bacterial blight are well known field problems throughout the country. Chickpea which occupies about 72 percent of the area under the pulse crop is often destroyed by the chickpea blight. Due to absence of a systematic seed production, the crop production continues to suffer and during 1994/95, about 170.95 and 20.50 million (Pak Rs) were spent only on import of chickpea and mashbean, respectively (Anon 1995 c).

Most of the diseases of legume crops are known to be seed-borne in nature. Therefore, investigations on pathological aspects of seeds were carried out to know the type and extent of seed mycoflora of major legume crops and results are presented in this paper.

Materials and Methods

Collection of seed samples. A total of 145 seed samples of major legume crops (chickpea, mung, lentil, pea and soybean) were collected from main seed sources (public and private sectors) of the country during the 1994/95 season. The samples were collected and working sample was prepared according to the procedures as laid down in ISTA rules (Anon 1985).

Detection of fungi. Four hundred seeds of each legume crop were tested using standard blotter method (Anon 1985). For small seeded crops (lentil and mungbean), twenty seeds were placed on three layers of moistened blotter paper in 9 cm diameter pyrex glass petri-dishes and for bold seeded crops (chickpea, pea and soybean), 16 seeds were plated in each petri-dish. Seeds were incubated for eight days at 20°C ±2 under alternating cycles of 12 hours day and night fluorescent tubes. Seeds were examined under stereomicroscope (Wild Heerburgg with two lights sources at magnification of 6-50). Fungi were identified based on colony habit characters, and species were confirmed after culturing the fungus on potato

dextrose agar medium, with the help of compound microscope (Leitz Laboralux-D), where deemed necessary (Barnett 1960; Ellis 1971; Nelson *et al* 1983).

Results and Discussion

A total of twenty four fungi belonging to different genera were detected from 145 seed sampls of major legume crops using standard blotter method. Percentage ranges of seed infection due to fungi, varied from crop to crop (Table 2). All the fungi reported in this study are known to be seed-borne in nature (Richardson 1990).

Thirteen seed-borne fungi were detected from 57 seed samples of chickpea. *Fusarium semitectum* and *F moniliforme* were found dominant followed by *Cephalosporium* spp, *F solani* and *F oxysporum* and others. Ilyas (1990) reviewed that ten fungal, two viral and one nematode diseases are reported to be prevalent in gram crop. Out of these, four pathogens are reported to be seed-borne. These are gram wilt, *Ascochyta* blight, *Phoma* blight and *Botrytis* grey mold. The most important, *Ascochyta* blight has caused losses upto 70 per cent in epidemic year 1981/82 in Pakistan (Qureshi *et al* 1985). In all these diseases, seeds play key role in primary infection and usually introduce pathogen inoculum to new areas (Halfon-Meiri 1970; Maden *et al* 1975). To check the seed-borne inoculum, seeds must be tested against these seed-borne pathogens in seed production system of the country (Bhutta *et al* 1992).

Four pathogenic fungi known to be responsible for causing various field diseases at different plant growth and development stages, were found associated with lentil seeds. Out of 26, seven seed samples were found infected with *Ascochyta lentis* with per cent ranges from 0.5 to 8.0. Lentil blight caused reduction in photosynthetic area of the plant which ultimately leads to poor yield (Gossen and Morall 1983). Two species of *Fusarium* (*F. oxysporum* and *F. semitectum*) are identified. Similarly, the infection of *Fusarium* spp on lentil seeds has been reported (Khare *et al* 1979; Bashir *et al* 1984). These fungal pathogens are reported to be very destructive and cause wilt and root rot diseases (Ujevic *et al* 1965; Khan *et al* 1983; Sexena and Khare 1988). Another fungus, *Phoma* spp. was found in three seed samples with infection percentage ranging from 0.5 to 5.0. This fungus is known to be responsible for causing root rot and *Phoma* blight in vegetable and chickpea (Bashir and Malik 1988; Wahid and Ali 1990) but it has not yet been reported on lentil crop in Pakistan.

The results have demonstrated the association of the seven seed-borne fungi from twenty seed samples of mungbean. *A. alternata* along with *Curvularia enagrostides* and *D. tetramera* cause foliage diseases (Gupta 1970; Ilyas 1990). *F moniliforme*, *F. oxysporum*, *F. semitectum* and *F. solani* were found to be associated with mungbean seed ranging from 0.5-3.0 per cent. *Fusarium* spp. are known to be responsible for wilt and root rot of mungbean (Qureshi *et al* 1985; Ilyas *et al* 1990). *M. phaseolina*, the cause of charcoal rot disease of a number of cultivated and wild plant species, was detected up to 1 per cent from mungbean seeds. Its association with mungbean seeds has been reported and it causes a progressive

Table 1
Major legume crops and their important seed-borne diseases in Pakistan

Crop	Disease (common name)	Casual organism
Gram, chickpea (*Cicer arietinum* L)	*Ascochyta* blight or gram blight,	*Ascochyta rabiei*
	Gram wilt	*Fusarium oxysporum* f.sp *ciceri*
	Phoma blight	*Phoma medicaginis*
	Botrytis grey mold	*Botrytis cinerea*
Lentil, Masoor	Lentil blight/wilt	*Ascochyta lentis*
(lens culinaris)	Wilt	*F. oxysporum*, f.sp *lentis*
(med)	leaf spot	*Alternaria alternata*
	Lentil viruses	*PsbMv&CMV*
Mungbean,	*Fusarium* wilt,	*F. oxysporum*,
Green gram	Root-rot	*F. solani*
*(Vigna radiata*L.)	Leaf spot	*Alternaria alternata*
and Urbean, Arhar,	Seedling blight	*Macrophomina*
Pigeonpea	and root-rot	*phaseolina*
(*Cajanus cajan* L)	Bacterial blight of Mungbean	*Xanthomonas campestris pv. Vignicola*
	Urdbean virus	*ULCV*
Common bean	Anthracnose	*Colletotrichum*
(Phaseolus vulgaris)	Halo blight of bean	*lendemuthianum* *Pseudomonas syringae* pv. *phaseolicola*
Pea (*Pisum sativum*)	*Ascochyta* blight	*Ascochyta pisi*
	Leaf spot	*Alternaria alternata*
	Downy Mildew	*Peronospora pisi*
	Bacterial blight	*Pseudomonas*
	Pea brown mosaic	*syringae* pv. *pisi* *PBMV*
Cow pea *(Vigna*	Anthracnose	*Colletotrichum*
unguicalata L)	Cow pea virus	*dematium ULCV*
Soybean	Purple stain seed	*Cercospora kikuchii*
(*Glycen max* L)	Pod and stem blight	*Diaporthe phaseolorum* var *sojae*
	Charcoal rot	*M. phaseolina*
	Anthracnose	*Collectorichum*
	Downy mildew	*truncatum*, *Peronospora*
	Soybean mosaic	*manshurica SMV*

Table 2

Seed mycoflora found associated with seeds of major legume crops in Pakistan, during the season 1994/1995

Crop	No. of samples		Detected fungi	Infection range (%)	Fungi noted but not counted
	Tested	Infected			
Gram	57	15	*Ascochyta rabiei*	0.5-1.5	*Aspergillus* spp.
Chickpea		5	*Botrytis cinerea*	0.5-1.0	*Cladosporium* spp.
(Cicer arietinum)		10	*Cephalosporium* spp.	3.0-10.0	*Penicillium* spp.
		5	*Fusarium equiseti*	0.5-3.0	*Mucor* spp.
		30	*F. moniliforme*	0.5-20.0	*Rhizopus* spp.
		7	*F. oxysporum*	0.5-4.5	
		20	*F. semitectum*	1.0-22.5	
		6	*F. solani*	1.5-6.5	
		1	*Macrophomina phaseolina*	1.0	
		5	*Myrothecium roridum*	0.5-1.0	
		3	*Phoma* spp.	0.5-1.0	
		1	*Rhizpoctonia* spp.	0.5	
		3	*Verticillium* spp.	0.5-1.0	
Lentil, Masoor	26	7	*Ascochyta lentis*	0.5-8.0	*Aspergillus* spp.
(Lens culinaris)		5	*F. oxysporum*	0.5-4.0	*Rhizopus* spp.
		10	*F. semitectum*	1.0-7.0	*Chaetomium* spp.
		3	*Phoma* spp.	0.5-5.0	
Mungbean	20	7	*Alternaria alternata*	1.0-7.0	*Aspergillus* spp. *Mucor* spp.
(Vigna radiata)		2	*Drechslera tetramera*	0.5-1.0	*Rhizopus* spp.
		4	*F. moniliforme*	0.5-2.0	*Trichoderma* spp.
		2	*F. oxysporum*	0.5-1.0	
		5	*F. semitectum*	1.0-3.0	
		2	*F. solani*	0.5-1.0	
		2	*M. phaseolina*	0.5-1.0	
Pea	15	9	*A. alternata*	1.0-6.0	*Aspergillus* spp.
(Pisum sativum)		7	*Ascochyta pisi*	0.5-3.5	*Cladosporium* spp.
		5	*F. moniliforme*	1.0-3.0	*Mucor* spp. *Penicillium* spp.
		1	*F. osysporum*	1.0	*Rhizopus* spp.
		8	*F. semitectum*	1.0-7.0	
Soybean	27	10	*A. alternata*	0.5-5.0	*Aspergillus* spp.
(Glycin max)		5	*Cercospora kikuchii*	0.5-3.0	*Cladosporium* spp.
		2	*Colletotrichum dematium*	0.5-2.0	*Penicillium* spp.
		8	*C. trunctatum*	0.5-5.0	
		1	*Curvularia lunata*	1.0	
		2	*F. equiseti*	0.5-5.0	
		5	*F. moniliforme*	0.5-5.0	
		2	*F. oxysporum*	0.5-3.0	
		3	*F. semitectum*	1.0-2.5	
		4	*M. phaseolina*	0.5-2.5	
		2	*Myrothecium verrucaria*	1.0	
		1	*Phoma* spp.	0.5-2.0	
		2	*Phomopsis sojae*	1.0-2.0	
		3	*Stemphylium botrysum*	1.0-2.0	

wilting, plugging and defoliation of mature plants and ultimately is responsible for a loss of vigour and yield reduction (Ilyas 1990).

Fifteen pea seed samples were assayed to determine the presence of five seed-borne fungal pathogens. The seed infection percentage of these pathogens ranged from 1.5 to 7.0 per cent. *A. alterna* responsible for *Alternaria* leaf blight, was isolated in nine seed samples. *Ascochyta pisi* was detected up to 3.5 per cent and reported to cause primary infection which leads to damping off (Molinero *et al* 1993). Major infection due to *Mycosphaerella pinodes* (sexual stage of *A pisi*) can reduce yield up to 50-75 per cent (Sheridian 1973). *Ascochyta* diseases are wide spread in Europe and temperate regions (Tivoli *et al* 1992). Infection potential and wide distribution of this fungus necessitate testing of all the seed consignments meant for import/export. Three *Fusarium* spp (*F. moniliforme, F. oxysporum* and *F. semitectum)* were detected in pea seeds ranging from 1.0-7.0 per cent. *F. oxysporum* spp is known to be responsible for causing wilt (Hagedorn 1984). This fungus was isolated from root knot nemadote (*Meloidogyne incognita*) damaged pea roots (Anwar *et al* 1994). These observations warrant a synergistic relationship between *M. incognita* and *F. oxysporum* which needs further study to confirm this relationship. All the seed-borne fungi detected in present study, were also isolated from pea seed by Khanzada *et al* (1989) and Wahid and Ali (1990).

A total of fourteen fungi were detected from 27 soybean seed samples. The dominating fungi, *A. alternata* and *Collectotrichum* spp were isolated from 0.5-5.0 per cent each in 10 soybean seed samples out of 27 samples tested. *A. alternata* infected seeds become rotted (Mishra and Praksh 1975; Anwar *et al* 1995). The seed infected with *Collectotrichum dematium* and *C. truncatum* failed to germinate or germinated seedlings died due to damping-off affect of the fungus (Anwar *et al* 1995). *Cercospora kikuckii* was isolated up to 3.0 per cent in five seed samples. It is reported that infected seeds germinate late as compared to non-infected seeds and produce diseased seedlings (Hepperly and Sinclair 1981). *M. phaseolina*, the cause of charcoal rot and *Phomapsis sojae*, the cause of pod and stem blight, were isolated up to 2.5 percent from soybean seeds. It was observed during blotter test that infected seeds failed to germinate or produce seedlings that may die soon after emergence. Hepperly and Sinclair (1978) observed that infected seelings under severe conditions lead to poor crop stand and reduction in yield.

All the four species of *Fusarium* which were found to be associated with soybean seeds are pathogenic as they cause seedling disease complex (Sahnaran and Gupta 1972; Summer and Minton 1987). Infection percentage ranged from 0.5 to 8.0 *F. oxysporum* causes root rot and wilt of seedlings and young plant, which become very severe in association with *Rhizoctonia solani* (Datnoff and Sinclair 1998) and *M incognita* (Goswami and Agrawal 1978) due to synergistic relationship with these two pathogens. This inter-relationship under our local conditions need further investigation. *Myrothecium verrucaria, Phoma* spp and *Stemphylium botrysum* were recorded in lower percentage. These fungi were also reported by Anwar *et al* (1995) but so far, no field disease was observed to be caused by these fungi in Pakistan.

In addition to these 24 fungi detected in this study, seven fungi, generally not considered field fungi in many legume crops, were also observed but not counted. These fungi are known to be involved in deterioration of seeds and production of *mycotoxins* (Milner and Geddes 1964; Dhingra *et al* 1973; Ellis *et al* 1974; Hashmi and Thrane 1990).

Conclusion

This study revealed that *A. alternata, Ascochyta* spp, *Colletotrichum* spp, *Fusarium* spp and *M. phaseolina* are found associated with legume seed samples. They are also known as common pathogenic fungi in all legume crops. Therefore, these fungi need special attention in producing disease free seeds for healthy crops and movement of seeds during international germplasm exchange and grain trading.

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