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Diversity of Seed-Borne Mycoflora in Relation to Different Chickpea Varieties in Uttar Pradesh, India

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A B S T R A C T

Chickpea (*Cicer arietinum*.) is grown extensively in India as an important legume crops. A quality seed is required to increase the production of chickpea qualitatively and quantitatively. Hence it is imperative that seeds must be tested for seed-borne mycoflora (seed-health), before they are sown in the field. In the context, a study was conducted to work out the diversity of seed-borne mycoflora in relation to different chickpea varieties. Seeds of ten varieties of chickpea were subjected to Standard Purity Work Board technique and Standard Blotter Paper Method technique (ISTA 2007) for the detection and isolation of seed-borne mycoflora. After incubation of seeds, fungi developed on each seed were examined. Standard Purity Work Board technique results revealed that among the different varieties tested, variety KGD 11 was rated as resistant to seed-born mycoflora with 370 healthy seeds, 11 deformed seeds, 10 wrinkled seeds, 30 discoloured seeds and 13 fruiting body seeds followed by Awarodhi with 364 healthy seeds, 25 deformed seeds, 18 wrinkled seeds, 36 discoloured seeds and 21 fruiting body seeds. Variety JG 315 and PUSA 362 was rated as susceptible in respect to physical seed abnormalities with 169 healthy seeds, 58 deformed seeds, 107 wrinkled seeds, 231 discoloured seeds, 72 fruiting body seeds and 203 healthy seeds, 63 deformed seeds, 85 wrinkled seeds, 197 discoloured seeds, 68 fruiting body seeds, respectively. It was evident with the experimentation that chickpea wilt disease causing pathogen *Fusarium oxysporum* which is the most yield limiting factors in chickpea was found associated with the seeds of all the widely adopted chickpea varieties *viz*., L 550, BG 3004, Pragati, BGD 72, JG 11, RSG 807, PUSA 362 and JG 315 except two varieties namely KGD 11 and Awarodhi. Variety KGD 11 was found more tolerant for the per cent infection of seed that is 10.00 % under pre-treated (PT) condition and 21.50 % under untreated (UT) condition with 99 % germination (UT), 100 % germination (PT) and five seed associated fungi *viz.,Alternaria alternata*, *Curvularia lunata*, *Fusarium semitectum*, *Aspergillus niger* and *Rhizopus arrhizus*.

Introduction

Pulses play an important role in Indian agriculture to the maintaining soil fertility and supplying protein to the large vegetarian population of the country. Nearly 11,000 species of legumes are known and many are of importance as industrial, medicinal of food plants. Considering the nutritional, agronomical and industrial value of pulses and yield of legumes the present study is aimed to work out the diversity of seed-borne mycoflora in relation to different chickpea varieties in Uttar Pradesh.

Chickpea (*Cicer arietinum* L.) commonly known as 'gram' is the most important legume grown in India and grown over 6.66 m ha of land (Kochhar, 2009). It has been found to be attacked by 172 pathogens including 67 species of fungi (Nene *et al.,* 1996). Chickpea suffers from a large number of fungal diseases namely Ascochyta blight (*Ascochyta rabiei*), Fusarium wilt (*F. oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) Alternaria blight (*Alternaria alternata*), Colletotrichum blight (*Colletotrichum dematium*), Stemphylium blight (*Stemphylium sarciniforme*), powdery mildew (*Leveillula taurica*), Sclerotinia stem rot (*Sclerotinia sclerotiorum*), wet root rot (*Rhizoctonia solani*) and foot rot (*Operculella padwickii*) (Singh and Sharma, 2005; Dubey *et al.,* 2007).

Out of many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the production potential of its genetic levels. Some of these fungi produce afla-toxins which damage the liver and induce carcinogenic, mutagenic and teratogenesis (Pereyra *et al.,* 2008). Therefore, control of seed-borne fungi is extremely important. Therefore, the study was undertaken to investigate diversity of incidence of seed-borne fungi associated with chickpea seeds to protect these seeds from fungal diseases.

Materials and Methods

The study was conducted to work out the distribution of pathogenic mycoflora associated with the seed of five most widely cultivated varieties of chickpea in Directorate of Seed Research, Mau and National Bureau of Agriculturally Important Microorganisms, Mau. The material used and methods followed are as under. Seed samples of nine most widely cultivate d varieties of chickpea *viz.,* L 550, BG 3004, KGD11, Pragati, Awarodhi, BGD 72, JG 11, RSG 807 and PUSA 362 and one susceptible standard check for *Fusarium oxysporum* f. sp. *ciceri* namely JG 315 were collected from AICRP on National Seed Project (Crops) of Directorate of Seed Research, Mau in sterilized polythene bags. Composite sample of each varieties were prepared by following the method described by Paul and Neergard (1977). The collected seeds were preserved at 05 C to avoided further contamination and for subsequent experimentation.

Standard purity work board technique

All the samples were subjected to the visual inspection by naked eye, under stereoscopic binocular microscope added with cool-light condition and by modified purity work board. Seeds with black point disease can be easily differentiated by the pronounced appearance of brown to dark brown or blackish discoloured areas. Symptoms on the seeds, having elliptical to oblong lesionswith lighter in center represent the presence of *Bipolaris sorokiniana*. *Curvularia lunata* resulted brown coloration of seed coats. *Alternaria alternata* was identified by the presence of dark brown and long conidial chain on incubated seed surface.

Standard blotter paper method technique

The stored seed samples of chickpea were subjected to Standard Blotter Paper Method (ISTA, 1996), a seed associated mycoflora detection techniques for detection of seed-borne pathogen. Two hundred seeds of all the samples were used in one replication under CRD of Standard Blotter Paper Method, each for un-treated (UT) seeds and pre-treated (PT) seeds after treatment with 0.1 % mercuric chloride ($HgCl_2$) solution. The seeds were placed in petridishes containing three layers of moistened blotter with defined quantity of streptopenicillin to avoid growth of bacteria as contamination. The petridishes were incubated at 24 ± 1 ^{0}C under 24 hours alternating cycle of light and darkness for 7 days in the incubation chamber. The single spore isolation techniques were followed to purify the culture for further studies.

Potato Dextrose Agar (PDA) and CzapakDox Agar (CDA), and Spezieller Nahrst of farmer Agar (SNA) medium as per described by Nirenberg 1976 were use to maintain the mycoflora. The sterilized media was poured into 9 cm diameter, petridishes. *Fusarium spp*. were placed in the centre of the plates and was incubated at 25± 1 ^{0}C.Sporulating cultures were obtained by placing autoclaved filter paper strip at the periphery of actively growing colonies. The isolated strains were identified after growth on CzapakDox Agar and Potato Dextrose Agar (Lacaz, *et al.*, 1991). Identification keys developed byBaijal and Mehrotra, 1980; Bissett, 1991; Domsch, *et al.*, 1993; Pitt, 1998; Hammil, 1970; Raper and Fennell, 1975; Refai, 1969; Samuels *et al.*, 1998; and Sutton, 1980were used to identify the seed-borne mycoflora.

Results and Discussion

The experiments were conducted to work out the diversity/load of seed-borne mycoflora on commercially cultivated chickpea seeds of ten different varieties. Seed samples of all the commercially cultivated varieties of chickpea were collected in sterilized polythene bags and stored under low temperature. Further the samples were analyzed for the associated mycoflora by employing the standard techniques with pre-treated and untreated seeds. Microscopic characteristics including colour, texture, appearance and diameter of the fungal colonies were compared for the identification of mycoflora.

Detection of seed health by Standard Purity Work Board Technique

The commercially cultivated chickpea seed samples of all the varieties were analyzed on standard purity work board under dry conditions. Healthy and diseased seeds were sorted out by visual examination. Diseased seeds were categorized as deformed, shrivelled, deshaped and discoloured seed. All the samples were examined for the associated mycoflora in which the dry seeds were subjected to the visual inspection by necked eye, under stereoscopic binocular microscope added with cool-light condition and by modified purity work board. Seeds with wilt disease can be easily differentiated by the pronounced appearance of profuse mycelial growth of the *Fusarium oxysporum* f. sp. *Cicero* fungus on seed surface and pink discolourations. Seeds infected with Anthracnose disease of chickpea produces symptoms of *Colletotrichum* necrotic lesions on seed coat and also shrivelled in shape, resulting significant dockage on seed. Chickpea *Ascochyta* blight infected seeds exhibited black, brown or grey necrotic lesions on the seed-coat due to presence of *Ascochyta rabiei* fungus. Graymold disease of chickpea caused by *Botrytis cinerea* may be confirmed in the presence of gray/brown lesions on seeds, and are often covered with a gray mass of fungal hyphae and spores. The

size of infected seeds was also reduced up to the significant level and badly discoloured. The associations of above mentioned mycoflora were finally confirmed after plating of seeds (stored sample) by using standard blotter method. The results of other examinations by using Standard Purity Work Board Technique (Table 1), revealed that maximum fruiting body seed was recorded 96.00 seeds with the variety Pragati followed by 72.00 seeds (JG 315), 68 seeds (PUSA 362) and 28 seeds with variety BG 3004. The minimum fruiting body seed was recorded 13 seeds with the variety KGD 11. Two more varieties namely RSG 807 and L 550 were found statistically at par in respect to minimum fruiting body seeds as 14 seeds and 17 seeds, respectively.

The difference between maximum and minimum fruiting body seed was statistical significant for determining the resistant and susceptible variety on the basis of fruiting body seeds. The minimum discoloured seed was recorded 30 seeds with the two variety KGD 11 and statistically at par with the variety Awarodhi with 36 seeds and variety L 550 with 39 seeds. Two more variety JG 11 (42 seeds) and BGD 72 (49 seeds) also recorded less than 50 discoloured seeds and statistically at par with the variety KGD 11. Whereas maximum discoloured seed was observed 231 seeds with the variety JG 315 followed by 197 seeds with the variety PUSA 362 and 187 seeds with the variety Pragati. The difference between maximum and minimum discoloured seeds was also found statistical significant for determining the resistant and susceptible variety on the basis of discoloured seeds. The maximum wrinkled seed was recorded 107 seeds with the variety JG 315 followed by 85 seeds (PUSE 362) and 81 seeds (Pragati). Whereas the minimum wrinkled seed was recorded 10 seeds with the variety KGD 11 followed by 11 seeds (L 550) and 13 seeds (BGD 72). The maximum and minimum differences of wrinkled seed in the studied varieties were also statistically significant to each other. In case of deformed seed, 63 seeds were found as maximum deformed seeds with the variety PUSA 362 followed by 58 seeds (JG 315) and 56 seeds (Pragati) and statistically non-significant with the minimum deformed seed of 09 with the variety RSG 807 followed by 11 seeds (KGD 11) and 14 seeds (L 550).

The maximum healthy seeds of 370 was recorded with the variety KGD 11 followed by 364 seeds (Awarodhi) and 361 seeds (L 550) and statistically significant with the minimum of 169 healthy seeds with the variety JG 315 followed by PUSA 362 (203 seeds) and Pragati (213 seeds).Overall under the dry seed examination (a non-injuring seed health test) method of detection, variety KGD 11 was rated as resistant to seed-born mycoflora with 370 healthy seeds, 11 deformed seeds, 10 wrinkled seeds, 30 discoloured seeds and 13 fruiting body seeds followed by Awarodhi with 364 healthy seeds, 25 deformed seeds, 18 wrinkled seeds, 36 discoloured seeds and 21 fruiting body seeds. Variety JG 315 and PUSA 362 was rated as susceptible in respect to physical seed abnormalities with 169 healthy seeds, 58 deformed seeds, 107 wrinkled seeds, 231 discoloured seeds, 72 fruiting body seeds and 203 healthy seeds, 63 deformed seeds, 85 wrinkled seeds, 197 discoloured seeds, 68 fruiting body seeds, respectively.

Detection of seed health by standard blotter paper method technique

Among the ten most widely cultivated varieties of chickpea seeds tested, the variety KGD 11 was found more tolerant for the per cent infection of seed that is 10.00% under pre-treated (PT) condition and 21.50 % under untreated (UT) condition with 99 % germination (UT), 100 % germination (PT) andfive seed associated fungi *viz.*, *Alternaria*

alternata, Curvularia lunata, Fusarium semitectum, Aspergillus niger and *Rhizopus arrhizus* (Table 2). Except susceptible standard check for *Fusarium oxysporum*f. sp. *ciceri* variety JG 315, variety Pragati was found more susceptible for the per cent infection of seeds in both PT (42.00 % seeds) and UT (55.60 % seeds) condition with 99 % germination (UT), 100 % germination (PT) and 12 seed associated fungi *viz.*, *Alternaria alternata, Botrytis cinerea, Colletotrichum truncatum, Ascochyta rabiei, Fusarium oxysporum, F. semitectum, Aspergillusflavus, A. niger, A. fumigatus, Mucor*species, *Penicillium notatum, and Rhizopus arrhizus*. The variety Awarodhi and JG 11 was also rated as tolerant for the per cent infection of seed. Awarodhi exhibited 11.25 % (PT)and 24.74 % seed infection with 93.00 % germination (UT), 99.00 % germination (PT) and six seed associated fungi *viz.*, *Alternaria alternata, Curvularia lunata, Fusarium semitectum, Aspergillus niger, A. flavus* and *Rhizopus arrhizus* whereas JG 11 exhibited 14.35 % (PT) and 30.56 % seed infection with 91.00 % germination (UT), 99.00 % germination (PT) and nine seed associated fungi *viz., Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Fusarium moniliforme, M. phaseolina, Trichoderma harzanium, A. niger, Mucor*species, *Rhizopus arrhizus.* Chickpea *Ascochyta* blight caused by *Ascochyta rabiei* was found associated with the seeds of only two varieties namely JG 315 and Pragati whereas one of the most widely spread and destructive diseases of commonly grown cultivars of *chickpea* wet root rot caused by *Rhizoctonia solani* pathogen was found associated with the seeds of three varieties namely JG 315, RSG 807 and BGD 72. *Macrophomin aphaseolina* pathogen causing most devastating disease of charcoal rot and root rot diseases in various economically important crops was found associated with the seeds of two varieties namely JG 315 and RSG 807. It was evident with the experimentation that chickpea wilt disease causing pathogen *Fusarium oxysporum* which is the most yield limiting factors in chickpea was found associated with the seeds of all the widely adopted chickpea varieties *viz*., L 550, BG 3004, Pragati, BGD 72, JG 11, RSG 807, PUSA 362 and JG 315 except two varieties namely KGD 11 and Awarodhi.

Maximum of hundred per cent germination under pre-treated condition of seeds was recorded with the variety KGD 11 whereas 99.00 % germination recorded with the three vanities namely BG 3004, Awarodhi and BGD 72. Minimum 52.00 % seed germination under pre-treated condition with the variety JG 315 followed by 58.00 % with the variety PUSA 362.

Un-treated seed condition revealed minimum of 34.00 % germination with the variety JG 315 followed by 48.00 % with the variety PUSA 362 whereas under pre-treated seed condition recorded minimum of 52.00 % germination with the variety JG 315 followed by 58.00 % with the variety PUSA 362.

Seed-borne diseases are regarded as major limiting factor for chickpea production. Healthy and pathogen free seed is the basic key for disease free crop. Seed-borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure 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). Experimental result showed that saprophytic fungi viz., *A. flavus* and *A. niger*were predominant among the fungi isolated. Such similar reports have been made by Rasheed *et al.,* (2004) 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) (Fig. 1–4).

Table.1 Standard dry seed examination for detection of FBS, DS, WS, DFS and HS of ten commercially cultivated chickpea varieties

S.No.	Variety	SE	FBS	DS	WS	DFS	HS
1	L 550	400	017	039	011	014	361
2	BG 3004	400	028	058	021	023	342
3	KGD 11	400	013	030	010	011	370
4	Pragati	400	096	187	081	056	213
5	Awarodhi	400	021	036	018	025	364
6	BGD 72	400	026	049	013	020	351
7	JG 11	400	022	042	020	019	358
8	RSG 807	400	014	064	016	009	336
9	PUSA 362	400	068	197	058	063	203
10	***JG 315	400	072	231	107	058	196
Mean			37.7	93.3	35.5	39.8	309.40
SEm±			0.71	1.70	1.16	1.21	10.3
CD $_{0.01}$			2.07	4.83	3.37	3.46	30.6

SE = Seeds examined; FBS = Fruiting body seed; DS = Discoloured seed; WS = Wrinkled seed; DFS = Deformed seed;HS = Healthy seed

Table.2 Seeds associated mycoflora of commercially cultivated chickpea varieties by Standard Blotter Paper Method before and after surface sterilization

S. N.	Varieties	Infection (%)		Germination (%)		Pathogenic mycoflora associated with seed
		UT	PT	UT	PT	
1	L 550	25.75	14.75	94.00	98.00	*Alternaria alternata, Colletotrichum truncatum, Helminthosporiumsativum, Fusarium oxysporum, F. semitectum, Aspergillus flavus, A. fumigatus, Mucor*species and *Rhizopus arrhizus*.[9]
2	BG 3004	25.75	15.00	91.00	99.00	*Alternariaporri, Curvularia lunata, Helminthosporiumsativum, Fusarium oxysporum, F. semitectum, Aspergillus flavus, A. niger and Mucor*species.[8]
3	KGD11	21.50	10.00	99.00	100	*Alternaria alternata, Curvularia lunata,Fusarium semitectum, Aspergillusniger*and *Rhizopus arrhizus*. [5]
4	Pragati	55.60	42.00	51.00	63.00	*Alternaria alternata, Botrytis cinerea, Colletotrichum truncatum, Ascochytarabiei, Fusarium oxysporum, F. semitectum, Aspergillus flavus, A. niger, A. fumigatus, Mucor*species, *Penicillium notatum, and Rhizopus arrhizus*. [12]
5	Awarodhi	24.75	11.25	93.00	99.00	*Alternaria alternata, Curvularia lunata, Fusarium semitectum, Aspergillusniger, A.*

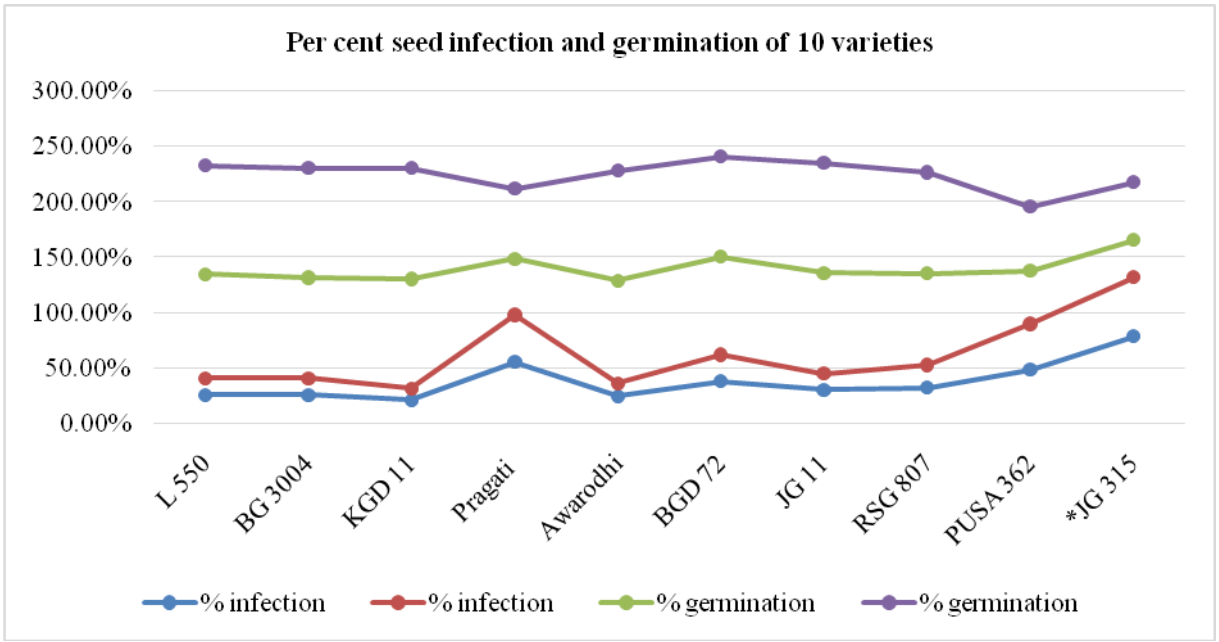
						flavus and *Rhizopus arrhizus*. [6]
6	BGD 72	38.24	23.50	89.00	90.00	*Alternaria alternata, Helminthosporium sativum, Fusarium oxysporum, F. semitectum, Colletotrichum truncatum, R. solani Penicillium notatum, Rhizopus arrhizus, Rhizopus sp. and M. phaseolina.* [10]
7	JG 11	30.50	14.35	91.00	99.00	*Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Fusarium moniliforme, M. phaseolina, Trichoderma harzanium, A. niger, Mucor* species*, Rhizopus arrhizus.* [9]
8	RSG 807	32.45	20.00	83.00	91.00	*Alternariaporri, Fusarium oxysporum, Fusarium moniliforme, R. solani, M. phaseolina Penicillium notatumand Aspergillus flavus.* [7]
9	PUSA 362	48.75	41.00	48.00	58.00	*Alternaria alternata, Helminthosporiumsativum, Botrytis cinerea, Colletotrichum truncatum,Fusarium moniliforme, Fusarium oxysporum, F. semitectum, Trichoderma harzanium, Aspergillus flavus, Mucor* species *and Rhizopus arrhizus*. [11]
10	***JG 315	78.50	53.00	34.00	52.00	*Alternaria alternate, Helminthosporium sativum, Botrytis cinerea, Colletotrichum truncatum, Ascochytarabiei, Fusarium oxysporum, F. semitectum, Fusarium moniliforme, R. solani, M. phaseolina, Trichoderma harzanium, Aspergillus flavus, A. niger, Mucor* species*,Penicillium notatum, and Rhizopus arrhizus.* [16]
Mean		38.18	24.98	77.30	84.90	
SEm±		2.57	1.27	3.63	4.59	
CD $_{0.01}$		7.63	3.74	10.8	13.6	

* JG 315 is used as standard check for *F. oxysporum* f. sp. *ciceri*
PT= Pre-treated condition of seed
UT= Un-treated condition of seed

Fig.1 Shrivelled discoloured seed of chickpea	**Fig.2** Fungal coating (*Botrytis)* in chickpea seed	**Fig.3** Fungal coating (*Fusarium)* on Chickpea seed



Fig.4 Effect of varieties on diversity of mycoflora and its impact on infection and germination in Sub-tropical India



These species have been reported to reduce the germination of seed and damage the seeds in storage (Christensen, 1973). 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 and Ghaffar (1991). It was also observed that surface sterilization of seed reduced the infection of *A. flavus* and *A niger* and increased the incidence of pathogenic fungi. Such similar report has 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. *A. flavus* mycotoxins producing fungi 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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