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RESEARCH ARTICLE

MYCOFLORA ASSOCIATED WITH FARMER STORED SEEDS OF CHICKPEA AND PIGEON PEA COLLECTED FROM SATARA

*Geetha Menon

Postgraduate Department of Botany, R. K. Talreja College, Ulhasnagar, Maharashtra

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ABSTRACT

Seeds harbor a considerable load of several fungal inoculums, responsible for various seed-borne diseases and damage. Cereals and pulses constitute the regular basic food of the masses that supply both carbohydrates and protein in their regular diet. Seeds of two pulses *Cicer arietinum* L. (Chickpea) & *Cajanus cajan* (L.) Millsp (Pigeon pea) were collected from the farmers of Satara (Maharashtra), India and studied for the associated fungal flora using standard Blotter method, Agar plate (Czapek Dox medium) and Seed Washing Methods. On the unsterilized seeds of *Cicer arietinum* L. (Chickpea) twenty three species of fungi belonging to ten genera and on the seeds of *Cajanus cajan* (L.) Millsp (Pigeon pea) twenty species belonging to two genera were observed. The most commonly isolated genera were *Aspergillus*, *Alternaria*, *Fusarium* and *Penicillium* while other prominent fungi detected on the pulse crops were *Cladosporium*, *Curvularia*, *Verticillium* and *Drechslera*.

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INTRODUCTION

India is the largest producer as well as the consumer of pulses. In India, pulses can be produced with a minimum use of resources and hence are less expensive and can be cultivated as an inter-crop and also as mixed crop. Mostly the cultivation of pulses are under rain fed conditions and therefore do not require intensive irrigation facility. Pulses are considered as the principal source of protein and contain around 25% protein, twice the protein contained by wheat and thrice the amount in rice. Chickpea (*Cicer arietinum* L.) is the most important pulse crop; and India is the largest producer and consumer of chickpea in the world. Chickpea, the cheapest source of protein is a prime pulse crop of Rabi and is the inseparable part of the daily diet of every Indian. It also has carbohydrates, Zinc, folic acid (Jukanti *et al.*, 2012). *Cajanus cajan* (L.) Millsp (Pigeonpea) commonly known as tur, is a very old crop of this country. It is the second most important pulse crop in the country after Gram. It is a rich source of protein and supplies a major share of the protein requirement of the vegetarian population of the country. Seeds of Pigeon pea are also rich in iron, iodine, essential amino acids like lysine, tyrosine, cystine and arginine (Arun and Mathew, 1991). The significance of sustainable agricultural production is hidden in the use of quality seed and it determines the limits

of productivity to be realized in a given cropping system. Seed-borne diseases have been found to affect the growth and productivity of crop plants. Presence or absence of seed borne fungi on seed surface is one of the important aspects that determine the quality of seed (Weber *et al.*, 2001). In view of this, the present investigation was aimed at detecting seed-borne fungal pathogen on farmer saved seeds of Chickpea and Pigeonpea from the district of Satara in Maharashtra (state), India.

MATERIALS AND METHODS

Satara is the one of the districts of Maharashtra, located at 16^o.50' to 18^o.10' N latitude and 73^o.45' to 75^o.0' E longitude. Stored Pulse seed samples of *Cajanus cajan* (L.) Millsp. (Pigeon pea) and *Cicer arietinum* L. (chickpea) were collected from farmers belonging to different villages in and around Satara. The seed samples of each crop plant were mixed to form a composite sample and stored in sterilized airtight containers (Neergard, 1973). In order to isolate the endophytic (internal) seed mycoflora, seeds were treated with 0.1% solution of mercuric chloride (HgCl₂) for two minutes, thoroughly washed thrice with sterile distilled water. Both unsterilized (seeds without any such pretreatment) and surface sterilized seeds were separately placed on agar plates and employed for the study of total (internal and external) seed mycoflora. The occurrence of different seed borne fungi was detected by employing Standard Blotter Method (SBM), Agar

*Corresponding author: Geetha Menon,

Postgraduate Department of Botany, R. K. Talreja College, Ulhasnagar, Maharashtra

Plate Method (APM) using Czapek Dox medium and Seed Washing Method (SWM). All materials except seeds, which were used in this experiment, were sterilized using 70% ethyl alcohol (ISTA, 1966). The fungal colonies emanating from seeds and seed washing were observed on 8th day after incubation (22±2° C, under the alternative cycles of 12hrs. of natural light and darkness), five replication were maintained for confirmation of fungal colonies. During the present investigation ectophytic as well as endophytic mycoflora associated with all the seeds selected were screened to study the association. The exposed seeds were examined on the 9th day under stereo binocular microscope for the presence of seed borne fungi. The incident fungi both endophytic and ectophytic found on the seeds were recorded and the isolated fungi were identified with the help of the keys, monograph and literature provided by Raper and Fennell (1965); Booth (1971); Ellis (1971) & Barnett and Hunter (1972). Germination percentage of each test species was also recorded and the data were statistically analyzed by Student's t test using XLSTAT program.

RESULTS AND DISCUSSION

Germination studies of the two test seed samples (chickpea and pigeon pea) both surface sterilized (treated) and nonsterilized were conducted in petriplates (Table no.1). The germination percentage of both the treated samples were comparatively higher than the untreated samples though the difference in chickpea was significant (t= 49.045; P= 0.05)

while in pigeonpea the difference was insignificant (t=40.036; at P= 0.05). In the present study three different methods were employed to isolate the seed mycoflora. The untreated seeds of chickpea were loaded with twenty three species of mycoflora belonging to 10 genera while on the treated seeds only nine species belonging to six genera were detected. The ten genera recorded on the chickpea were *Aspergillus*, *Alternaria*, *Fusarium*, *Rhizopus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Mucor*, *Penicillium* and *Verticillium*. The genus *Aspergillus* was represented by nine species, while *Alternaria* and *Fusarium* were represented by three species each, genus *Rhizopus* with two species. Other genera like *Cladosporium*, *Curvularia*, *Drechslera*, *Mucor*, *Penicillium*, and *Verticillium* were represented by one species each (Table no.2). There were twenty five species of fungi belonging five genera associated with the untreated pigeonpea seeds while with the treated seeds there were eleven species belonging to only two genera of fungi of which *Aspergillus* was predominant. The dominant fungal flora on the untreated Pigeonpea was *Aspergillus* with eighteen species, in addition to *Mucor abundans*, *Fusarium* (*F. oxysporum*, *F. moniliforme* & *F. solani*), *Rhizopus stolonifer* & *Curvularia lunata*; while on the treated seeds, *Aspergillus* (ten species) and *Penicillium oxalicum* were detected (Table 3). Occurrence of different species of *Aspergillus* on both the untreated pulse seeds, Chickpea and Pigeonpea samples were very high. The Blotter method was useful to detect only three genera of mycoflora on the untreated chickpea seeds viz *Aspergillus*, *Alternaria* and *Drechslera*; with two species of genus *Aspergillus* (*A. niger* & *A. oryzae*) one species of

Table 1. Germination Percentage of Surface Sterilized and Unsterilized Seeds from Satara

S.No	Germination Percentage in seeds			
	Chickpea		Pigeonpea	
	Unsterilized	Sterilized	Unsterilized	Sterilized
1.	53.33	76.66	43.33	63.33
2.	56.66	73.33	46.66	66.66
3.	53.33	70.00	43.33	66.66
Mean	54.44	73.33	44.44	65.55

Table 2. Mycoflora Associated With Chickpea Seeds from Satara

S. No.	Name Of Fungus	Unsterilized	Sterilized
•	<i>Alternaria dianthicola</i> Neergaard.	+	+
•	<i>A. tenuis</i> Auct.	+	-
•	<i>A. tenuissima</i> (Kunze ex Pers) Wilts.	+	-
•	<i>Aspergillus candidus</i> Link ex Fries.	+	-
•	<i>A. flavus</i> Link ex Fries.	+	+
•	<i>A. flavipes</i> (Bain. and Sart.) Thom and Church.	+	+
•	<i>A. fumigatus</i> Fresenius	+	-
•	<i>A. niger</i> VanTieghem.	+	+
•	<i>A. niveus</i> Blochwitz.	+	-
•	<i>A. oryzae</i> (Ahlburg in Korschelt) Cohn.	+	+
•	<i>A. parasiticus</i> Speare.	+	-
•	<i>A. terreus</i> Thom.	+	-
•	<i>Cladosporium cladosporioides</i> (Fr.) de Vries.	+	-
•	<i>Curvularia lunata</i> (Wakker) Boedijn.	+	+
•	<i>Drechslera australiensis</i> . (Bugni.) Sub. & Jain.	+	-
•	<i>Fusarium oxysporum</i> Schl.ex Fries.	+	+
•	<i>F. moniliforme</i> Schleldon	+	-
•	<i>F. solani</i> (Mart.) Sacc.	+	-
•	<i>Mucor abundans</i> Povah.	+	-
•	<i>Penicillium purpurenium</i> Stoll.	+	-
•	<i>Rhizopus nodosus</i> Namyslowski.	+	-
•	<i>R. stolonifer</i> (Her.ex Link) Lind.	+	+
•	<i>Verticillium</i> sp.	+	+

Table 3. Mycoflora Associated with Pigeonpea Seeds from Satara

S. No	Mycoflora	Unsterilized	Sterilized
•	<i>Aspergillus alliaceus</i> Thom and Church.	+	-
•	<i>A. amstelodami</i> (Mang) Thom and Church.	+	+
•	<i>A. chevalieri</i> (Mang) Thom and Church	+	+
•	<i>A. flavus</i> Link ex Fries.	+	-
•	<i>A. flavipes</i> (Bain. and Sart.) Thom and Church.	-	+
•	<i>A. fresenii</i> Subram.nom.nov.	+	-
•	<i>A. insecticola</i> Subram. nom. nov.	+	+
•	<i>A. japonicus</i> Saito.	+	+
•	<i>A. niger</i> Van Tieghem.	+	+
•	<i>A. niveus</i> Blochwitz.	+	-
•	<i>A. oryzae</i> (Ahlburg in Korschelt) Cohn.	+	+
•	<i>A. parasiticus</i> Speare.	+	-
•	<i>A. proliferens</i> G. Smith.	+	-
•	<i>A. sydowi</i> Thom and Church.	+	+
•	<i>A. unguis</i> (Emil-Weil and Gaudin) Thom & Raper.	+	+
•	<i>A. ustus</i> (Bainier) Thom and Church.	+	-
•	<i>A. violaceo-fuscus</i> Gasperini	+	+
•	<i>A. wentii</i> Wehmer.	+	-
•	<i>Curvularia lunata</i> (Wakker) Boedijn.	+	-
•	<i>Fusarium oxysporum</i> Schl.ex Fries.	+	-
•	<i>F. moniliforme</i> Schleldon	+	-
•	<i>F. solani</i> (Mart.) Sacc.	+	-
•	<i>Mucor abundans</i> Povah.	+	-
•	<i>Penicillium purpurgenum</i> Stoll.	+	-
•	<i>P.oxalicum</i> Currie and Thom.	-	+
•	<i>Rhizopus stolonifer</i> (Her.ex Link) Lind.	+	-

Table 4. Mycoflora incident on Surface Sterilized and Unsterilized Pulse Seeds from Satara

S. No	Name of Fungus	Mycoflora Incident (%) On Seeds											
		Pigeonpea						Chickpea					
		Endophytic		Ectophytic		Total		Endophytic		Ectophytic		Total	
		US	S	US	S	US	S	US	S	US	S	US	S
1.	<i>Alternaria dianthicola</i>	-	-	-	-	-	-	19.8	11.3	21.2	3.33	40	14.6
2.	<i>A. tenuis</i>	-	-	-	-	-	-	13.8	-	-	-	13.8	-
3.	<i>A. tenuissima</i>	-	-	-	-	-	-	9.1	-	-	-	9.1	-
4.	<i>Aspergillus alliaceus</i>	13.3	1.23	-	-	13.3	1.23	-	-	-	-	-	-
5.	<i>A. amstelodami</i>	11.7	-	17.5	1.3	29.2	1.3	-	-	-	-	-	-
6.	<i>A. awamori</i>	-	-	1.33	-	-	1.33	-	-	-	-	-	-
7.	<i>A. carbonarius</i>	1.23	-	-	-	1.23	-	-	-	-	-	-	-
8.	<i>A. chevalieri</i>	12.6	-	13.5	3.33	26.1	3.33	-	-	-	-	-	-
9.	<i>A. candidus</i>	4.13	1.2	-	-	4.13	1.2	-	-	-	-	-	-
10.	<i>A. flavus</i>	-	-	-	-	-	-	38.6	12.3	42	34.2	80.6	46.5
11.	<i>A. flavipes</i>	-	-	23.7	9.2	23.7	9.2	40	11.5	48.3	13.3	88.3	24.8
12.	<i>A. fresenii</i>	7.2	-	-	-	7.2	-	-	-	-	-	-	-
13.	<i>A. fumigates</i>	-	-	22	6.4	22	6.4	23.68	3.33	28.4	1.23	52.08	4.56
14.	<i>A. insecticola</i>	18.5	3.33	-	-	18.5	3.33	-	-	-	-	-	-
15.	<i>A. japonicas</i>	17.6	1.2	-	-	17.6	1.2	-	-	-	-	-	-
16.	<i>A. lutescens</i>	8.5	-	-	-	8.5	-	-	-	-	-	-	-
17.	<i>A. nidulans</i>	-	-	-	-	-	-	-	-	1.33	-	-	1.33
18.	<i>A. niger</i>	26.4	3.33	28.5	9.6	54.9	12.93	45.5	40.5	39.8	18.5	85.3	59
19.	<i>A. niveus</i>	-	-	-	-	-	-	-	-	4.2	-	-	4.2
20.	<i>A. oryzae</i>	22.8	4.2	19.6	7.2	42.4	11.4	43.3	36.2	35	14.5	78.3	50.7
21.	<i>A. parasiticus</i>	11.3	1.23	-	-	11.3	1.23	-	-	23.3	1.33	23.3	1.33
22.	<i>A. phaeocephalus</i>	-	-	-	-	-	-	-	-	1.33	-	1.33	-
23.	<i>A. proliferens</i>	5.3	1.2	-	-	5.3	1.2	-	-	-	-	-	-
24.	<i>A. quercinus</i>	3.3	-	-	-	3.3	-	-	-	-	-	-	-
25.	<i>A. repens</i>	-	-	1.33	-	1.33	-	-	-	-	-	-	-
26.	<i>A. sydowi</i>	14.4	6.2	-	-	-	6.2	-	-	-	-	-	-
27.	<i>A. terreus</i>	-	-	17.2	1.33	17.2	1.33	-	5.65	-	-	5.65	-
28.	<i>A. unguis</i>	14	5.3	-	-	14	5.3	-	-	-	-	-	-
29.	<i>A. ustus</i>	1.3	-	-	-	1.3	-	-	-	-	-	-	-
30.	<i>A. versicolor</i>	2.7	-	-	-	2.7	-	-	-	-	-	-	-
31.	<i>A. violaceo-fuscus</i>	5.3	-	-	-	5.3	-	-	-	-	-	-	-
32.	<i>A. wentii</i>	9.3	-	-	-	9.3	-	-	-	-	-	-	-
33.	<i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	18.2	15.4	19.8	2.2	38	17.6
34.	<i>Curvularia lunata</i>	-	-	-	-	-	-	-	-	23.6	4.5	23.6	4.5
35.	<i>Drechslera australiensis</i>	-	-	-	-	-	-	12.2	3.4	-	-	12.2	3.4
36.	<i>Fusarium oxysporum</i>	14.5	11.2	16.4	3.3	30.9	14.5	7.6	4.5	19.9	3.33	27.5	7.83
37.	<i>F. moniliforme</i>	-	-	11.2	1.22	11.2	1.22	20.3	16.4	22.6	4.5	42.9	20.9
38.	<i>F. solani</i>	-	-	7.6	-	7.6	-	11.8	5.7	13.6	1.33	25.4	7.03
39.	<i>Mucor abundans</i>	1.8	0.9	2.6	-	4.4	0.9	16.5	14	18.4	3.33	34.9	16.83
40.	<i>P. oxalicum</i>	-	-	-	-	-	-	3.33	2.1	14.8	4.4	18.13	6.5
41.	<i>P. purpurgenum</i>	-	-	3.7	-	3.7	-	-	-	-	-	-	-
42.	<i>R. oryzae</i>	3.33	1.23	-	-	3.33	1.23	-	-	-	-	-	-
43.	<i>R. nodosus</i>	-	-	-	-	-	-	10.8	10.5	18.6	6.4	29.4	16.9
44.	<i>R. stolonifer</i>	-	-	1.3	-	1.3	-	15.5	12.3	11.6	2.33	27.1	14.63
45.	<i>Verticillium</i> sp.	-	-	-	-	-	-	17.6	15.2	19.7	4.33	37.3	19.53

Alternaria (*A. dianthicola*); Genus *Drechslera* (*D. australiensis*) on the chickpea. While the only genus detected on untreated pigeonpea was *Aspergillus* with seven species (*A. amstelodami*, *A. oryzae*, *A. unguis*, *A. niger*, *A. insecticola*, *A. japonicus* and *A. violaceo-fuscus*) (Fig.1). Using the Agar plate method seven genera of endophytic mycoflora could be isolated from the untreated chickpea, including the single genus detected on pigeonpea. The eight genera observed were *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Rhizopus* and *Verticillium*. In chickpea genus *Aspergillus* with nine species (*A. flavipes*, *A. flavus*, *A. fumigates*, *A. candidus*, *A. niveus*, *A. parasiticus*, *A. terreus*, *A. oryzae* & *A. niger*); genus *Alternaria* three species (*A. dianthicola*, *A. tenuis* and *A. tenuissima*); single species of genus *Cladosporium* (*C. cladosporioides*); one species of *Curvularia* (*C. lunata*); three species of *Fusarium* (*F. oxysporum*, *F. moniliforme* and *F. solani*); single species of *Mucor* (*M. abundans*); two species of *Rhizopus* (*R. nodosus* and *R. stolonifer*) and single species of genus *Verticillium* *sps.* On the pigeonpea the endophytic mycoflora detected was *Aspergillus* with five species (*A. amstelodami*, *A. oryzae*, *A. Chevalieri*, *A. sydowi* & *A. flavipes*) (Fig.1).

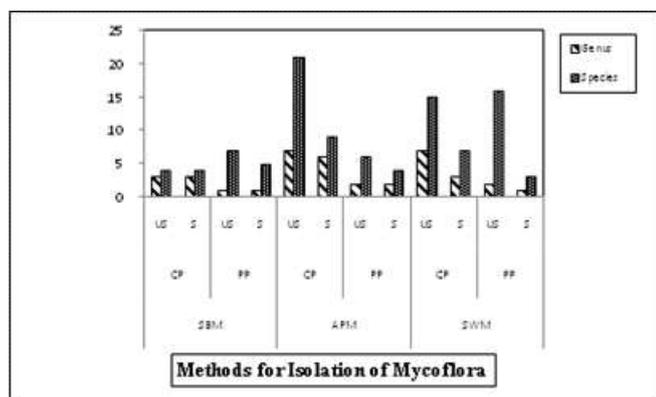


Figure 1. Isolation of seed mycoflora by different methods

The mycoflora isolated by using Seed washing method (SWM) were ectophytic form. On the untreated chickpea seeds seven genera of fungi were detected viz. *Aspergillus* (*A. flavipes*, *A. flavus*, *A. fumigates*, & *A. niger*); *Alternaria* (*A. dianthicola*, *A. tenuis* and *A. tenuissima*); *Cladosporium* (*C. cladosporioides*); *Fusarium* (*F. oxysporum*, *F. moniliforme* and *F. solani*); *Mucor* (*M. abundans*); *Rhizopus* (*R. nodosus* and *R. stolonifer*) and genus *Verticillium* *sps.*; while on the untreated pigeon pea two genera of fungi were detected, fifteen species of *Aspergillus* (*A. alliaceus*, *A. amstelodami*, *A. chevalieri*, *A. fresenii*, *A. lutescens*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. proliferans*, *A. quercinus*, *A. sydowi*, *A. unguis*, *A. ustus*, *A. versicolor* & *A. wentii*) and *Penicillium oxalicum* (Fig 1). Due to surface sterilization of seeds the incidence of ectophytic mycoflora decreased, though could not be totally eradicated. The percentage incidence, total of both endophytic and ectophytic mycoflora in unsterilized chickpea (Table no.4) were very high especially *Aspergillus* (*A. niger* (85.3%), *A. flavus* (80.6 %), *A. oryzae* (78.3%), followed by *Alternaria alternata* (40 %); *Fusarium moniliforme* (42.9%), *Mucor abundans* (34.9%) etc, while in the pigeonpea few species of *Aspergillus* [*A. oryzae* (42.4 %), *A. niger* (54,9 %)] were in the higher range followed by other species of *Aspergillus* in the range of 30-20% while all other mycoflora were between 3-18%. After treating the seeds with mercuric chloride, in

chickpea the endophytic mycoflora reduced to 59.79%, ectophytic to 28.7%, while in pigeonpea it was 18% and 23% respectively (Table 4) indicating the significance of surface sterilization.

Germination of seed serves as an index of seed health. In the present study, due to high fungal infestation in the untreated seeds of pigeonpea, poor germination percentage (<50%) was recorded though the germination percentage did not improve significantly post treatment (Table no 1). The fungi associated with seeds under study was the storage fungi belonging to *Aspergillus* and *Fusarium* genus and these are known to cause deterioration of seed quality, affect the viability and reduce germination. The storage fungi reduce seed germination probably by producing toxins (Hashmi and Thrane, 1990). Scussel (1998) observed that *Fusarium*, *Penicillium* and *Aspergillus* strains especially *A. flavus*, *A. niger*, *A. parasiticus* were also responsible for the production of aflatoxin in most seeds. Desjardins *et al.* (2006) reported that *Fusarium* spp produced Zeralenone mycotoxin capable of causing haemorrhage and necrosis in bone marrow. Present result showed that *Aspergillus* were the predominant fungi in both pigeonpea and chickpea. *P. oxalicum* was specific to pigeon pea seeds and *P. purpurgenum* specific to chickpea seeds.

Conclusion

Moreover, endophytic fungi like *Fusarium*, *Rhizopus* and *Penicillium* though present within the range of 3-25%, also have possibly inhibited the germination of the untreated chickpea (54.44 %), thereby reducing the germination percentage as compared to the treated seeds (> 73 %). These fungi probably are found internal to the seeds and therefore, could not be controlled by surface sterilization. Several other workers Ghangaokar and Kshirsagar (2013) and Javaid *et al* (2005) also have reported similar findings. Moreover the predominance of *Aspergillus* and *Fusarium* on the pulse seeds in the present study, may not only be limited to loss in yield, but also accounts for the build-up of mycotoxins in infected grains thereby rendering the seeds unhealthy for human consumption too. The findings of this study are therefore, important as they emphasize the need for effective measures aimed at reducing seed-borne infection of both chickpea and pigeonpea seeds from Satara.

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