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Seed mycoflora of pigeonpea (*Cajanus cajan* (L.) Mills.) and it's management

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ABSTRACT
Seeds of thirty-five genotypes were collected from RRS, GKVK, Bangalore, during *Kharif* 2001 and analyzed for seed mycoflora by employing standard blotter technique. The studies revealed that pigeonpea seeds were infecting with six fungi *Viz., Alternaria* sp, *Aspergillus flavus, A. niger*, *Helminthosporium* sp. *Fusarium* sp. and *Cladosporium* sp. Amongst these six fungi infected in pigeonpea seeds, *Alternaria* sp had a maximum of 75% infection in PUSA2001-1 genotype followed by *Aspergillus flavus* (72% infection in S_1) *A. niger*, (Maximum of 16% infection in AL 201) *Helminthosporium* sp. (Maximum of 60% infection in PUSA 2001-1) and *Cladosporium* sp (Maximum of 76% infection in TT 101) and least was *Fusarium* spp. (Maximum of 40% infection in H 88-25). The seed treatments like hot water treatment solar heat treatment and chemical treatments were studied, it reveals that hot water treatment of seeds at 35 ^{0}C for 30 minutes found effective in reducing seed infection. Solar heat treatment on terraces for 2 hours reduces the seed infection. Emisan (2 g/Kg) seed and mancozeb (2 g/kg) seed completely eliminated seed infection by the pathogen.

Key words : Pigeonpea, Seed mycoflora, Alternaria, Aspergillus.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Mills.) is being grown in tropical and subtropical parts of the world and has got high protein content of 21 per cent. The literature revealed that more than hundred pathogens were known to attack the crop (3). Among them *Cercospora* leaf spot, *Alternaria* leaf spot, Phyllody, sterility mosaic disease, *Fusarium* wilt and *Rhizoctonia* root rot are common. Incidentally only few of the seed borne pathogens known to cause economic loss. Among the pathogens except *Fusarium udum,* the seed borne nature of any of these pathogens is not yet recorded. The present paper reveals the fungi associated with the seeds of pigeonpea genotypes.

MATERIALS AND METHODS

Assessment of seed mycoflora:

Seeds of thirty-five genotypes were collected from RRS, GKVK, Bangalore, during *Kharif* 2001 and analysed for seed mycoflora by employing standard blotter technique as per the International Seed Testing Rules (2). Four hundred seeds of each sample was plated on three layers of moist blotters placed in sterile Petriplate of 90 mm diameter at the rate of 10 seeds per plate and the plates were incubated for seven days at temperature of 28 ± 1^{0}C. After incubation, seed mycoflora were recorded on eighth day by observing fungal growth on seeds with the help of a stereo binocular microscope. Further the species were confirmed by conidial structure and their frequency of occurrence was expressed in percentage. Mean time the percent germination and percent decayed /abnormal seeds were also recorded.

Management of seed borne inoculum on pigeon pea seeds:

To control the seed infection of pigeon pea highly infected seed sample of the cultivar Pusa 2001-2 were selected and the seeds were subjected to different methods of treatment *viz*., physical and chemical methods. A control was maintained for comparison without subjecting the seeds to any treatment. Four hundred seeds were used in each treatment. Seeds were incubated at a temperature of 28 °C with relative humidity of 70 per cent on moist blotters as per the standard blotter method with 4 replications under each treatment and examined for the effect of seed treatment on seed germination and per cent infection by the mycoflora was recorded on 8th day under stereo binocular microscope in all treatments. Physical seed treatment methods: *viz*., hot water treatment and solar heat treatment were employed to control the seed infection.

Hot water treatment:

Pigeon pea seeds were subjected to hot water treatment at 30, 35, 40 and 45°C with time interval of 10, 20 and 30 minutes at each temperature level. Seeds were shade dried and incubated for seven days on moist blotters by employing standard blotter method. Observations were recorded on 8th day.

Solar heat treatment:

The Pigeon pea seeds were subjected to solar heat treatment for two hours by keeping them on different substrates *viz*., white paper, black paper, sand, concrete and asbestos. The seeds were incubated for seven days by employing the standard blotter method and the seeds were examined for the development of the fungus. The observations were recorded on 8th day and expressed in percentage.

Chemical seed treatment:

Pigeon pea seeds were treated with five fungicides *viz*., emisan, chlorothalonil, mancozeb, thiophanate methyl and captafol at the rate 2 g/kg seed. Four hundred Pigeonpea seeds in each treatment were plated on standard blotters and incubated for 7 days. On eighth day observations were recorded and expressed in percentage.

RESULTS AND DISCUSSION

Six fungi *Viz., Alternaria* spp. *Aspergillus flavus, A. niger, Helminthosporium* spp. *Cladosporium* spp. and *Fusarium* spp. were found associated with pigeonpea seeds.

The maximum infection of *Alternaria* spp. (75.00%) was recorded in PUSA-2001-1 and minimum was found in TT-102 (0.00%). Maximum infection of *Aspergillus niger* was observed in S-1 (72.00%) followed by H-88-22 (50.00%) and TT-102 was free from the infection. Maximum of infection of *Helminthosporium* spp., (60.00%) was found in the seeds of PUSA-2001-1, whereas PA-237, H-88-20 and H-88-25 were free from infection.

In certain genotypes *Fusarium* spp. association was recorded with maximum infection of 40.00 per cent in H-88-22 and least infection of 2.00 per cent was recorded in PUSA-991. Some genotypes were free from *Fusarium* spp infection *Aspergillus niger* was found up to 16.00 per cent in AL-2001, while PUSA-2003, PA-266 etc., were free from *A. niger.* Maximum recovery of *Cladosporium* spp. (76.00%) was observed in TT-101, where as AL- 2001, PA-266, H-88-22, H-88-25 etc., were free from infection. *Aspergillus nigerwas* also found frequently and there was significant reduction in germination (1-10%) Where ever *Aspergillus niger* was dominant the seeds were unable to germinate due to mycelial coverage and rotting.

Management of Alternaria alternata on Pigeon pea seeds

Hot water treatment*:*

When seeds were treated with hot water at a temperature of

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Table 1: Seed mycoflora of different pigeonpea genotypes.

Genotypes	Per cent species of Mycoflora						Per cent Germination
	Alternaria spp	*Aspergillus niger*	*A. flavus*	*Cladosporium* spp	*Fusarium* spp	*Helminthosporium* spp	
UPAS 120	33.60	33.20	5.40	21.00	0.00	16.80	90.00
AL 201	70.00	20.00	0.00	0.00	20.00	40.00	85.00
PUSA2003	68.00	12.00	0.00	14.00	0.00	36.00	86.00
PUSA2001-1	75.00	05.00	0.00	45.00	0.00	60.00	90.00
PUSA2001-2	62.50	15.00	2.50	12.50	15.00	40.00	80.00*
PUSA2001-3	64.00	10.00	6.00	12.00	10.00	16.00	92.00
PA 262	63.30	16.60	3.30	6.60	0.00	26.60	825.00
PA 266	65.00	02.50	0.00	0.00	2.50	27.50	91.00
PA273	16.00	40.00	0.00	2.00	6.00	0.00	90.00
H 88-22	60.00	50.00	0.00	0.00	40.00	0.00	89.00
H 88-25	60.00	05.00	5.00	0.00	0.00	0.00	88.00
H90-13	57.50	25.00	5.00	5.00	10.00	15.00	86.00
ORGK 108	43.00	18.00	1.00	14.00	4.00	13.00	92.00
TT 103	41.25	11.25	3.75	0.00	0.00	13.70	92.00
AL 1419	35.00	30.00	0.00	0.00	0.00	15.00	90.00
GC 11-39	35.00	43.33	1.66	5.00	0.00	1.66	91.00
PA 234	25.00	10.00	0.00	0.00	0.00	12.50	92.00
PUSA2001	57.50	17.50	0.00	5.00	5.00	2.50	87.00
AL 2001	36.00	06.00	16.0	0.00	0.00	4.00	90.00
JSMP 8	15.00	27.00	0.00	0.00	0.00	40.00	91.00
TT101	56.00	14.00	0.00	76.00	0.00	23.00	84.00
S1	09.00	72.00	0.00	59.00	0.00	17.00	86.00
JJA 65	16.00	07.00	0.00	42.00	0.00	2.00	91.00
BSR 1	09.00	16.00	0.00	0.00	27.00	5.00	92.00
IPA 04	19.00	11.00	0.00	64.00	0.00	18.00	90.00
TT 102	0.000	00.00	0.00	0.00	17.00	4.00	96.00
PA 128	30.00	03.30	3.30	10.00	0.00	6.60	85.00
AL201 (CH)	46.50	25.00	0.00	0.00	7.50	7.50	86.00
PUSA 992	36.75	23.30	0.00	0.00	0.00	0.00	90.00
PUSA 991	42.00	10.00	0.00	0.00	2.00	6.00	89.00
AL 1430	62.00	36.00	0.00	0.00	0.00	16.00	85.00
KSMR 88	62.00	30.00	0.00	0.00	0.00	20.00	83.00
TTB 7	64.00	46.00	4.00	0.00	8.00	8.00	84.00
BAHAR	35.00	7.50	0.00	0.00	25.00	0.00	85.00
BRG 1	45.00	30.00	0.00	0.00	5.00	0.00	86.00

30°C for 10 min. 39 per cent infection and 85 per cent germination was recorded whereas hot water treatment for 20 min and 30 min recorded 33 per cent seed infection with 88 per cent germination and 29.24 per cent infection with 90 per cent germination respectively. Hot water treatment of 40°C at 10 min, 20 min and 30 min and 45°C at 10 min, 20 min and 30 min completely eliminated seed infection but drastically reduced germination (Table 2).

Solar heat treatment:

Minimum seed infection of 26.25 per cent with 92 per cent germination was observed when seeds were treated with solar heat for 2 hours on terrace followed by 37.5 per cent infection with 70 per cent germination. When seeds were treated on asbestos, 32.25 per cent infection with 90 per cent germination were recorded when seeds were treated on black papers. While 35.75 per cent infection with 86 per cent germination was recorded on sand compared to 37 per cent infection with 81per cent germination on white papers (Table 3).

Chemical seed treatment:

Chemical treatment at the rate of 2 g/kg seed with chlorothalanil and captofol completely eliminated seed infection with 90 per cent and 85 per cent seed germination respectively whereas mancozeb treatment recorded 10 per cent infection with 75 per cent germination. Emisan treatment recorded 15 per cent seed infection with 70 per cent germination compared to 80 per cent germination by thiophanate methyl treatment (Table 4).

Screening of pigeonpea seeds for mycoflora revealed the presence of *Alternaria* spp, *Aspergillus flavus*, *A.niger*, *Helminthosporium* spp, *Cladosporium* spp and *Fusarium* spp. *Alternaria* spp was observed in the seeds of all the genotypes tested except TT-102.

Maximum infection of *Alternaria* sp. was found in PUSA 2001 (75.00%) followed by AL-201 (70.00%). *Aspergillus flavus* was another common pathogen found on all the genotypes except TT-102 with maximum infection were found in S-1 (72.00%) followed by H-88-22 (50.00%) and TTB-7 (46.00%).

Aspergillus niger was found only in few genotypes with maximum infection in AL-2001 (16.00%). Maximum infection of *Cladosporium* sp. was found in TT- 101 (76.00%) followed by IPA 04 (64.00%) and few genotypes were free from infection. The infection of *Helminthosporium* sp. was found in almost all the genotypes with maximum infection in PUSA-2001-1 (60.00 %) followed by AL-201 (40.00%) and PUSA-2001-2 (40%), whereas PA-273, H-88-22 and

Table 2 : Effect of hot water treatment for the control of seed mycoflora

Sl.No.	Water temperature ^{0}C	Time (min.)	Seed infection (%)	Seed germination (%)
1	30	10	39.00 (6.03)*	85.00
	30	20	33.00 (5.87)	88.00
	30	30	29.24 (5.34)	90.00
2	35	10	16.35 (4.08)	90.00
	35	20	10.00 (3.64)	92.00
	35	30	00.00 (0.71)	87.00
3	40	10	00.00 (0.71)	66.00
	40	20	00.00 (0.71)	00.00
	40	30	00.00 (0.71)	00.00
4	45	10	00.00 (0.71)	00.00
	45	20	00.00 (0.71)	00.00
	45	30	00.00 (0.71)	00.00
5	Control	--	40.00 (6.34)	86.00
	Sem±		0.306	
	CD at 0.05% Cultivar PUSA2001-2		0.703	

* Figures in the parenthesis are square root transformed values

H-88-25 were free from infection. *Fusarium* spp., were found in few genotypes with maximum infection in BSR-1 (27.00%) followed by AL-201 (20.00%). Agarwal and Gupta (1) observed the association of M*acrophomina phaseolina, A. flavus* and *Fusarium* sp. in all the 16 varieties of soybean seed they tested. Teggi and Hiremath (4) reported the association of *Alternaria alternata. Cladosporium fulvum, F. moniliforme*, *A. flavus, A. niger* and *Trichothecium roseum* on the seeds of greengram.

When seeds were treated with hot water at a temperature of 30°C for 10 min 39 per cent infection and 85 per cent germination was recorded whereas hot water treatment for 20 min and 30 min recorded 33 per cent seed infection with 88 per cent germination with 90 per cent germination and 29.24 per cent infection with 90 per cent germination respectively. Hot water treatment of 40°C at 10 min, 20 min and 30 min and 45°C at 10 min, 20 min and 30 min completely eliminated seed infection but drastically reduced germination.

Table3 : Effect of solar heat treatment for the control of seed mycoflora

Sl.No.	Substrate	Seed infection (%)	Seed germination (%)
1	Terrace	26.25 (4.39)	92.00
2	Asbestos	37.50 (7.25)	70.00
3	Black paper	32.25 (5.55)	90.00
4	Sand	35.75 (5.58)	86.00
5	White paper	37.00 (6.07)	81.00
6	Control	45.00 (6.61)	85.00
	Sem±	0.436	
	CD at 0.05%	1.608	
	Cultivar PUSA2001-2		

* Figures in the parenthesis are square root transformed values

Table 4: Effect of chemical treatment for the control of seed mycoflora

Sl.No.	Chemical	Seed infection (%)	Seed germination (%)
1	Emisan	15.00	70.00
		(0.71)	
2	Mancozeb	10.00	75.00
		(0.71)	
3	Chlorotalanil	08.75	90.00
		(3.03)	
4	Captafol	09.75	85.00
		(3.20)	
5	Thiophonate methyl	16.25	80.00
		(4.08)	
6	Control	40.00	60.00
		(6.61)	
	Sem±	0.474	
	CD at 0.05%	1.062	
	Cultivar PUSA2001-2		

* Figures in the parenthesis are square root transformed values

Note: Seed treatment at the rate of 2 g/Kg seed.

Minimum seed infection of 26.25 per cent with 92 per cent germination was observed when seeds wee treated with solar heat for 2 hours on terrace followed by 37.5 per cent infection with 70 per cent germination. When seeds were treated on asbestos, 32.25per cent infection with 90 per cent germination was recorded when seeds were treated on black paper. While 35.75 per cent infection with 86 per cent germination was recorded on sand compared to 37 per cent infection with 81 per cent germination on white papers. Investigations were not available in respect of physical methods of control of the pathogen.

Chemical seed treatment at the rate 2 g/kg seed with Chlorothalanil and capyofol eliminated seed infection to maximum extent with 90 per cent and 85 per cent seed germination respectively whereas emisan treatment recorded 15 per cent infection with 70 per cent germination. Mancozeb treatment recorded 10 per cent seed infection with 75 per cent germination compared to 80 per cent germination by thiophanate methyl treatment.

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