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To identify the seed borne mycoflora associated with pigeonpea seeds by Standard Blotter Method

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ABSTRACT

The study aims at identifying seed borne fungi associated with *Cajanus cajan* L. Mill sp. Nineteen varieties of pigeanpea were collected from various places in different agroclimatic zones for the detection of seed mycoflora associated with them. The seed born fungi was screened by using Standard blotter plate method from selected untreated and treated seeds. A total of 14 fungal species were found associated with pigeonpea seeds of different varieties with varying degree of incidence in standard blotter method (Untreated). The detected fungi were *Fusarium monillifoarmae, Alternaria alternate, Aspergilus flavus, Aspergilus niger, Aspergilus fumigates, Aspergilus candidus, Cladosporium cladosporoides, Curvularia lunata, Rhizoctonia solani, Drechsera tetramera, Penicillium oxalicum, Mucor* spp., *Rhizopus nigricans* and *Cheatomium globosum.* The maxium number of fungal species were associated with seeds of variety UPAS-120 .In this method (Pre-treated) 11 fungal species were detected. The Treatment reduced the average number of colonies of each fungus associated with seeds of different varieties considerably while 4 fungal species such *as Penicillium oxalicum, Mucor* spp. *Rhizopus nigricans* and *Chaetomium globosum.*

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INTRODUCTION

As major source of dietary protein, the pulses are of utmost importance for vegetarian people of the world. The pulses have got the ability of fixing atmospheric nitrogen through symbiosis with *Rhizobium* sp. And thus fulfil the nitrogen requirement to a great extent. Pigeonpea [*Cajanus cajan* (L.) Mill sp.] is one of the major pulse crops of the tropics and sub tropics including America, India, Australia, Hawaii, Uganda, Italy, East and West Indies and South-East Africa. The country's total area coverage and production of Arhar has been about 44 Lha and 42 Lt, respectively. As usual Maharashtra has contributed >28 per cent of area and 25 per cent of total production during this period. (Anonymous, 2017-18).

There are several factors which are responsible for

their low production. Among them, diseases play an important role (Nine, 1986 and Pal, 1996). Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Williams and McDonald, 1983; Kubiak and Korbas, 1999; Dawson and Bateman, 2001 and Islam *et al.*, 2009)

MATERIAL AND METHODS

Nineteen varieties of pigeonpea were collected and studies were made with reference to the mycoflora associated with them. The research work was carried out in the Department of Agricultural Botany, S.D.J. Post Graduate College, Chandeshwar, Azamgharh. The seed samples of 19 Varieties of pigeonpea were collected from various places in different agro-climatic zones for the detection of seed mycoflora associated with them. The presence of fungal flora Associated with pigeonpea seed was detected by using the ISTA (1985) by Standard blotter method.

Standard blotter method (Untreated seeds):

By this method, seed were tested in 4 replication using one hundred seed per replication thus, a total of 400 seeds per sample. Randomly selected 10 seeds were placed on three layered moist blotters at equal distance with the help of sterilized forceps in each petridish of 9.0 cm in diameter. These seeds were then incubated at a temperature of 25± 1^0C for 7 days using twelve hour alternating cycles of light and darkness. These plated seeds were examined for the presence of seed borne mycoflora under stereoscopic binocular and compound microscope. The associated mycoflora were observed and recorded in percentage from different seed samples.

Pre-treated seeds:

A total of 400 seeds from each samples were pre-treated by dipping the seeds, separately, in 1 per cent solution of chlorine for a period of 10 minutes. Then they were examined by standard blotter method as described earlier. At the end of incubation period, the fungal species growing on the seeds were transferred and cultured on 2.0 per cent PDA medium and purified by single spore isolation techniques, pure culture, obtained were stored on potato dextrose Agar medium in culture tubes in refrigerator at 5-8 Celsius.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Detection of mycoflora associated with pigeonpea seed was carried out and findings are presented as below:

The experimental findings on detection of seed borne mycoflora by standard blotter method (Table 1) showed the presence of 14 fungal species belonging to 11 generad with varying degree of incidence. These fungal species were *Fusarium moniliformae, Alternaria alternate, Aspergilus niger, A. flavus, A. fumigates, A. candidaus, Cladosporium cladosporoids, curvularia lunata, Rhizcotonia solani, Drechselra teramera, Penicillium oxalicum, Mucor* sp. *Rhizopus nigricans* and *Chaetomium globusum.*

Standard blotter method (Pretreated seeds) from preated seeds only 10 fungal species belonging to 7 genera were isolated as compared to 14 fungal species obtained in untreated seeds. It indicates the effect of chlorine pretreatment on the occurrence of fungi associated with seeds. The average number of colonies of all fungal species associated with pigeonpea seeds was found reduced due to chlorine treatment. In Pretreated seeds certain fungal species were not recorded, such as *Rhizopus nigricans, Penicillium oxalicum, Mucor* sp. and *Chaetomium globusum.* The Rapidly growing fungi *viz., Chaetomium globusum, Rhizopus nigricans, Mucor* sp. were probably eliminated or suppressed while slow growing fungi faced less competion. And consequently developed on more number of pigeonpea seed. *Fusarium moniliformae* was detected from seeds of 9 varieties namely UPAS-120, Pant A-3,GT-1, MA-97,T-21,C-11, Narendra-1 and Bahar. Of 19 pigeonpea varieties 16 showed the presence of *Alternaria alternate,* the three varieties devoid of this fungal species were BDN2, ICP-335 and NP(WR)15. Fungal species *Aspergillus flavus* and *A. niger* were detected from seeds of 12 varieties of pigeonpea. The 7 varieties were not infected by *Aspergilus flavus* were T-7, C-11, BDN2, Bahar, ICP-335, Mukta and NP-15. *A. niger* was observed in the varieties UPAS-120, MA-97, T-21, ICP-151, T-17, Pusa -9, Manak, Pusa-74, Narendra-1,

Table 1 : Frequencies (%) incidence of fungal species associated with seeds of different varieties of pigeonpea seeds by standard blotter method

Sr. No.	Varieties	*Fusarium moniliformae*	*Alternaria alternata*	Aspergilus flavus	*A.niger*	*A.fumigatus*	*A.candidatus*	*Cladosporium claedosporiodes*	*Curvularia*	*Rhizocotonia*	*Dreschlera*	*Penicillum*	*Mucor*	*Rhizopus nigricans*	*Chaetomium globusom*	Total no. of fungal spp.	Total % of fungal colonies
1.	UPAS-120	8	15	10	8	-	-	5	3	2	2	1	1	2	2	12	59
2.	Pant A-3	2	10	9	5	-	-			4						5	30
3.	GT-1	2	4	3	1	1	1									6	12
4.	MA-97	1	10	7	4	-			2							6	26
5.	T-21	6	12	8	6	3	3	2	3				1			10	46
6.	ICP-151	3	6	7	4			1								4	20
7.	T-7	1	3	9	2							1	1			7	18
8.	T-17	2	7	4	7							1	1			6	22
9.	Pusa-9		11	5	3				1							4	20
10.	ICP-8862		14	6									1			3	21
11.	Manak			6	9		3									3	18
12.	C-11		3	5	1										1	4	10
13.	Pusa-74	1	5	4	3		5									5	18
14.	Narendra-1	4	9	8	5											4	26
15.	BDN2			5	2						4					3	11
16.	Bahar			4												2	5
17.	ICP-335		5			1										3	7
18.	Mukta		7					1		1			1			3	9
19.	NP(WR)15														3	1	3
	Range of incidence	1-8	3-15	3-10	1-9	1-3	1-5	1-5	1-3	1-4	2-4	1-1	1-1	1-2	1-3		

BDN2, Bahar, ICP-335.

In this experiment, 19 varieties of pigeonpea were studied among them UPAS-120 Carried 9 fungal sp. Out of total 10 species detected in pretreated seeds. *Aspergilus candidatus*, was not found on pretreated seeds of UPAS-120. The variety T-21 showed 7 fungal species while GT-1 showed 5 fungal species. Each of the varieties Pusa-9, Pant A-3, MA-97 Manak, Pusa-74, Narendra-1, carried 4 fungal species on pre-treated seeds. No fungal sp. was detected on pretreated seeds of variety NP(WR)-15.

Conclusion and Discussion:

Seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant; a necessary condition for good yields (Diaz *et al.,* 1998). The standard blotter method (Table 1) showed the presence of 14 fungal species belonging to 11 generad with varying degree of incidence. These fungal species were *Fusarium moniliformae*, *Alternaria alternate*, *Aspergilus niger*, *A. flavus*, *A. fumigates*, *A. candidaus*, *Cladosporium cladosporoids*, *curvularia lunata*, *Rhizcotonia solani*, *Drechselra teramera*, *Penicillium oxalicum*, *Mucor* sp. *Rhizopus nigricans* and *Chaetomium globusum.* In this method (Pre-treated) 11 fungal species were detected. The Treatment reduced the average number of colonies of each fungus associated with seeds of different varieties considerably while 4 fungal species such as *Penicillium oxalicum, Mucor* spp., *Rhizopus nigricans* and *Chaetomium globosum.* The Standard Blotter method was found suitable for detection of fungi like *Alternaria alternate, Asspergilus*

flavus, Aspergilu niger, Curvularia lunata.

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