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Detection of seed mycoflora of pigeonpea by seed health testing methods

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Abstract
An investigation was conducted to detect the associated seed mycoflora in pigeonpea and its control. A total of 5 varieties of pigeonpea seed were collected from Agriculture Research Station, Badnapur. Standard agar plate methods and modified PDA method were used for detection of seed mycoflora of pigeonpea seeds. Across the two methods adopted, a total of ten fungal genera including *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Macrophomina phaseolina, Fusarium oxysporum* f. sp. *udum, Alternaria alternata, Botrytis cinera, Aspergillus* sp., *Trichoderma* sp. and *Cladosporium* sp. with the seeds of pigeonpea were detected. The fungi detected were identified based on their cultural and morphological characteristics. Among the ten fungal species detected the occurrence of *A. niger* was observed (57.75, 53.58, 37.32, 35.20 and 32.67%, respectively) in cultivars ICP-8863, ICP-2376, BSMR-736, BDN-708 and BSMR-853, followed by the fungi *A. flavus* (31.10, 17.15, 16.33, 14.60 and 12.05%, respectively) in cultivars ICP-8863, BSMR-736, BSMR-853, BDN-708 and ICP-2376 in Standard agar plate method. In modified PDA method maximum frequency of *Botrytis cinerea* was observed (55.00, 52.50, 30.00 and 25.80%, respectively) in var. BSMR-736, BSMR-853, ICP-2376 and ICP-8863, followed by fungi *A. niger* (40.10, 37.45, 32.50, 10.00 and 0.00,respectively) in the cultivars ICP-2376, ICP-8863, BDN-708 and BSMR-736, Per cent infectivity of seed mycoflora varied across the methods adopted and varieties tested. The highest per cent infectivity of 57.75% was observed with the fungus *A. niger* on ICP-8863 in Standard agar plate methods. In modified PDA method the highest per cent infectivity of 55.00% was observed with the fungus *Botrytis cinerea* on BSMR-736.

Keywords: Pigeonpea, seed mycoflora, standard agar plate method, modified PDA method

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 intercrop and also as a mixed crop. Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a legume belonging to family of fabaceae. Other common names are "Red gram, Arhar, Tur, Congo pea, Gunga pea, turvarica, thogari or ganduland No-eye pea" (Sheela, 2013) [12]. It is an important grains legume crop of rainfed agriculture in the tropics and subtropics. Compared with other grains legumes, pigeonpea ranks only sixth in area and production, but it is used in more diverse ways than other. Pigeonpea is a versatile crop grown primarily as a vegetable and a multi-use green crop (dhal) in India. Pigeonpea seed is composed of cotyledons (85%), embryo (1%) and seed coat (14%) (Faris and Singh, 1990) [4]. In India during 2017-18, pigeonpea was cultivated on an area of 4.43 M ha with a production of 4.25 million MT (Anonymous, 2018) [2]. In Maharashtra, pigeonpea is cultivated on an area of 12.29 lakh/ha with the production of 10.59 Lakh/tones. Pulse seeds are reported to carry many molds both in field and during storage. The association of fungi adversely affects quality and health of the seeds. The term "seed mycoflora or seed borne fungi" is used for both qualitative as well as quantitative analysis of fungi occurring on or in the seeds. The fungi associated with seeds at the stage of harvest and under storage bring about several undesirable changes making them unfit for consumption and sowing (Patil, 2012) [9]. Seeds are regarded as means of transporting plant pathogen (Agarwal and Sinclair, 1996) [1]. Seedborne pathogens may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage (Khanzada *et al.*, 2002) [6]. Seed mycoflora is a major factor affecting seed health. Wide ranges of fungi have been encountered with pigeonpea seed. Seed mycoflora of pigeonpea as well as their culture filtrates caused considerable reduction in germination per cent as compared to untreated control (Patil *et al.*, 2012) [9]. Hence, it is imperative that seed must be tested before they are sown in the field.

Seed health testing methods like agar plate methods and modified PDA method have been employed for detection of internal and external seed borne mycoflora (Meena Kumari, 2002) [8]. Considering these issues, present study was planned and conducted with the aim to detect and determine frequency of various seedborne fungi of pigeonpea.

Materials and Methods

A total of five varieties of pigeonpea seed were collected Agricultural Research Station, Badnapur. The seeds were collected in polythene bags and stored at room temperature of 25±2^{0}C. In Standard agar plate method four hundred each seeds of pigeonpea var. ICP-8863, ICP-2376, BSMR-853, BSMR-736 and BDN-708 were placed at the rate of (10 seeds / petriplate) containing 20 ml of two per cent water agar and incubated at 27±2^{0}C, for 7 days. After seven days of incubation, these plates were observed under stereo-binocular microscope to ascertain growth of various fungi associated with seeds of pigeonpea. Based on cultural characters, various fungi appeared in petriplates were aseptically isolated individually onto autoclaved and cooled PDA medium in separate petriplates and incubated further for a week. After a week of incubation, well developed fungal colonies appeared. By applying hyphal tip technique, these fungi were transferred aseptically onto autoclaved and cooled PDA slants in test tube, incubated to proliferate and stored in refrigerator for further studies.

In modified PDA method four hundred each seeds of pigeonpea var. ICP-8863, ICP-2376, BSMR-853, BSMR-736 and BDN-708 were placed at the rate of (10 seeds / petriplate) containing 20 ml of autoclaved and cooled acidified Potato Dextrose Agar (pH 4.5). Seeds were placed after pre-treatment with 2-3% sodium hypochlorite solution for 3 to 5 minutes, washed in three sequential changes of sterile distilled water and the plates were incubated at 26±2^{0}C, for a week. After a week of incubation, the fungal colony growth was examined under stereo-binocular microscope.

Result and Discussion

The analysis five varieties of pigeonpea using standard agar plate methods and modified PDA method showed the association of ten fungal species. The fungi detected were identified based on their cultural and morphological characteristics. The fungal species detected through standard agar plate methods and modified PDA method includes *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Macrophomina phaseolina, Fusarium oxysporum* f. sp. *udum, Alternaria alternata, Botrytis cinera, Aspergillus* sp., *Trichoderma* sp. and *Cladosporium* sp.(Table 1and 2).

Per cent infectivity of seed mycoflora varied across the methods adopted and varieties tested. In standard agar plate method (Table 1 and Fig.1),results revealed that, association of seven major fungi *viz*., *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Macrophomina phaseolina, Fusarium oxysporum* f. sp. *udum, Alternaria alternate* and *Trichoderma* sp., with the seeds of pigeonpea var. ICP-8863, ICP-2376, BSMR-853, BSMR-736 and BDN-708, respectively. *Botrytis cinerea, Aspergillus* sp. and *Cladosporium* sp. were absent in standard agar plate method. Germination per cent varied from 67.97 to 75.08%. In all the varieties, maximum seed germination (75.08%) was observed in var. BSMR-853 and minimum seed germination (67.97%) was observed in var. ICP-8863. The frequency (Table 1and Fig.1) association / incidence of all the seven fungi was found maximum infected (100, 100, 100, 100 and 87.5%) and healthy seeds were 00.00% to 12.5% in the seeds of all cultivars of pigeonpea *viz*., ICP-8863, ICP-2376, BMSR-853, BSMR-736 and BDN-708, respectively.

The results from Fig. 1 revealed that, among all the cultivars, highest frequency of various seed mycoflora was observed in ICP-8863 i.e., 57.75 and 31.10% of *A. niger*and *A. flavus*, respectively. However, among these seven fungi comparatively maximum frequency of *A. niger* was observed. Among all the mycoflora associated, highest frequency was observed of *A. niger* (43.30%), followed by *A. flavus* (avg. 18.24%), *A. alternata* (avg. 13.06%), *Trichoderma* sp. (avg. 8.32%, *R. stolonifer* (avg. 6.37%), *M. phaseolina* (5.52%) and minimum frequency was *of Fusarium oxysporum* f. sp. *udum* (avg. 4.70%), respectively.

Results of the present studies were in consonance with those reported earlier by the several workers Lokesh and Hiremath (1992) [7] reported that, agar plate method was found efficient in enumeration of seed mycoflora of pigeonpea. Rathod *et al.*, (2012) [11] employed various methods to detect legumes seedborne mycoflora and reported most reliable and faster. Sheela, (2013) [12] reported that, agar plate method was found to be most suitable techniques for detection of fungi in pigeonpea. Pradhan, (2015) [10] reported that, eight fungal flora were detected and isolated from five pigeonpea varieties by agar plate method. Geetha, (2017) [5] reported the association of fungal flora using agar plate method in pigeonpea and chickpea.

In modified PDA method Results (Table 2 and Fig.2) revealed that, in modified PDA method germination per cent, healthy and infected seeds and association of ten major fungi *viz*., *Aspergillus niger, A. flavus, Fusarium oxysporum* f. sp. *udum, Macrophomina phaseolina, Alternaria alternata, Botrytis cinera, Trichoderma sp., Aspergillus sp., Rhizopus stolonifer* and *Cladosporium* sp. with the seeds of pigeonpea ICP-8863, ICP-2376, BSMR-853, BSMR-736 and BDN-708 cultivars, respectively. Results (Table 2 and Fig. 2) revealed that, in modified PDA method, germination per cent was ranged between 99.87 to 55.12% in the cultivar BSMR-736, BDN-708, BSMR-853, ICP-2376 and ICP-8863, respectively. Healthy seeds ranged in the cultivars ICP-8863(00.00), ICP-2376(00.00), BSMR-853(00.00), BSMR-736(00.00) and BDN-708 (27.5), respectively.

The results revealed that, among these ten fungi comparatively maximum frequency of *Botrytis cinerea* was observed i.e. 55.00, 52.5, 30.00 and 25.80% in var. BSMR-736, BSMR-853, ICP-2376 and ICP-8863, followed by the fungi *viz*., *A. niger* i.e. 40.10, 37.45, 32.5 and 10.00% in var. ICP-2376, ICP-8863, BDN-708 and BSMR-736, *Aspergillus sp.* i.e. 21.5, 12.5 and 7.5% in var. ICP-2376, BSMR-853 andBSMR-736, *A. flavus* i.e. 20.50, 7.50, 3.80 and 2.50% in var. ICP-8863, BDN-708, ICP-2376 and BSMR-853, *A. alternata* i.e. 15.00, 7.60 and 7.50% in var. BSMR-853, BSMR-736 and BDN-708, *M. phaseolina* i.e. 15 and 8.10% in var.BSMR-736 and BDN-708, *Fusarium oxysporum* f. sp. *udum* i.e. 15.50 and 2.5% in var.BDN-708 and ICP-2376 and *Cladosporium* sp. i.e. 7.50, 1.50 and 1.40% in var.BSMR-853, BSMR-736 and BDN-708, respectively. In modified PDA method, highest frequency was observed of *Botrytis cinerea* (avg. 32.66%), followed by *A. niger* (avg. 24.01%) and *Aspergillus* sp. (avg. 8.30%) and *A. flavus* (avg. 6.86%) and *A. alternata* (avg. 6.0%) and *M. phaseolina* (avg. 4.62%) and *Fusarium oxysporum* f. sp. *udum* (avg. 3.60%) and *Cladosporium* sp. (avg 4.78%), respectively and very less

frequency was observed in *Trichoderma* sp. (avg. 2.10%), respectively.

Result also indicated that, in modified agar plate method all seed mycoflora associated externally and internally with the seed was detected.

This method was found most useful for detection of seedborne pathogens of pigeonpea. Similar results were found by, Chaudhari and Sharma, (2015) [3] reported that, using blotter and agar plate methods as recommended by ISTA, the seed mycoflora of different pigeonpea seed samples were examined. Nine fungi were isolated from the seeds of different pigeonpea varieties.

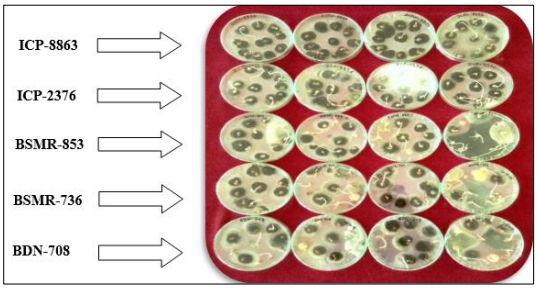

Fig 1: Detection of Seedborn Mycoflora by Standard Agar Plate Method in Different Cultivars of pigeonpea

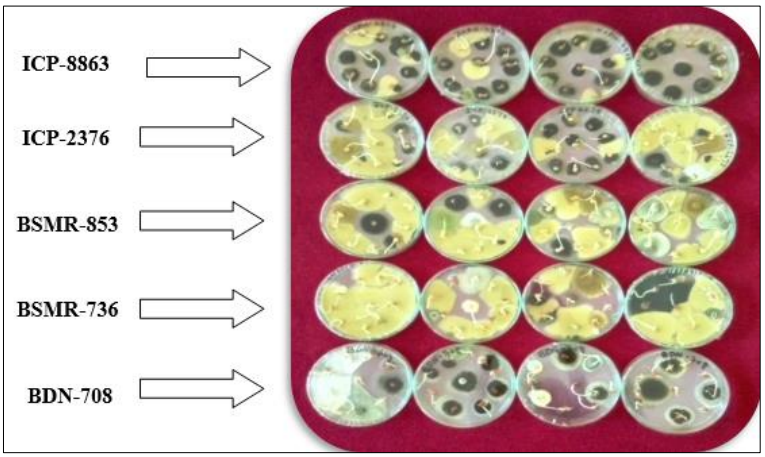

Fig 2: Detection of Seedborn Mycoflora by Modified PDA Method in Different Cultivars of pigeonpea

Table 1: Per cent frequency of various fungi associated with pigeonpea seeds by Standard agar plate method

Varieties	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed Mycoflora (%)									
				Aspergillus niger	*Aspergillus flavus*	*Rhizopus stolonifer*	*Macrophomina phaseolina*	*Alternaria alternata*	*Fusarium oxysporum* f. sp. *Udum*	*Botrytis cinerea*	*Trichoderma* sp.	*Aspergillus* sp.	*Cladosporium* sp.
ICP-8863	67.97	0.00	100	57.75	31.10	10.75	0.00	0.00	0.00	0.00	11.15	0.00	0.00
ICP-2376	68.07	0.00	100	53.58	12.05	5.54	0.00	0.00	17.69	0.00	11.18	0.00	0.00
BSMR-853	75.08	0.00	100	32.67	16.33	6.25	15.50	23.90	0.00	0.00	5.35	0.00	0.00
BSMR-736	70.27	0.00	100	37.32	17.15	4.15	12.10	17.50	0.00	0.00	11.17	0.00	0.00
BDN-708	69.15	12.50	87.50	35.20	14.60	5.20	0.00	23.92	5.85	0.00	2.75	0.00	0.00
Mean	70.10	2.50	97.50	43.30	18.24	6.37	5.52	13.06	4.70	0.00	8.32	0.00	0.00

Table 2: Per cent frequency of various fungi associated with pigeonpea seeds by Modified PDA method

Varieties	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed Mycoflora (%)									
				Aspergillus niger	*Aspergillus flavus*	*Rhizopus stolonifer*	*Macrophomina phaseolina*	*Alternaria alternate*	*Fusarium oxysporum* f. sp. *udum*	*Botrytis cinerea*	*Trichoderma* sp.	*Aspergillus* sp.	*Cladosporium* sp.
ICP-8863	55.12	0.00	100	37.45	20.50	5.75	0.00	0.00	0.00	25.80	10.50	0.00	0.00
ICP-2376	80.05	0.00	100	40.10	3.80	2.10	0.00	0.00	2.50	30.00	0.00	21.50	0.00
BSMR-853	82.62	0.00	100	10.00	2.50	0.00	0.00	15.00	0.00	52.50	0.00	12.50	7.50
BSMR-736	99.87	0.00	100	0.00	0.00	0.00	15.00	7.60	0.00	55.00	0.00	7.40	15.00
BDN-708	85.25	27.50	72.50	32.50	7.50	0.00	8.10	7.50	15.50	0.00	0.00	0.00	1.40
Mean	80.58	5.50	94.50	24.01	6.86	1.57	4.62	6.00	3.60	32.66	2.10	8.30	4.78

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