IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION OF MYCOFLORA ASSOCIATED WITH PIGEONPEA [CAJANUS CAJAN (L). MILLSP.

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Abstract: Pigeonpea [Cajanus cajan (L.) is major pulse crop grown in India. The study aims at identifying seed borne fungi associated with stored pigeogen seeds. Seed health testing is a pre-requisite for seed improvement, seed production, seed certification and trade in seed. Using blotter and agar plate methods as recommended by ISTA, the seed mycoflora of different pigeonpea seed samples were examined. Fungi were isolated from the seeds of different pigeonpea varieties. Most dominant fungal species were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium spp.* And *Penicillium spp.*

Key words: Seed borne fungi, Pigeonpea, agar plate, blotter paper

Introduction:

Cajanus cajan is one of the important leguminous crop of the tropics and subtropics. It is a major pulse crop of India. Dry dehulled split seeds are used as “Dal” for preparing “Varan”, “Dal”, and “Sambar”. Green seeds as vegetable, crushed dry seed as animal feed, green leaves as fodder and stem as fuel wood are being used, Pigeonpea [Cajanus cajan (L.) is economically and nutritionally an important legume and is a major source of protein for the poor communities of many tropical and subtropical regions of the world.

Production:

Pigeonpea seeds have 21% protein. Most of the world area under pigeogenpea is occupied by India with 78.6% of production of Pigeonpea by India alone. Major pigeonpea growing states of India include Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh and Gujarat. Though Maharashtra ranks first in area and production, productivity of Maharashtra is only 663 kg/ha. India has largest acreage under pigeonpea (3.90 M ha) with a total production and productivity of 2.89 mt and 741 kg/ha, respectively DAC, 2011. In Gujrat, pigeonpea crop in an area of 2, 77,000 ha with the production of 2, 73,000 tonnes Anon, 2011.

Losses:

Seeds are regarded as means of transporting plant pathogens. Seeds borne diseases affect growth and productivity of crop plants. Major cause of low productivity is the losses due to diseases. Among diseases wilt and sterility mosaic are important. Recent surveys have indicated that major losses in the Pigeonpea are due to wilt disease which caused by *Fusarium udum* Butler var. cajani. Losses ranging between 0.2 to 100% have been estimated from India (Butler 1906, Patil 1984, Kannaiyan et al., 1984 and Gade 2002). Kannaiyan et al., (1984) recorded maximum mean losses to the tune of 22% from Maharashtra.

The term “seed mycoflora or seed borne fungi” is used for both qualitative and quantitative analysis of fungi occurring on or in the seeds. The fungi associated with the seeds at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for human consumption and sowing Patil et al,. 2012. A seed borne pathogen may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage Jalander and Gachande, 2011. Leguminous crops commonly carry seed borne diseases. Present investigation was undertaking to find out the mycoflora associated with the seeds of different pigeogen varieties.

MATERIAL AND METHODS:

Experimental location

Experimental location the experiment was conducted in the Department of Botany, Shivaji College Udgir, Maharashtra .Sources of experimental materials Seed samples of pigeonpea were collected for the isolation and identification of seed-borne fungi from Dharashiv and Beed district of Maharashtra farm.

Farmers were selected randomly for sampling. From each seed sample, an amount of 250g seeds were taken and kept separately in labelled, pre-sterilized polythene bags. Plating of the seed component standard blotter paper method and agar plate method as described by the International seed Testing Association (ISTA) 1996, was used for the isolation of the seed borne fungi associated with the pigeonpea seed samples. In the blotter paper method, pair of sterile white papers of 8.5 cm diameter was soaked in sterile distilled water and were placed in pre-sterilized petri plates of 90 mm diameter. Ten seeds per petriplates, in order to isolate only internal seed mycoflora, were surface sterilized for 2 minutes with 1% sodium hypochlorite solution followed by three subsequent washings in sterilized distilled water to remove sodium hypochlorite solution from seeds and non-surface sterilized, were place at equal distance on three layers of properly moistened sterilized blotters. These plates were incubated at a temperature of 25±20C for 12 hrs in alternating cycles of light and darkness. The seeds were examined regularly for the growth of fungi over the seed. Whereas, in Agar plate method, pre-sterilized petriplate were poured with 20 ml of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantantly placed aseptically. Incubation and other details of the study were same as described in blotter test method.

Examination of incubated seeds:

Sampling for identification of fungi was done at seventh days. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the stereo zoom compound microscope. The samples of fungus were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi. The binocular compound microscope was used to determine the type of fungus in each plate. The seed-borne fungi were identified using identification keys and cross-checked for each seed plates to identify the type of fungus growing on each seed. After seven days of incubation, fungal species found growing on the surface of seeds, were identified and their percentage frequency (PF) of occurrence of fungal was calculated by applying the following formula: PF = (No. of seeds on which fungus appear / Total number of seeds) X 100.

Isolate no.	Fungi	Standard blotter paper method		PDA plate	
		SS	US	SS	US
1.	*Aspergillus niger*	_	*	-	*
2.	*Aspergillus flavus*	_	*	*	*
3.	*Fusarium spp.*	*	_	*	_
4.	*Fusarium monilliforme*	*	_	*	_
5.	*Fusarium oxysporum*	*	_	*	_
6.	*Rhizopus spp.*	_	*	_	*
7.	*Penicillium spp.*	*	*	*	*

Table I. Fungal association with naturally infected pigeonpea seeds * Present –Absent SS- Surface sterilized US- Un-sterilized

Seed-borne fungi associated with naturally infected pigeonpea seeds

Isolate no.	Name of Fungi	Percent Frequency of Mycoflora	
		Standard blotter paper method	Agar plate method
1.	*Aspergillus flavus*	16.67	15
2.	*Aspergillus niger*	17.65	16.42
3.	*Fusarium spp.*	10.78	10.71
4.	*Fusarium moniliforme*	14.70	12.46
5.	*Fusarium oxysporum*	12.74	12.14
6.	*Rhizopus spp.*	3.92	6.42
7.	*Penicillum spp.*	7.84	5.89

Table II. Percentage frequency of seed-borne fungi associated with naturally infected pigeon pea seeds

Result and Discussion:

Isolation of seed mycoflora from pigeonpea was done critically and results are depicted in Table 1. Total seven fungi with six genera were isolated by blotter paper and agar plate method i.e., *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium spp.*, *Rhizopus sp.*, *Aspergillus niger* and *Aspergillus flavus*. These results are in agreement with those of Patil, et al., 2012 and Kandhare, 2014. In Standard blotter paper method, the percent incidence of *Aspergillus niger* (17.65%) was highest followed by *Aspergillus flavus* (16.67%), *Fusarium oxysporum* (12.74%), *Fusarium moniliforme* (14.70%), *Fusarium spp.* (10.78%), *Penicillium* (7.84%) *and Rhizopus* sp. (3.92%) were found (Table 2).

Whereas, in Agar plate method, the percent incidence of *Aspergillus niger* (16.42%) was highest followed by *Aspergillus flavus* (15.00%), *Fusarium oxysporum* (12.14%), *Fusarium moniliforme* (12.46%), *Fusarium spp.*

(10.71%), *Penicillium* (5.89%) and *Rhizopus sp*. (6.42%) were found (Table 2). Our results indicated that these seed borne fungi could be the main seed borne pathogen affect the seed viability.
Such similar results were observed by Patil, et al., 2012. He found the highest incidence of *Aspergillus flavus* (30.00%) and *Aspergillus niger* (25.00%) in pigeonpea seeds and *Aspergillus flavus* (25.00%) and *Aspergillus niger* (20.00%) in chickpea seeds. Rathod, et al., 2012 also recorded the maximum incidence of Aspergillus flavus and *Aspergillus niger* in both in Standard blotter paper as well as Agar plate method in pigeonpea, gram, green gram, black gram and groundnut. Kandhare, 2014 also recorded maximum incidence by Aspergillus flavus and *Aspergillus niger* in pigeonpea seeds. Seed sample of green gram recorded that higher incidence of mycoflora by Fusarium oxysporum (22.50%), Aspergillus flavus (9.50%), while least incidence found in *Curvularia lunata* (4.50%) by Ashwini, and Giri 2014 in Standard blotter paper method.

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