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REVIEW OF RESEARCH

# COLLECTION OF SEED

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**ABSTRACT :**

*The present investigation was carried out in the phytopathological laboratory and glass house in the department of Botany of K. S. Saket P.G. College, Faizabad (U.P) with eleven varieties / lines of pigeonpea. The details of materials used, experimental procedures followed and techniques adopted are described in this chapter.*

**KEYWORDS :** *Collection of Seed , phytopathological laboratory , materials used, experimental procedure.*

## 1. Collection of seed samples:

Eleven seed samples of pigeonpea, each from one variety / lines were collected from Farmer's field, seed companies and pulse section of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (Uttar Pradesh) (Table 1) for the detection of seed mycoflora associated with them. These samples were stored in screw tight plastic bottles at room temperature (20-35O C) for further studies.

## 2. Detection of mycoflora associated with collected samples:

ISTA 1985 method was followed for testing of the samples for the presence of mycoflora associated with pigeonpea seeds which included four techniques.

l. Inspection of dry seeds.
ll. Seed washing test.
Ill. Standard blotter method without and with pretreatment with I.O percent chlorine solution.
IV. Agar plate method with potato dextrose agar medium.

## (2) (i) Inspection of dry seeds:

Dry seeds were examined for the presence of fungal fructifications such as sclerotia, perithecia, Acervuli, Pycnidia etc. and symptoms like discolouration, deformation and spots on its surface by naked eyes and with

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| Serial No. | Varieties / Lines |
|---|---|
| 1. | Prabhat |
| 2. | T-21 |
| 3. | Pant A-3 |
| 4. | NDA 91-2 |
| 5. | T-7 |
| 6. | T-17 |
| 7. | Pusa-33 |
| 8. | NDA 91-13 |
| 9. | NDA 91-14 |
| 10. | Bahar |
| 11. | NDA 91-1 |

the help of magnifying hand lens. Further, seeds were categorised in three categories.

**1 Apparently healthy looking.**
II.Discoloured.
III. Shrivelled seeds.

On the basis of their size, shape and appearance, seeds of each category were further divided into two groups.

(a) Under sized (having diametre less than 3.5mm)
(b) Normal sized (having diametre 3.5 mm or more)

Two hundred seeds from each category were tested by standard blotter method to assess the extent of fungi associated with them.

**2(II) Seed washing test :**

This method was used to detect the external mycoflora on pigeonpea seeds. Fifty randomly selected seeds from each sample were divided into two groups, each was suspended in 10ml. of sterilized distilled water in the conical flasks, separately.

The flasks were shaken by hand for 10 minutes. After shaking, equal volume of this suspension was transferred into two centrifugal tubes. These tubes were kept in centrifuge and rotated at 2500-3000 r.p.m

For 15 minutes. The supernetant liquid was decanted off from each tube and sediment from respective tubes was throughly mixed in 2.0 ml lactophenol (Agarwal, 1976) and examined under compound microscope for the presence of fungal spores, fructifications and mycelial fragments .

**(2) (III) a. Standard blotter method (untreated seeds)**

Four hundred seeds per samples in four replications, each of one hundred seeds were tested by this method. Ten randomly selected seeds were plated on three layered moist blotters at equal distance with the help of sterilized forceps in each petridish (9.0 cm diametre). These seeds were then incubated at a temperature of 28 + t 10c under twelve hours in alternating cycles of light and darkness for 7 days. These plated seeds were examined for the presence of seed-borne mycoflora under sterioscopic binocular and with the help of compound microscope.

**(2)(III) b. Standard blotter method (Pretreated seeds) ;**

Four hundred seeds from each sample were pretreated by dipping the seeds, separately, in 1.0 percent solution of chlorine for a period of 10 minutes and 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 two percent.Potato dextrose agar medium and purified by single spore isolation and hyphal tip isolation techniques.

The pure cultures, thus obtained were stored on PDA in culture tubes (i e. slants) in the refrigerator at 5-8°C for further studies.

**(2) (IV) Agar plate method:**

Four hundred seeds per sample in four replication, each of one hundred seeds were pretreated with 1.0 percent chlorine as described in standard blotter method and plated on PDA at the rate of 5 seeds per petridish. The seeds were incubated at 28 + 10c for 7 days under twelve hours alternating cycles of light and darkness and examined macroscopically by naked eyes for the presence of fungal colonies on seeds. Their specific identification was made with the help of standard identification manuals under sterioscopic binocular and compound microscope.

**(3) Comparative seed health testing methods for the detection of Individual pathogens:**

For comparative study five different seed health testing methods (incubation methods) viz. standard blotter method without or with pretreated seed, deep freezing blotter method 2,4-D blotter method, agar plate method with PDA and ulster method were adopted for pathogens only. They were compared for their suitability for the detection of individual pathogen. In one set of standard blotter method, the seeds were pretreated with 1.0 percent chlorine prior to plating. Randomly selected two hundred seeds of Prabhat and T-21 on the basis of pathogenic fungi found associated with seeds of these varieties were tested by each method. The procedures adopted is given below except standard blotter method and agar plate method with PDA which were earlier described.

**(3) (I) Deep freezing blotter method :**

This method is modification of blotter method (limonard 1966) and consists of plating of the seeds on three- layered moist blotters in petridishes @ 10 seeds per dish. These petridishes were incubated at 28 +1oc for 24 hours in complete darkness and then transferred into deep freez at 20°C for another 24 hours in darkness. The dishes were then taken out and subsequently incubated at a tempreture of 28 + 10c for 5 days under twelve hours alternative cycles of light and darkness The frozen seeds did not germinate and provide a better environment for proper development of fungus/fungi on seeds are easily visible under sterioscopic binocular microscope.

**(3) (II) 2, 4-D Blotter method :**

This is another modification of blotter method proposed by Neergaard and Saad (1962) which was later accepted by ISTA (1985) The method consisted to plating the seeds in petridishes on three layered blotters moistened in 0.001 percent solution of 2, 4-D (Sodium salt of 2, 4-Dichlorophenoxy acetic acid) @ 10 seeds per dish. These dishes were well wrapped in blotter paper in order to reduce evaporation of the 2.4- D and incubated for 7 days at 28 + 10c under twelve hours alternating cycles of light and darkness. The seeds were examined on 8th day for the presence of mycoflora on seeds.

**(3) (III) Ulster method:**

Muskett and Malone (1941) proposed this method which is a modification of agar plate method with PDA. In this method, malt extract agar medium was used in place of PDA and seeds were plated on the medium @ 5 seeds per dish. These dishes were incubated at 28 + 10c twelve hours alternating cycle of light and darkness for 7 days. After incubation period, the seeds were exmined macroscopically and under compound microscope for recording the pathogens associated with pigeonpea seeds.

**(4) Mycoflora associated with different categories of seeds:**

The seeds of pigeonpea cultivar Prabhat showing maximum number of fungal species on blotters, were separated into three categories viz. apparently healthy looking, discoloured and shrivelled seeds. Further seeds of all categories were put under two sets i. e. under size and normal size as described earlier. Two hundred seeds

of each category were tested in four replication, each treatment of having fifty seeds. The observations of the fungi present on the seeds were recorded after 7 days of incubation.

**(5) Fungi associated with internal tissues of seeds:**

Four hundred seeds in four replications, each of one hundred seeds were surface sterilized in 0.1 percent mercuric chloride solution for 3 minute. The seeds were subsequently washed in sterilized distilled water for 2 to 3 times in order to remove the traces of mercury on seed surface and tested by standard blotter method for the presence of internally seed borne fungi as described earlier.

**[5](I) Location of fungi in seed components:**

The experiment was conducted to know, the exact location of fungi in seeds by component plate technique (Madenet. al. 1975) one hundred seeds, each of Prabhat and T-21 were soaked in sterilized water, individually for more than one night and each seed was dissected aseptically into components, i.e. seed coat, cotyledons and embryonal axis under sterioscopic binocular microscope. Surface contaminants were eliminated by dipping seed components in 1.0 percent chlorine solution for 10 minutes. All the components of each seed were then plated directly on three layered moist blotters in a petridish and incubated at 28 + 10c for 7 days under twelve hours alternating cycles of light and darkness and examined under sterioscopic binocular microscope for the fungal infection in different components.

**[6] Growth habit, cultural and morphological characters of isolated fungi leading to their specific identification:**

The growth characters of each fungal species as it appeared and looked on seeds were recoreded under sterioscopic binocular microscope (6.4 to 40 x magnification). The cultural and morphological characters of each fungus were studied individually either on malt extract agar or on potato dextrose agar after 7-10 days of incubation. The morphological characters, i. e, shape, size and colour of the sexual and asexual structures of fungi were recorded and measured under compound microscope. The size of fungal spores or fructifications or mycelia was Based on measurements of fifty counts and standard nomenclature for colour according to (Ridgway, 1912). The species of fungus were identified with the help of standard identification manuals or monographs.

**[7] Methods of studying pathogenic behaviour of isolated fungi:**

Pathogenicity test in the pots was carried out to detect the pathogenic nature of isolated fungal species.

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