INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 13 | ISSUE 1 | APRIL, 2020 | 40-44

● ISSN-0974-2670 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/13.1/40-44

Detection of seed mycoflora associated with Indian bean cultivars

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ARITCLE INFO

Received : 20.01.2020
Revised : 21.02.2020
Accepted : 05.03.2020

KEY WORDS :
Indian bean, Cultivars, Seed mycoflora, Agar plate method, Blotter paper method

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ABSTRACT

Seed mycoflora detected in agar plate method was *Rhizopus stolonifer*, *Fusarium oxysporum*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Penicillium* sp. in five different Indian bean cultivars. Overall 5.35 to 23.94 per cent seeds showed various seed mycoflora. *Aspergillus niger* developed maximum colonies and *Penicillium* sp. the least. Similarly seed mycoflora was detected in blotter paper method, 13.49 to 35.90 per cent seeds showed various seed mycoflora. *Aspergillus niger* developed maximum colonies and *Rhizopus stolonifer* the least. Per cent seed mycoflora was high in blotter paper mehod compared to agar plate method.

How to view point the article : Prajapati, Dhara R. and Patel, P.R. (2020). Detection of seed mycoflora associated with Indian bean cultivars. *Internat. J. Plant Protec.*, **13**(1) : 40-44, **DOI : 10.15740/HAS/IJPP/13.1/40-44,**

INTRODUCTION

Indian bean (*Lablab purpureus* L.) (Sweet) usually known as *Dolichos* bean, Hyacinth bean or Field bean is one of the most ancient crop among cultivated plants. It is a bushy, semi-erect, perennial herb, mainly cultivated either as a pure crop or mixed with finger millet, groundnut, castor, corn, *Bajra* or sorghum in Asia and Africa. It is a multipurpose crop grown for pulse, vegetable and forage purpose (Gowda, 2013).

In India, *Lablab* is a field crop mostly confined to the peninsular region and cultivated to a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra. Karnataka contributes a major share, accounting for nearly 90 per cent in terms of both area and production in the country. Outside India, the crop is cultivated in East Africa, with similar uses and in Australia as a fodder crop (Gowda, 2013).

Indian bean has been reported to suffer from various types of disease and majority of them are known to be caused by fungi which are seed borne in nature. Among them, anthracnose caused by *Colletotrichum* sp., blight caused by *Alternaria* sp., vascular wilt caused by *Fusarium* sp. and other molds like *Rhizopus* sp. and *Mucor* sp. are major seed borne mycoflora observed in Indian bean (Saxena and Kumari, 2017). Seed borne fungi are the most important plant pathogens that cause direct and indirect losses of the bean crop throughout the world (Schwartz and Galvez, 1980).

Indian bean is affected by total twenty two fungal species. Among all, Important seed borne fungal diseases are blight (*Alternaria alternata* Fr. Keissl.), (*Curvularia lunata* Wakker.), seed rot/vascular wilt (*Fusarium*

oxysporum Schltdl.), (*Rhizopus stolonifer* Ehrenb.), *(Mucor speciosus* Oudem.), (*Penicillium notatum* Westling.), (*Cladosporium* Link.) bean anthracnose (*Colletotrichum dematium* Pers.) and seedling blight/ charcoal rot (*Macrophomina phaseolina* Tassi.) were dominant pathogens which caused seed borne diseases. These all species were found to cause considerable amount of loss, spoilage of storage grains and also produce mycotoxins. Among them, *Alternaria* spp., *Aspergillus flavus* (Link.), *Cheatomium* spp. and *Curvularia* spp. were associated with the seeds while remaining fungal species are mostly storage fungi (Saxena and Kumari, 2017).

MATERIAL AND METHODS

The experiment was undertaken at the Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari during 2018-19.

Collection of seed samples:

Seeds of Indian bean cultivars collected from major Indian bean growing areas of Navsari as well as Surat district and also from the Pulse Research Station, Navsari Agricultural University, Navsari. There were five major varieties were collected named GV 1, GV 2, GNIB 21, GNIB 22, VAL 125-26. The trial was laid out in Completely Randomized Design under laboratory condition.

Detection and isolation of pathogens:

For detection and isolation of seed mycoflora from Indian bean seeds two different seed health testing methods *viz.*, standard blotter method and agar plate method (ISTA,1985) were used.

Standard blotter method (ISTA, 1985):

Three layers of sterilized blotter paper disc of 90mm diameter were placed in sterile Petri plates (90mm diameter) and moistened with sterile distilled water. The excess water was drained off from the plates. Ten seeds of each variety were placed at equal distance on moistened blotter in Petri plates by having one seed at the center and nine seeds at the periphery. They were inoculated under 12/12hr alternating light and darkened period at 25±2ºC for seven days in BOD incubator. Ten repetitions were taken for the confirmation of presence or absence of fungi associated with it, developing fungal growth on each of them were observed regularly and identified. The total number of fungal colonies were calculated and expressed in percentage.

Agar plate method (ISTA, 1985):

The collected seeds of each of the five varieties *viz.,* VAL-125-36, GV 2, GNIB 22, GNIB 21 and GV 1 were used separately in the experiment. Ten seeds of each variety were taken and then transferred aseptically in Petri plates containing 20ml solidified potato dextrose agar (PDA) medium at equal distance per plate by keeping one seed at the center and nine seeds at the periphery. Then seeds were incubated under 12/12hr alternating light and darkened period at 25±2ºC for seven days. Four hundred seeds from each sample were used. The total fungal colonies were calculated and Frequency of occurrence of fungal species was calculated as per Butt *et al.* (2011).

$$\text{Total fungal colonies (\%)} = \frac{\text{No. of seeds colonized in each plate by fungal species}}{\text{Total no. of seeds in each plate}} \times 100$$

Further isolated fungi were pure cultured and stored in PDA slants under 5^0C for further use.

RESULTS AND DISCUSSION

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

Assessement of Seed mycoflora by standard blotter method:

Detection of per cent seed mycoflora load on different Indian bean cultivars was carried out by standard blotter method under laboratory condition. Results showed that 7 fungal species belonging to 5 genera were associated with different five Indian bean cultivars, but per cent incidence of seed mycoflora load on seeds was higher in all five Indian bean cultivars as compared to Agar plate method (Table 1).

Result revealed that *Aspergillus niger* was found significantly highest (25.30%) in GV 1(35.90%) followed by GV 2(33.89%) cultivar of Indian bean. Whereas, significantly lowest (13.35%) recorded in VAL 125-36. The frequency of *Fusarium oxysporum* was significantly observed maximum in GV 2(31.71%) and GV 1(29.30%)

followed by GNIB 22 and VAL 125-36 (24.83% and 20.71%), respectively, which was minimum (14.15%) in GNIB 21. *Curvularia lunata* recorded significantly maximum (30.25%) in GNIB 22 followed by GNIB 21 (29.62%), while it was minimum (19.16% and 16.22%) in VAL 125-36 and GV 1, respectively (Fig. 1).

The present findings are in conformity with the earlier findings of Shovan *et al.* (2008) who reported that blotter method was found effective for detection of seed borne fungi in soybean and observed *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp. and *Rhizopus stolonifer* in soybean.

Most of the fungal species detected in the present study was reported earlier by Saxena and Kumari (2017) who reported seed mycoflora of Indian bean *viz.*, *Aspergillus* sp., *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Rhizopus stolonifer, Mucor* sp., *Cheatomium* sp., *Cladosporium* sp., found associated with the seeds as important seed borne fungi isolated using both blotter paper method and agar plate method.

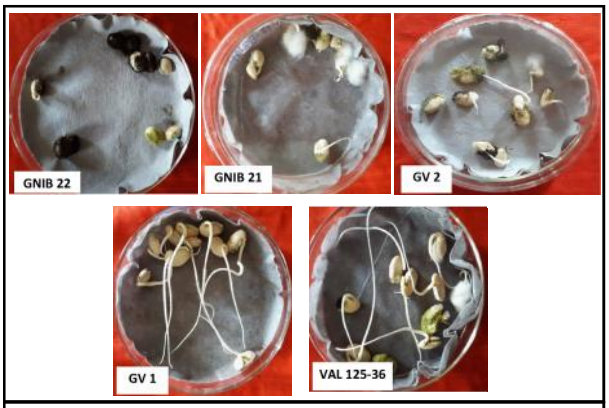

Plate 1: Seed mycoflora by standard blotter method

Assessement of Seed mycoflora by agar plate method:

Detection of per cent seed mycoflora load on different Indian bean cultivars was carried out by agar plate method under laboratory condition. Results showed that 7 fungal species belonging to 5 genera were associated with different five Indian bean cultivars. Seed mycoflora load on Indian bean seeds were observed *i.e.*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *curvularia lunata, Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Penicillium* sp. (Table 2).

Result revealed that overall *Aspergillus niger* was found significantly highest (23.04%) and also in GV 2 (35.55%) followed by GV 1(32.80%) cultivar of Indian bean whereas, significantly lowest (11.70%) recorded in VAL 125-36. The frequency of *fusarium oxysporum* was significantly observed maximum in GV 2(28.69%) followed by GV 1(28.40%), which was minimum

Table 1 : Occurrence of seed mycoflora on seeds of Indian bean cultivars by blotter paper method

Fungi	Per cent seeds showing seed mycoflora					Mean
	Cultivars					
	GV 1	GV 2	GNIB 21	GNIB 22	VAL 125-36	
Aspergillus niger	35.90* (34.54)	33.89 (31.12)	27.87 (21.86)	15.50 (7.15)	13.35 (5.35)	25.30 (20.00)
Fusarium oxysporum	29.30 (24.09)	31.71 (27.66)	14.15 (5.98)	24.83 (17.64)	20.71 (12.52)	24.14 (17.58)
Curvularia lunata	16.22 (7.81)	23.19 (15.52)	29.62 (24.43)	30.25 (25.38)	19.16 (6.53)	23.69 (15.93)
Aspergillus flavus	20.01 (11.73)	21.05 (12.92)	24.48 (17.17)	24.88 (17.71)	0.00 (0.00)	18.08 (10.46)
Rhizopus stolonifer	0.00 (0.00)	12.89 (4.99)	15.61 (7.24)	20.79 (12.60)	0.00 (0.00)	9.86 (3.06)
Alternaria alternata	0.00 (0.00)	0.00 (0.00)	13.49 (5.45)	13.72 (5.64)	0.00 (0.00)	5.44 (1.36)
Penicillium sp.	0.00 (0.00)	0.00 (0.00)	20.77 (12.58)	20.71 (12.51)	0.00 (0.00)	8.30 (3.15)
S.E.±	0.15	0.19	0.18	0.23	0.11	-
C.D. (P=0.05)	0.45	0.58	0.53	0.67	0.35	-
CV%	2.11	2.25	1.74	2.12	2.42	-

*Figures outside parentheses are arcsine transformed value. Figures in parentheses are original values

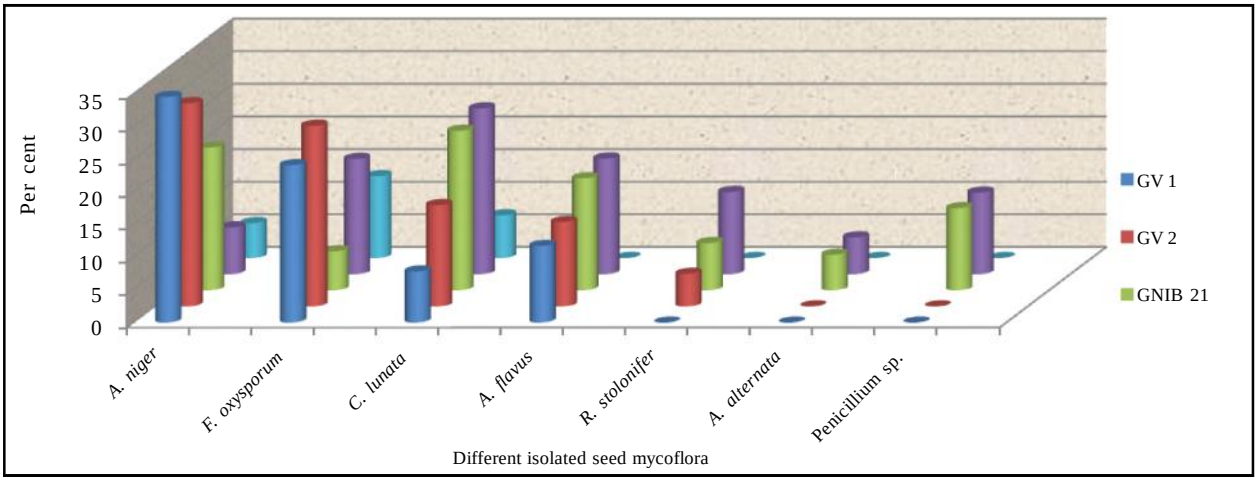

Fig. 1: Per cent seed mycoflora on blotter paper method

(11.40%) in VAL 125-36. *Curvularia lunata* recorded significantly maximum (31.07%) in GNIB 22 followed by GNIB 21 (25.51%), while it was minimum (9.72%) in VAL 125-36 (Fig. 2).

The present findings are also in conformity with the reports of Saxena and Kumari (2017) who found twenty two seed mycoflora of Indian bean using agar plate method. Among them *Aspergillus* sp., *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Rhizopus stolonifer* and *Mucor* sp. were found most dominant fungi.

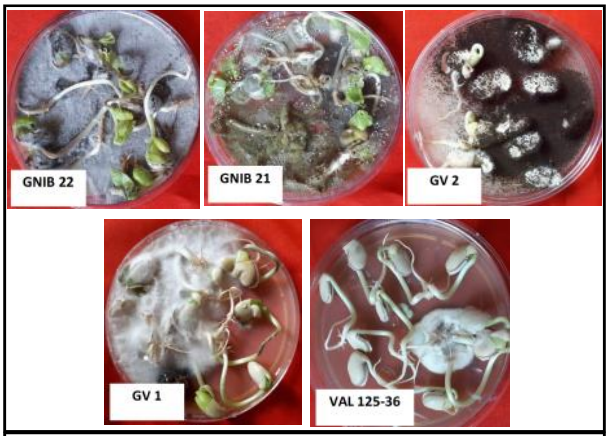

Plate 2 : Seed mycoflora by agar plate method

Conclusion:

Overall seven fungal species belonging to five

Table 2 : Occurrence of seed mycoflora on seeds of Indian bean cultivars by agar plate method

Fungi	Per cent seeds showing seed mycoflora					Mean
	Cultivars					
	GV 1	GV 2	GNIB 21	GNIB 22	VAL 125-36	
Aspergillus niger	32.80*	35.55	15.14	24.54	11.70	23.94
	(29.38)	(33.83)	(6.83)	(17.25)	(4.17)	(18.28)
Fusarium oxysporum	28.40	28.69	13.59	13.47	11.40	19.11
	(22.75)	(23.07)	(5.53)	(5.43)	(3.94)	(12.14)
Curvularia lunata	23.40	21.57	25.51	31.07	9.72	22.25
	(15.83)	(13.53)	(18.57)	(26.63)	(2.86)	(15.48)
Aspergillus flavus	17.90	19.43	27.07	27.34	0.00	18.34
	(9.49)	(11.08)	(20.73)	(21.10)	(0.00)	(12.48)
Rhizopus stolonifer	0.00	0.00	9.41	17.37	0.00	5.35
	(0.00)	(0.00)	(2.70)	(8.95)	(0.00)	(2.33)
Alternaria alternata	0.00	0.00	15.94	15.30	0.00	19.00
	(0.00)	(0.00)	(7.56)	(6.98)	(0.00)	(2.90)
Penicillium sp.	0.00	0.00	11.44	9.32	0.00	13.30
	(0.00)	(0.00)	(3.94)	(2.63)	(0.00)	(1.31)
S.E.±	0.20	0.16	0.16	0.32	0.03	-
C.D. (P=0.05)	0.61	0.5	0.24	0.96	0.10	-
CV%	2.81	2.24	2.00	3.30	1.54	-

*Figures outside parentheses are arcsine transformed value. Figures in parentheses are original values.

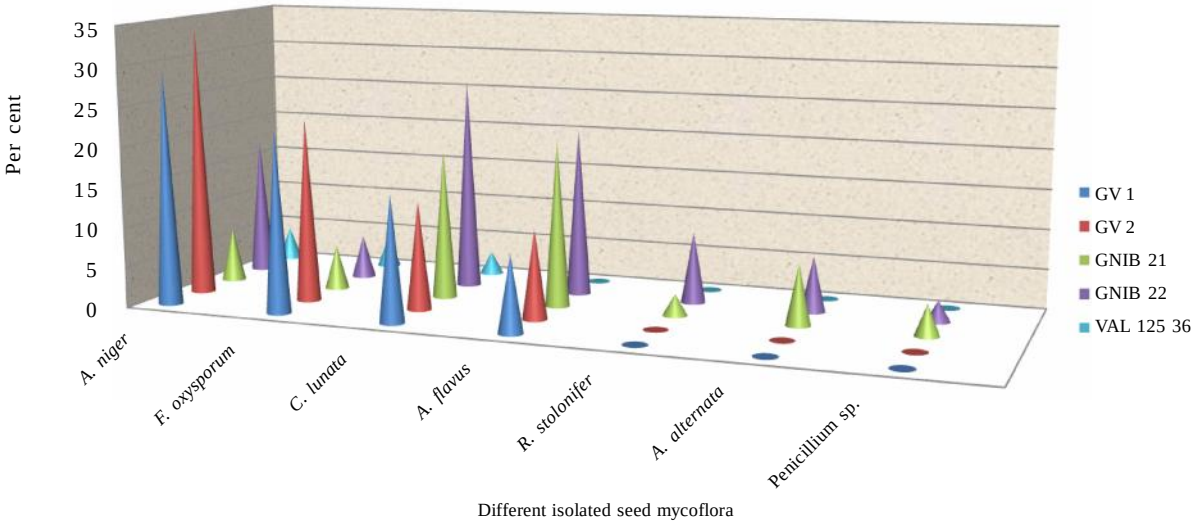

Fig. 2 : Per cent seed mycoflora on agar plate method

genera *viz.*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Penicillium* sp. detected by blotter paper method. *Aspergillus niger* was observed significantly highest in cultivar GV 1 (35.9%) and in GV 2(33.89%) followed by *Curvularia lunata* in cultivar GNIB 21(29.62%) and GNIB 22 (30.25%). *Alternaria alternata* showed significantly lowest (13.49%) incidence of seed mycoflora in GNIB 21.

Overall seven fungal species belonging to five genera *viz.*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Penicillium* sp. detected by agar plate method. The mean data indicated *Aspergillus niger* was found significantly highest (23.94%) in all five cultivars which was followed by *Curvularia lunata* (22.25%), *Aspergillus flavus* (18.34%) and *Fusarium oxysporum* (19.11%). *Penicillium* sp., *Alternaria alternata* and *Rhizopus stolonifer* fungi showed significantly lowest per cent incidence in all Indian bean cultivars (13.30, 19.00 and 5.35%), respectively.

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