

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.099>

Prevalence of Seed mycoflora of Mung bean (*Vigna radiata* L.) in Karnataka, India

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ABSTRACT

Keywords

Mung bean,
Seed,
Mycoflora,
Infection,
Districts etc.

Article Info

Accepted:
14 May 2017
Available Online:
10 June 2017

Eighteen samples of mung bean seeds collected from thirteen districts of Karnataka viz., Bengaluru, Chikkaballapura, Tumakuru, Mysuru, Mandya, Hassan, Chitradurga, Chamarajanagar, Dharwad, Kalaburgi, Raichur, Davanagere and Bagalkot were tested for seed mycoflora by employing standard blotter method as per the International rules for seed testing. Maximum seed infection was recorded in Kalaburgi district (25.73%) followed by Raichur (24.08%), Bagalkot (16.33%) and least infection were recorded in Tumakuru district (6.83%). Maximum association of seed mycoflora was by *Fusarium oxysporum* (39.72%) followed by *Penicillium notatum* (38.50%), *Aspergillus niger* (19.18%) and least with *Chaetomium globosum* (1.7%). In total twelve fungi belonging to ten genera viz., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus candidus*, *Alternaria alternata*, *Penicillium notatum*, *Rhizopus tolonifer*, *Cladosporium* sp, *Fusarium oxysporum*, *Mucor* sp, *Curvularia lunata*, *Chaetomium globosum*, and *Macrophomina phaseolina* were recorded on mung bean seeds collected from different districts of Karnataka.

Introduction

Mung bean [*Vigna radiata* L. Wilczek, syn. *Phaseolus aureus* Roxb, *P. radiates* L.] is the third most important pulse crop among the thirteen food legumes grown in India. It is also commonly known as green gram, which is an ancient and well known leguminous crop of Asia. In India, the name green gram is more commonly used than mung bean (Chatterjee and Randhawa, 1952). This crop is native to Indo-Burma region of South-East Asia. It is cultivated extensively in the Indo-Burma-Thailand region of Asia.

Mung bean contains about 24 per cent protein, which is about two third of the protein content of soybean, twice that of wheat and thrice that of rice.

The protein present in mung bean is comparatively rich in lysine, an amino acid that is deficient in cereal grains which are rich in methionine, cystine and cysteine, the sulphur bearing amino acids. Therefore, a diet combining mung bean and cereal grains form a balanced amino acid diet.

Mung bean is grown mainly as a Kharif crop. However, its cultivation in Rabi is restricted to the eastern and southern parts of the country. The major mung bean growing states are Odisha, Maharashtra, Andhra Pradesh, Rajasthan, Karnataka and Gujarat. It ranks third among all pulses grown in India after chickpea and pigeon pea. In India, the total

production of mung bean is 15 lakh tonnes from an area of 34.40 lakh ha with a productivity of 406.98 kg ha⁻¹, of which Karnataka occupies 5.28 lakh ha with a production of 1.08 lakh tonnes and average yield of 204.55 kg ha⁻¹ (Anonymous, 2011).

Among the various factors responsible for low yields, biotic and abiotic stresses take a heavy toll of the crop, out of which diseases cause an estimated yield loss of 20 to 30 per cent (Singh, 1995). Green gram suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among the fungal diseases, powdery mildew, anthracnose, *Cercospora* leaf spot, web blight and dry root rot are the most prevalent diseases. Apart from foliar and soil borne diseases, same fungi are also causing qualitative and quantitative loss in the storage.

Seed is the focal point in agriculture development without which an agriculture system is meaningless (Schwin, 1994) and high quality seed is an important pre-requisite for sustainable and profitable crop production. Seed health is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigour and reduced yield (Van Gastel *et al.*, 1996).

Diseases and injuries to seeds are caused by micro-organisms including virus, bacteria, fungi and nematodes. Among the parasitic organisms, the fungi are frequently encountered on seeds (Neergaard, 1977). The control of seed borne pathogens is the first step in any agricultural crop production and protection programme.

Storage fungi do not invade before the harvest, but they may be found on the seeds in low percentages often below one per cent. Seeds are known to carry a considerable amount of microorganisms. Some of these cause various diseases. Pathogens are

associated with seeds in the form of contaminants, externally and internally as seed borne. These organisms become active under favourable condition and affect seed germination which results in lower plant population and abnormal seedlings in field, thereby causing considerable reduction in yield (Christensen and Lopez, 1963).

Invasion by fungi in storage might result in the discoloration of the seeds, rise in temperature, mustiness, loss in weight and various changes in the seed constituents. Some of the seeds infecting fungi produce mycotoxins such as aflatoxin, patulin, citrinine and ochratoxin (Bilgrami *et al.*, 1979).

The seed mycoflora are carried over from year to year and from one place to another with the seeds which serve as primary source of infection for subsequent crops. Management of seed borne diseases has been reasonably achieved through fungicides and biological agents. Seed treatment is one of the important methods in the integrated management of any disease and has provided excellent results in reducing losses caused by diseases with increase in quality and quantity of seed, although chemicals have been successful in managing the diseases but they are hazardous to the environment apart from the possibility of the toxicity getting into the growing plant and finally into the food chain. Therefore, seed must be “substantially free” from inoculum with high level of germination and purity before sowing.

Presently, the information on the mung bean seed mycoflora is meagre. Hence, there is a need to generate information on the prevalence of seed mycoflora. Keeping this in view, present investigation was envisaged.

Materials and Methods

The present investigations on seed mycoflora

of mung bean (*Vigna radiata* L.) included identification of seed mycoflora on mung bean samples collected from different districts of Karnataka. The investigations were carried out at the College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru during 2014-2016.

Collection of mung bean seeds for the identification of seed mycoflora in different districts of Karnataka

To identify seed mycoflora prevailing in different districts, eighteen seed samples were collected from thirteen different districts viz., Bagalkot, Bengaluru, Chamrajanagar, Chikkballapur, Chitradurga, Davanagere, Dharwad, Hassan, Kalaburgi, Mandya, Mysuru, Raichur and Tumakuru of Karnataka. While two samples were collected from Davanagere (Harihar and Davanageretaluk), Chikkabalapura (Gauribidanur and Chikkabalapurataluk), Mysuru (Hunsur and Mysurutaluk), Raichur (Lingsugur and Raichurtaluk), Tumakuru (Tiptur and Tumakurutaluk) districts; only one sample was collected from Bagalkot, Bengaluru, Chamrajanagar, Chitradurga, Dharwad, Hassan, Kalaburgi, Mandya districts.

Seed mycoflora

Eighteen seed samples of mung bean were collected from different districts of Karnataka and assayed for seed mycoflora by employing standard blotter method as per the International Rules for Seed Testing. According to ISTA (Anonymous, 1996), four hundred seeds of each sample were placed equidistantly, aseptically on three layers of moist blotters moistened with sterile distilled water in sterile Petriplates of 90 mm diameter at the rate of twenty-five seeds per plate and the plates were incubated for seven days under diurnal cycles of 12 h light and 12 h darkness at room temperature of 28 ± 1 °C.

After incubation seed mycoflora was recorded on eighth day by observing fungal growth on seeds under stereo binocular microscope. Further, the species were confirmed by preparing slides and their frequency of occurrence was expressed in percentage.

Identification of seed mycoflora

The seed mycoflora were identified by studying the morphological characters of the fungus and referring to the “The genera of fungi sporulating in pure culture (Von Arx and Cramer, 1981), Hyphomycetes taxonomy and biology (Subramanian, 1983), the genus *Fusarium* (Booth, 1971) and Illustrated genera of imperfect fungi (Burnett, 1972).

Results and Discussion

Seeds samples collected from different districts were plated on moist blotters by following the standard blotter method. The observations were recorded and the data is presented in table 1, figure 1 and plate 1. Twelve fungi belonging to ten genera viz., *Aspergillus niger*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus candidus*, *Penicillium notatum*, *Rhizopus stolonifer*, *Cladosporium sp.*, *Fusarium oxysporum*, *Mucor sp.*, *Curvularia lunata*, *Macrophomina phaseolina* and *Chaetomium globosum* were observed in all the eighteen mung bean seed samples collected from different districts.

Prevalence of seed mycoflora in seed samples revealed that seed sample collected from Bagalkottaluk of Bagalkot district, recorded maximum association of *P. notatum* (46 %) followed by *F. oxysporum* (38 %), *M. phaseolina* (30 %), *A. niger* (22 %), *Cladosporium sp.* (18 %), *A. flavus* (14 %), *A. candidus* (9 %), *A. alternata* (7 %), *C. lunata* (4 %), *R. stolonifer* (4 %), *Mucor sp.* (2 %) and *C. globosum* (2 %). Obtained results were in conformity with Tripti Agarwal *et al.*,

(2011), they isolated *A. alternata*, *Chaetomium* spp., *Penicillium citrinum*, *Aspergillus niger*, *A. flavus*, *Rhizopus nigricans*, *Fusarium oxysporum* from the collected samples of chickpea, lentil and black gram seeds. Mukhtar *et al.*, (2007) also reported association of similar mycoflora in lentil seeds.

Seed sample collected from Bengaluru north taluk of Bengaluru district showed prevalence of seed mycoflora with maximum association of *F. oxysporum* (59 %) followed by *P. notatum* (34 %), *A. flavus* (21 %), *Cladosporium sp.* (16 %), *A. niger* (14 %), *M. phaseolina* (12 %), *A. alternata* (4 %), *R. stolonifer* (4 %), *Mucor sp.* (3 %), *C. lunata* (2 %) and *A. candidus* (2 %). Rauf (2000) also recorded *Alternaria alternata*, *Ascochyta* spp., *Colletotrichum* spp., *Fusarium* spp. and *Macrophomina phaseolina* as the most frequent fungi in legume crop seeds.

Seed sample collected from the Chamarajanagar taluk of Chamarajanagara district recorded maximum association of *F. oxysporum* (50 %) followed by *P. notatum* (46 %), *Cladosporium sp.* (24 %), *A. niger* (18 %), *A. flavus* (16 %), *M. phaseolina* (14%), *R. stolonifer* (4 %), *C. lunata* (3 %), *C. globosum* (3 %), *A. alternata* (2 %) and *Mucor sp* (1 %).

Similar mycoflora association was also reported by Chakravarthy *et al.*, (2001), Tomar *et al.*, (2002), Raj Naik and Papanna (2001) and Ramesh and Avitha Marihal, (2002).

Out of two seed samples collected from Chikkaballapura district, sample from Gauribidanur taluk recorded maximum association of seed mycoflora. Maximum association was by *F. oxysporum* (40 %) followed by *P. notatum* (38 %), *A. niger* (17 %), *M. phaseolina* (14%), *Cladosporium sp.*

(8 %), *A. flavus* (8 %), *C. globosum* (3 %), *A. candidus* (2 %), *A. alternata* (2 %) and *C. lunata* (1 %). Seed sample collected from Chikkaballapur taluk showed the maximum association of *P. notatum* (30 %), followed by *F. oxysporum* (24 %), *M. phaseolina* (20 %), *Cladosporium sp.* (14 %), *A. flavus* (12 %), *A. niger* (10 %), *Mucor sp.* (5%), *A. candidus* (4 %), *R. stolonifer* (2 %) and *C. globosum* (1 %). The findings of Shalini Verma and Dahroo (2003) were also the same type of mycoflora association with pea seeds. They recorded *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Pythium aphanidenriatum*, *Pythium ultimum*, *Gliocladium virens*, *Cladosporium herbarum*, *Rhizopus nigricans*, *Mucor sp*, *Dactylaria sp*, *Geotrichum* and *Rhizopus stolonifer*

Seed sample collected from Hiriyur taluk of Chitradurga district recorded maximum association of *F. oxysporum* (39 %) followed by *P. notatum* (32%), *A. niger* (27 %), *A. flavus* (20 %), *M. phaseolina* (12%), *Cladosporium sp.* (12 %), *R. stolonifer* (10 %), *A. alternata* (8 %), *C. lunata* (4 %), *A. candidus* (4 %) and *C. globosum* (2 %). Similar kind seed mycoflora association was noted by Dawar *et al.*, (2007) and Venugopal Rao *et al.*, (2015).

Out of two seed samples collected from Davanagere district, sample from Harihartaluk recorded maximum association of seed mycoflora. Maximum association was by *P. notatum* (54 %) followed by *F. oxysporum*. (36 %), *A. flavus* (22 %), *A. niger* (19 %), *A. candidus* (10 %), *M. phaseolina* (10%), *Cladosporium sp.* (6 %), *C. lunata* (4%), *R. stolonifer* (2 %) and *Mucor sp.* (1%). Seed sample collected from Davanageretaluk showed the maximum association of *F. oxysporum* (46 %), followed by *P. notatum* (39 %), *A. niger* (21 %), *Cladosporium sp.* (14 %), *A. flavus* (13 %), *M. phaseolina*

(10%), *R. stolonifer* (8 %), *A. candidus* (4 %), *A. alternata* (2 %), *Mucor* sp. (2 %) and *C. globosum* (1 %). Narayan and Ayodhya (2013) reported similar *Fusarium oxysporum*, *F. moniliform*, *F. solani*, *Chaetomium* sp, *Curvularia lunata*, *Macrophomina* sp., *Alternaria alternata*, *Chaetomium* spp., *Penicillium citrinum*, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Rhizopus nigricans*, *Monilia* sp., *Penicillium* sp., *Rhizoctonia* sp., and *Trichoderma* sp. mycoflora with pea, horse gram and green gram seeds. Seed sample collected from Dharwad taluk of

Dharwad district recorded maximum association of *F. oxysporum* (44 %) followed by *P. notatum* (28%), *A. niger* (23 %), *A. flavus* (18 %), *Cladosporium* sp. (15 %), *A. candidus* (12 %), *M. phaseolina* (6%), *R. stolonifer* (6 %), *C. lunata* (6 %), *A. alternata* (5 %), *C. globosum* (4 %) and *Mucor* sp. (2%). Ramesh *et al.*, also reported *M. phaseolina* (27.0 – 28.0%), *F. oxysporum* (5.0 - 5.5%), *A. flavus* (7.0%) and *A. niger* (3.5-4.0%) from the seed storage units and APMC market, Raichur in 2013.

Plate.1 Mycoflora on seeds after incubation on standard blotter

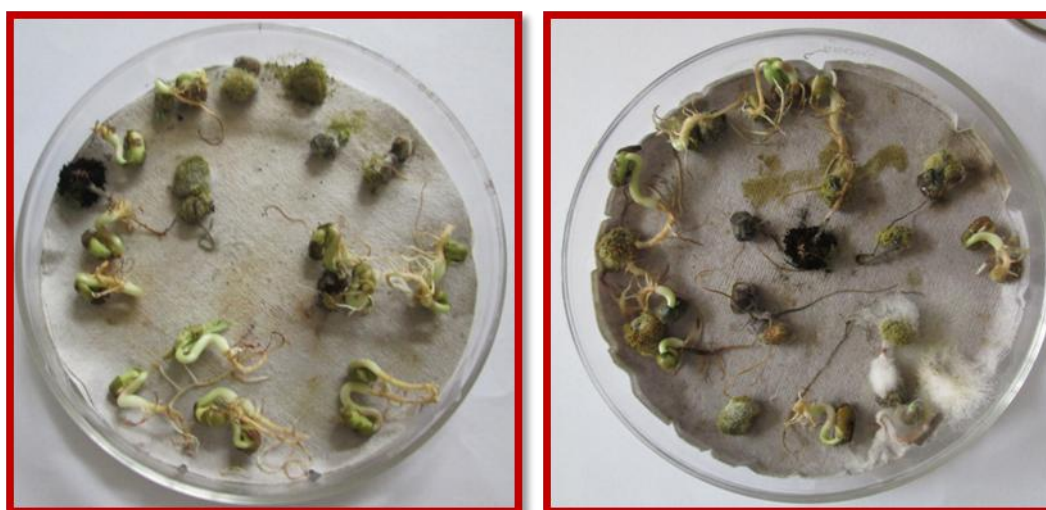


Fig.1 Mung bean seed mycoflora in Karnataka

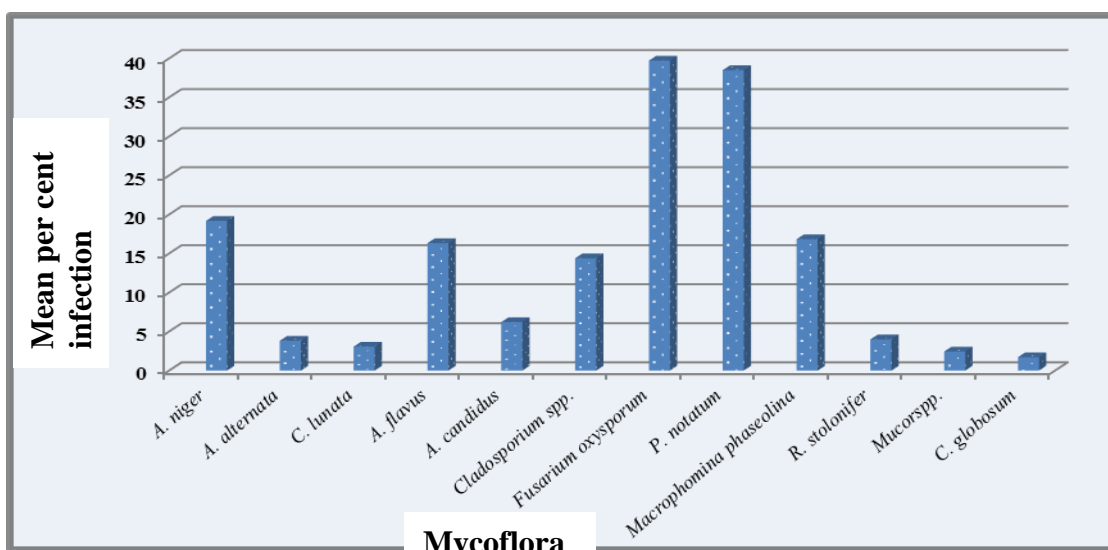


Table.1 Association of seed mycoflora in seeds of mung bean collected from different districts of Karnataka

Sl. No	Districts	Taluks	Seed germination (%)	Per cent seed mycoflora												
				<i>Aspergillus Niger</i>	<i>Alternaria alternate</i>	<i>Curvularia lunata</i>	<i>Aspergillus flavus</i>	<i>Aspergillus Candidus</i>	<i>Cladosporium spp.</i>	<i>Fusarium oxysporum</i>	<i>Penicillium notatum</i>	<i>Macrophomina phaseolina</i>	<i>Rhizopusstolonifer</i>	<i>Mucor spp.</i>	<i>Chaetomium globosum</i>	Mean
1.	Bagalkot	Bagalkot	81	22	7	4	14	9	18	38	46	30	4	2	2	16.33
2.	Bengaluru	Bengaluru North	91	14	4	2	21	2	16	59	34	12	4	3	0	14.25
3.	Chamarajnaragar	Chamarajnaragar	85	18	2	3	16	0	24	50	46	14	4	1	3	15.08
4	Chikkaballapura	Chikkaballapura	86	10	0	0	12	4	14	24	30	20	2	5	1	10.17
		Gauribidanur	95	17	2	1	8	2	8	40	38	14	0	0	3	11.08
5.	Chitradurga	Hiriyur	75	27	8	4	20	4	12	39	32	12	10	0	2	14.17
6	Davanagere	Harihar	88	19	0	4	22	10	6	36	54	10	2	1	0	13.67
		Davanagere	93	21	2	0	13	4	14	46	39	10	8	2	1	13.33
7.	Dharwad	Dharwad	87	23	5	6	18	12	15	44	28	6	6	2	4	14.08
8.	Hassan	Hassan	90	16	4	2	15	0	25	28	41	20	0	6	0	13.08
9.	Kalaburgi	Kalaburgi	78	36	10	8	30	14	26	65	62	46	12	8	2	25.73
10.	Mandya	Srirangapattana	87	18	0	1	12	6	10	22	24	12	2	1	0	9.00
11	Mysuru	Hunsur	89	20	0	3	11	3	6	39	28	10	1	6	2	10.75
		Mysuru	92	12	5	2	6	1	2	26	39	12	0	4	2	9.25
12	Raichur	Raichur	84	42	12	7	36	15	28	60	58	22	5	0	4	24.08
		Lingsugur	79	28	6	5	24	16	23	52	46	30	8	3	2	20.25
13	Tumakuru	Tumakur	92	8	2	0	10	8	8	29	28	8	2	0	2	8.75
		Tiptur	90	11	0	3	6	2	4	18	20	15	2	0	1	6.83
Mean				19.18	3.83	3.06	16.33	6.22	14.39	39.72	38.50	16.83	4.0	2.44	1.72	

From Hassan taluk of Hassan district, the seed sample collected showed the maximum association of *P. notatum* (41 %) followed by *F. oxysporum* (28 %), *Cladosporium sp.* (25%), *M. phaseolina* (20%), *A. niger* (16 %), *A. flavus* (15 %), *Mucor sp.* (6%), *A. alternata* (4 %) and *C. lunata* (2 %). The observed results were matched with Zaidi and Pathak (2013) they observed *Fusarium oxysporum*, *Alternaria alternata*, *Chaetomium spp.*, *Penicillium citrinum*, *Aspergillus niger*, *A. flavus* and *Rhizopus nigricans* relation with Mungbean seeds.

Kalaburgi district recorded maximum association of *F. oxysporum* (65 %) followed by *P. notatum* (62%), *M. phaseolina* (46%), *A. niger* (36 %), *A. flavus* (30 %), *Cladosporium sp.* (26 %), *A. candidus* (14 %), *A. alternata* (10 %), *C. lunata* (8 %), *Mucor sp.* (8%) and *C. globosum* (2%). Similar species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Rhizopus* and *Trichoderma* were reported by Ramesh and Avitha Marihal (2002) in association with the soybean crop seeds.

Seed sample collected from Srirangapattanaluk of Mandya district recorded maximum association of *P. notatum* (24 %) followed by *F. oxysporum*. (22 %), *A. niger* (18 %), *M. phaseolina* (12%), *A. flavus* (12%), *Cladosporium sp.* (10 %), *A. candidus* (6 %), *Mucor sp.* (6%), *R. stolonifer* (2 %), *Mucor sp.* (1%) and *C. lunata* (1 %). Tanuja (2015), Dawar *et al.*, (2007) and Ushamalini and ParameshaNaik (2008) found the similar findings during their studies on seed mycoflora.

Out of two seed samples collected from Mysuru district, sample from Hunsurtaluk recorded maximum association of seed mycoflora. Maximum association was by *F. oxysporum* (39 %) followed by *P. notatum*

(28 %), *A. niger* (20 %), *A. flavus* (11 %), *M. phaseolina* (10%), *Cladosporium sp.* (6 %), *Mucor sp.* (6 %), *A. candidus* (3 %), *C. lunata* (3 %), *C. globosum* (2 %) and *R. stolonifer* (1%). Seed sample collected from Mysurataluk showed the maximum association of *P. notatum* (39 %) followed by *F. oxysporum* (26%), *A. niger* (12 %), *M. phaseolina* (12 %), *A. flavus* (6 %), *A. alternata* (5 %), *Mucor sp.* (4 %), *Cladosporium sp.* (2 %), *C. globosum* (2 %), *C. lunata* (2%) and *A. candidus* (1 %). Krishnamurthy *et al.*, (2003) also reported many fungal species viz., *M. phaseolina*, *F. semitectum*, *F. moniliforme*, *A. alternata*, *A. terreus*, *A. flavus* and *F. solani* from mung bean seed samples collected from Chamarajanagar, Mysore and Gundlupettaluku of Karnataka.

Out of two seed samples collected from Raichur district, sample from Raichurtaluk recorded maximum association of seed mycoflora. Maximum association was by *F. oxysporum* (60 %) followed by *P. notatum* (58 %), *A. niger* (42 %), *A. flavus* (36 %), *Cladosporium sp.* (28 %), *M. phaseolina* (22%), *A. candidus* (15 %), *A. alternata* (12%), *C. lunata* (7 %), *R. stolonifer* (5 %) and *C. globosum* (4 %). Seed sample collected from Lingsugur taluk showed the maximum association of by *F. oxysporum* (52 %) followed by *P. notatum* (46 %), *M. phaseolina* (30 %), *A. niger* (28 %), *A. flavus* (24 %), *Cladosporium sp.* (23 %), *A. candidus* (16 %), *R. stolonifer* (8 %), *A. alternata* (6 %), *C. lunata* (5 %), *Mucor sp.* (3 %) and *C. globosum* (2 %). Ramesh *et al.*, (2013) also reported identical seed mycoflora from the samples collected from MARS and APMC units, Raichur.

Out of two seed samples collected from Tumakuru district, sample from Tumakurataluk recorded maximum association of seed mycoflora. Maximum

association was by *F. oxysporum* (29 %) followed by *P. notatum* (20 %), *A. flavus* (10 %), *A. niger* (8 %), *Cladosporium sp.* (8 %), *M. phaseolina* (8 %), *A. candidus* (8%), *A. alternate* (2 %), *R. stolonifer* (2 %) and *C. globosum* (2 %). Seed sample collected from Tipturtaluk showed the maximum association of *P. notatum* (20 %) followed by *F. oxysporum* (18 %), *M. phaseolina* (15 %), *A. niger* (11 %), *A. flavus* (6 %), *Cladosporium sp.* (4 %), *C. lunata* (3 %), *A. candidus* (2 %), *R. stolonifer* (2 %) and *C. globosum* (1%). Raij Naik and Papanna (2001) reported *Rhizopus sp.*, *Aspergillus sp.* and *Fusarium sp.* in association with green gram.

The predominant fungi detected in the order of prevalence were found to be *F. oxysporum* (65 %), followed by *Penicillium sp.* (62 %), *M. phaseolina* (46 %), *A. niger* (42 %), *A. flavus* (36 %), *Cladosporium sp.* (28 %), *A. candidus* (16 %), *Rhizopus sp.* (12 %), *Alternaria sp.* (12 %), *Mucor sp.* (8 %), *C. lunata* (8 %) and *C. globosum* (4 %). Highest seed mycoflora was recorded in Kalaburgi (25.73 %) followed by Raichur (24.08 %) and Bagalkot districts (16.33 %) and lowest seed mycoflora was seen in Tumakuru, Mandya and Mysuru districts of Karnataka.

Current study revealed the prevalence of seed mycoflora differs from districts to districts, this may be due to weather conditions prevailing in different districts which might have favoured association of certain mycoflora associated with the seed samples. Tanuja (2015) also observed prevalence of seed mycoflora differ from location to location.

During the present investigation also, similar fungi were associated with mung bean seeds in different districts. Seeds are the efficient carriers for survival, large scale and long distance spread of pathogens. Infected or contaminated seeds serve as major source of

inoculum for large number of plant pathogens which may infect the seeds and survive as spore or resting structures on or with in the seeds (Neergaard, 1977). This investigation opens the new avenues in studying and managing the seed mycoflora to get better management strategies.

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How to cite this article:

Devamani B.D., M. Saifulla, Jayappa and Jabbar Sab. 2017. Prevalence of seed mycoflora of Mung bean (*Vigna radiata* L.) in Karnataka, India. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 844-852. doi: <https://doi.org/10.20546/ijemas.2017.606.099>