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Studies of seed borne micoflora of mungbean and it's effect on seed germination and seedling vigour index

RM Dolas, SB Gawade and MC Kasture

Abstract
The present investigation was carried out on the seed borne mycoflora associated with the seeds of mung bean varieties and its individual effects on seed germination and seedling vigour index (*In vitro*). Similarly, different fungicides, bioagents and botanicals tested against different seed borne mycoflora and its positive effect on seed germination and seedling vigour index in mung bean were investigated. The seeds of varieties *viz.*, BPMR-145, Utkarsh, Kopergaon, BM-2002-1, AKM-8802, DGGV–2, SML-668 and Vaibhav were collected from Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar and Oilseed Research Station, Jalgaon. Six seed borne fungi *viz., Fusarium oxysporum, Macrophomina phaseolina, Curvularia lunata, Botrytis cinerea Aspergillus flavus* and *Aspergillus niger* were found associated with seeds of mung bean varieties externally and two seed borne fungi *viz., Fusarium oxysporum* and *Macrophomina phaseolina* were found associated internally. All the isolated pathogens were found pathogenic to seeds of mung bean resulting into reduction in seed germination and seedling vigour index. The seed borne mycoflora *viz., Fusarium oxysporum, Macrophomina phaseolina, Curvularia lunata, Aspergillus flavus* and *Aspergillus niger* were found more damaging. The pathogen *Macrophomina phaseolina* showed lowest seed germination and seedling vigour index over control. The seed germination and seedling vigour index due to *Macrophomina phaseolina* was 38 per cent and 741.0, respectively as against 85 per cent and 1962.4, respectively in control. The reduction in seed germination and seedling vigour index due to *Macrophomina phaseolina* was 55.29 and 62.24 per cent, respectively over control. The pathogens *viz., Fusarium oxysporum, Aspergillus flavus, Curvularia lunata, Aspergillus niger* and *Botrytis cinerea* also showed reduced seed germination and seedling vigour index. The reduction in seed germination was 47.05, 41.17, 36.47, 35.29 and 25.88 per cent while reduction in seedling vigour index was 55.05, 49.80, 48.71, 40.28 and 37.93 per cent, respectively for *Fusarium oxysporum, Aspergillus flavus, Curvularia lunata, Aspergillus niger and Botrytis cinerea* over control.

Keywords: borne micoflora, mungbean

Introduction
Green gram (*Vigna radiata* (L.) Wilczek) is commonly known as mung bean or mung. It is very ancient annual crop in Indian farming. Mung bean is especially grown in Southeast Asia but some are also grown in Africa and America. In India, it is one of the most important pulse crops. It is grown in almost all parts of the country. This crop is sown usually as dry land crop in almost all the states of India, namely Madhya Pradesh, Bihar, Uttar Pradesh, Andhra Pradesh, Rajasthan, Karnataka and Maharashtra. It is an excellent source of high quality protein and consumed in different ways. Ascorbic acid (Vitamin C) is synthesized in sprouted seeds of mung bean with increment in riboflavin and thiamine. Since mung bean is a leguminous crop, it has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop. Being a short duration crop it also provides an excellent green fodder to the animals.

Green gram is a highly nutritious containing 24 per cent of high quality protein, 1.3 per cent fats, 56.6 per cent carbohydrates and 3 per cent dietary fibers. It is rich in minerals having 140 mg calcium, 8.4 per cent iron and 280 mg phosphorous. It also contains 0.47 mg vitamin B_1, 0.39 mg vitamin B_2 and 2 mg niacin. It has calorific value of 334 calories per 100 g of edible protein (Baldev *et al.*, 2003) [4].

India is the world's largest producer as well as consumer of green gram. It produces about 1.5 to 2.0 million tons of mung bean annually from about 3 to 4 million hectares of area with an average productivity of 500 kg per hectare. Green gram output accounts for about 10-12 % of total pulse production in the country. Mung production in the country remained stable more than a decade through the 2000s at around 10 to 15 lakh tons. But a sudden jump in output was noted in 2010-11 to 1.75 million tonnes primarily on account of rise in production from Madhya Pradesh, Rajasthan and Tamil Nadu. In 2014-15 the mung bean production in India was 1.39 million tonnes in which, Maharashtra's contribution was about 20 %, while Rajasthan was highest having 26 % of the total production. Mung bean production in the country is largely concentrated in five states *viz*., Rajasthan, Maharashtra, Andhra Pradesh, Gujarat and Bihar. These five states together contribute for about 70 % of total Mung production in the country. There is a distinct change in production pattern of mung bean across states. Traditionally Rajasthan, Maharashtra, Andhra Pradesh are major mung bean producing states. But there is significant rise in production from other states in recent years particularly, from Tamil Nadu, Uttar Pradesh and Gujarat. Nevertheless, production remained volatile across the years with respect to most of the states. As per the latest available estimates, Rajasthan, Maharashtra occupies the first two positions, contributing over 45 %. Andhra Pradesh contributes about 10 % while together Gujarat and Bihar account for about 13 % of total production in the country (Anonymus, 2015) [3].

Seed borne diseases are regarded as major constraints in mung bean production. Infected seeds serve as the source for the spread of the pathogen in disease free area. Seed infection affects the import and export adversely because the seed affected with microbes is not acceptable in international market.

Seeds are the carrier of fungal flora either externally or internally. The variety and intensity of fungal flora changes area-wise and depends upon climate under which seed produced storage or in field, if, not controlled. Also they reduce seed quality i.e. seedling vigour and germination percentage. So it is necessary to control harmful fungi before causing damage by using suitable available measures i.e. chemicals or bio agents.

The seed borne pathogens associated with seeds externally or internally may cause seed rot, seedling blight and resulting into low germination. Some fungi are associated with testa and cotyledon of seeds infected in form of mycelium, pycnidium and conidia or spores, after germination the infection translation to hypocotyls and stem bases as well as dicotyledonary leaves of seedling. Some fungal seed borne pathogens having ability to kill the seedling or plants and substantially, reduce the productive capacity (Shamsur Rahman *et al.*, 1999) [17]. Seed mycoflora play an important role in determining the quality and longevity of seed.

As many as 16 diseases have been reported on mung bean, many of these diseases have been reported as seed borne. Seedling blight, root rot, stem rot, leaf and pod rot caused by *Macrophomina, Curvularia, Alternaria* are some major fungal diseases of mung bean. Species of *Alternaria, Cladosporium, Fusarium* and *Rhizoctonia* are known to cause seed rot and pre and post-emergence losses in green gram (Khare and Chaubey, 1978; Saxena, 1986 and Patil *et al.*, 1990) [9, 16, 14].

Several workers reported *Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Cladosporium* spp., *Colletotrichum* spp., *Curvularia lunata, Fusarium* spp.,

Macrophomina phaseolina, Phoma medicaginis and *Rhizopus* are seed borne and seed transmissible (Raut and Ahire, 1988; Patil *et al.*, 1990) [15, 14]. The above mentioned fungi are potentially harmful for cultivation of mung bean. So it is better to use some protective measures to control these pathogens.

The present investigation was undertaken to know the association of various fungi with mung bean seeds and their effect on seed germination and seedling vigour.

Material and Method

Mung bean seeds

To study the mycoflora associated with seeds of mung bean and to test the efficacy of bio agents, botanicals and fungicides on seed mycoflora, seed germination and seedling vigour index, the seeds of mung bean varieties were collected. The seeds of varieties *viz.*, BPMR-145, Utkarsh, Kopergaon, BM- 2002-1, AKM-8802, DGGV–2, SML-668 and Vaibhav were collected from Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar and Oilseed Research Station, Jalgaon.

Culture media

The commonly used laboratory medium i.e. potato dextrose agar (PDA) was used for detection and isolation of mycoflora associated with seeds of mung bean varieties.

Glass wares

The standard corning brand glasswares *viz.,* petriplates, conical flasks, slides and test tubes were used.

Equipments

The laboratory equipments *viz.,* autoclave, laminar flow cabinet, incubator, sterio-binocular microscope, research binocular microscope and weighing balance were used.

Incubation room

The incubation room was used for keeping the blotter plates. The temperature of incubation room was 20 ± 2 ^{0}C controlled automatically with alternate cycle of 12 hrs. light and 12 hrs darkness (Automatically controlled by electronic timer).

Miscellaneous material

Pointed needles, inoculating needle, forceps, blotting papers, scissor, glass marking pencil, glass rods, cover slips, towel papers, mercuric chloride, spirit lamp and sterilized water etc. were used.

Detection and isolation of seed borne mycoflora in mung bean

The following methods were used for detection of external and internal seed borne mycoflora in mung bean.

Detection of external seed borne mycoflora in mung bean

The ISTA's standard blotter test described by Neergaard (1979) [12] was used for detection of external seed borne fungi of mung bean. The varieties *viz.*, BPMR-145, Utkarsh, Kopergaon, BM-2002-1, AKM-8802, DGGV–2, SML-668 and Vaibhav were used for detection of external and internal seed borne mycoflora.

The protocol of standard blotter method used for this purpose is as below:

1. Blotter papers having size of 11 cm diameter were soaked in distilled water and placed in three layers in transparent petriplates (plastic) after draining off excess moisture.
2. A fixed number of seeds i.e. 10 per petriplate were placed equidistant from one another with 9 seeds in circle and

one at centre under aseptic conditions. Likewise 400 seeds were plated on petriplates for each variety.

3. After plating the seeds, the petriplates were incubated at 20 ± 2 ^{0}C in incubation room under alternate cycles of 12 hours light and 12 hours darkness for seven days.
4. The seeds were examined on 8th day under sterio-binocular microscope.
5. The mycoflora associated with seeds of mung bean varieties was identified mostly on the basis of morphological characters of fungi, fruiting body, conidiophores and conidia.
6. The mycoflora associated with seeds was also examined under research microscope for confirmation.
7. The per cent incidence of different seed borne fungi on seeds of mung bean was calculated on the basis of number of seeds infected with mycoflora.

Isolation of external seed borne mycoflora from seeds of mung bean

The fungal colonies of different fungi associated with seeds of mung bean varieties were picked up with the help of a sterilized inoculating needle and transferred on potato dextrose agar (PDA) slants, numbered and incubated at 26 ± 2^{0}C for seven days. The pathogens were purified and the pure isolates grown on potato dextrose agar slants were kept in refrigerator for further studies.

Detection of internal seed borne mycoflora of mung bean varieties

The internal seed borne mycoflora associated with seeds of mung bean varieties were detected by ISTA's standard agar plate test (Neergard, 1979) [12]. The method is described below:

1. The seeds of mung bean varieties were sterilized with 1.0 per cent NaOCl solution for 5 minute to prevent saprophytic development of pathogens. Three washings with sterilized water were given to remove corrosive sublimate.
2. Ten seeds of each variety were placed in each petridish containing 2 per cent water agar. These petridishes were incubated at 20 ± 2^{0}C in the incubation for 7 days. Total 100 seeds were plated on PDA plates aseptically under controlled condition.
3. The observations on seed borne mycoflora in seed of each variety of mung bean were recorded with the help of sterio-binocular microscope after 7 days of incubation.
4. The identification of fungi was done based on colony colour, colony size, type of mycelium, spreading habit, fruiting structures of fungi and by microscopy.
5. The per cent incidence of different seed borne fungi associated with seeds of mung bean varieties was calculated.

Isolation of internal seed borne mycoflora from seeds of mung bean

As soon as fungal colonies observed on seeds of mung bean varieties on plates were transferred aseptically on potato dextrose agar (PDA) slants with the help of sterilized inoculating needle under controlled condition. The isolates obtained were then maintained in pure form on potato dextrose agar slants, numbered carefully and preserved at low temperature in refrigerator for further studies.

Effect of seed borne mycoflora on seed germination and seedling vigour index in mung bean: Towel paper method (*In vitro*)

The effect of pathogenic seed borne mycoflora isolated from seeds of mung bean varieties on seed germination and seedling vigour index was studied by adopting the procedure of 'Between Paper' (rolled towel paper) method as described by ISTA's rules. Six important seed borne pathogens *viz., Fusarium oxysporum, Macrophomina phaseolina, Curvularia lunata, Botrytis cinerea, Aspergillus flavus* and *Aspergillus niger* isolated from the seeds of mung bean varieties were used for this study. The experiment was laid out in Completely Randomized Design (CRD) with four replications in laboratory.

Four hundred seeds of variety Vaibhav of mung bean were inoculated with the individual pathogen by dipping the seeds in concentrated suspension of spores (10^6 cfu/ml) for 12 hours. These seeds were then dried in shade for 12 hours (Agarwal and Sinclair, 1993) [2]. Fifty seeds were placed on one towel paper and rolled carefully avoiding disturbances of seeds from their places. For each pathogen 8 towel papers i.e. 50 seeds per towel paper (total 400 seeds) were used. The rolled towel papers were then kept in slanting position and incubated at 20-25 ^{0}C and RH above 85 per cent in seed germinator (Anonymous, 1999). A count of normal seedlings was recorded after 7 days. The seeds with full growth of plumule and radical were considered normal. The germination was expressed in percentage. The root and shoot length (cm) of randomly selected 10 normal seedlings from each towel paper were measured with the help of scale and seedling vigour index was computed by using formula given by Abdul-Baki and Anderson (1973).

SVI = [Mean root length (cm) + Mean shoot length (cm)] x Seed

Germination (%)

The per cent reduction in seed germination and seedling vigour index with the inoculation of pathogens over control was calculated. The data was subjected to statistical analysis (Panse and Sukhatme, 1985) [13].

Result and Discussion

Detection of external seed borne mycoflora associated with seeds of different mung bean varieties

The external seed borne mycoflora *viz., Fusarium oxysporum, Macrophomina phaseolina, Curvularia lunata, Botrytis cinerea, Aspergillus flavus* and *Aspergillus niger* were found associated with seeds of mung bean varieties, externally. Among the mung bean varieties, the incidence of seed borne mycoflora was found highest on seeds of variety Vaibhav i.e. 51.3 %, followed by Kopergaon (39.5 %), BM-2002-1 (38.9 %), Utkarsh (38.5 %), SML-668 (30.4 %), AKM-8802 (29.8 %), BPMR-145 (28.8 %) and DGGV-2 (27.9 %) respectively.

These results are more or less in agreement with Jharia (1970) [8], Agarwal *et al.* (1972) [1], Raut and Ahire (1988) [15], Chilkuri and Giri (2014) [5]. Jharia (1970) [8] found association of *Alternaria* spp., *Aspergillus flavus, A. niger, Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp., *Rhizoctonia* spp. and *Rhizopus* spp. with mung and urid seeds. Agarwal *et al.* (1972) [1] noted association of *Alternaria longissima, Cercospora* spp., *Colletotrichum truncatum, Curvularia lunata, Drechslera tetramera, Fusarium equiseti, F. moniliforme, F. semitectum, Macrophomina phaseolina, Myrothecium roridum* and *Phoma* spp. with green gram and black gram seeds. Raut and Ahire (1988) [15] noted association of 14 fungi with seeds of green gram *viz*., *Alternaria tenuis*, *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Cladosporium oxysporum*, *Drechslera rostrata*, *Fusarium moniliforme*, *F. semitectum*, *F. oxysporum*, *Macrophomina phaseolina*, *Nigrospora* spp., *Phoma medicaginis*, *Penicillium* spp. and *Rhizopus* spp. Chilkuri and Giri (2014) [5] examined seed samples of green gram by blotter method and showed association of 10 fungi belonging to eight genera *viz*., *Acremonium strictum*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Macrophomina phaseolina*, *Phoma medicaginis*, *Penicillium* spp. and *Rhizopus* spp.

Detection of internal seed borne mycoflora in mung bean varieties

The seed borne mycoflora *viz.*, *Fusarium oxysporum* and *Macrophomina phaseolina* was found associated with the seeds of mung bean varieties internally. Among the mung bean varieties, Vaibhav showed highest incidence of internal seed borne mycoflora i.e. 16.4 %. It was followed with seeds of varieties BPMR-145 (15.3 %), Kopergaon (11.7 %), BM-2002-1 (9.0 %), AKM-8802 (7.0 %), Utkarsh (6.8 %), SML-668 (5.5 %) and DGGV-2 (4.4 %).

These results are more or less in agreement with Nath *et al.* (1970), Chindhalore (1974) [6], Khare and Chaubey (1978) [9], Kumar and Singh (1996) [10] and Jaiman and Jain (2004) [7] Mandhare *et al.* (2009) [11]. Nath *et al.* (1970) detected various fungi of mung bean seeds by blotter and agar plate method *viz. Alternaria tenuis*, *Botrydiplodia palmatum*, *Cercospora kikuchii*, *Choanephora* spp., *Colletotrichum truncatum*, *Corynespora cassiicola*, *Curvularia lunata*, *Diaporthe phaseolorum* var. *sojae*, *Drechslera rostrata*, *Fusarium equiseti*, *F. moniliforme*, *F. semitectum*, *F. solani* and *Macrophomina phasiolina* were most predominant fungi and were found responsible for seed rot and loss of germination capacity. Chindhalore (1974) [6] showed internally seed borne infection of *Fusarium equiseti* and *Macrophomina phaseolina* in green gram and noticed that these fungi were highly pathogenic. Khare and Chaubey (1978) [9] reported *Fusarium equiseti*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum* and *Fusarium solani* to cause intra-embryonal infection in green gram. Jaiman and Jain (2004) [7] isolated 18 fungi by blotter and agar plate method including species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotricum*, *Curvularia*, *Drechslera*, *Fusarium*, *Gliocladium*, *Macrophomina*, *Myrothecium*, *Penicillium*, *Phoma*, *Pleospora* and *rhizopus*. Mandhare *et al.* (2009) [11] reported seed rot and seedling mortality of soybean resulting into reduced seed germination with seed borne infection of *M. phaseolina*.

Table 1: Detection and assessment of external seed borne mycoflora of mung bean varieties

Sr. No.	Varieties	Incidence of seed borne mycoflora (%)						Total incidence (%)
		F.o.	M.p.	C.l.	B.c.	A.f.	A.n.	
1	BPMR-145	6.7	7.3	3.1	-	3.5	8.2	28.8 (32.42)
2	Utkarsh	4.0	1.3	4.7	4.2	2.8	21.5	38.5 (38.35)
3	BM-2002-1	4.2	3.4	5.6	1.6	8.4	15.7	38.9 (38.63)
4	AKM- 8802	2.3	2.5	4.7	2.8	4.2	13.3	29.8 (33.04)
5	DGGV-2	2.0	1.7	2.3	1.6	2.4	17.9	27.9 (31.94)
6	Kopergaon	8.7	1.8	-	4.9	13.3	10.8	39.5 38.93
7	SML - 668	2.2	2.3	11.7	1.8	2.3	10.1	30.4 (33.45)
8	Vaibhav	6.8	9.6	5.7	3.9	12.1	13.2	51.3 (45.74)
	S. E. ±							0.61
	CD at 5 %							1.79
	C.V. (%)							3.36

Figures in parentheses indicates arc sin transformed values

Where,

F.o. : Fusarium oxysporum

M.p. : Macrophomina phaseolina

C.l. : Curvularia lunata

B.c. : Botrytis cinerea

A.f. : Aspergilllus flavus

A.n. : Aspergillus niger

Table 2: Detection and assessment of internal seed borne mycoflora of mung bean varieties

Sr. No.	Varieties	Incidence of seed borne mycoflora (%)		Total incidence (%)
		F.o.	M.p.	
1	BPMR-145	7.2	8.1	15.3 (22.96)
2	Utkarsh	4.5	2.3	6.8 (15.03)
3	BM-2002-1	5.1	3.9	9.0 (17.45)
4	AKM- 8802	3.3	3.7	7.0 15.34
5	DGGV-2	2.3	2.1	4.4 (12.22)
6	Kopergaon	9.3	2.4	11.7 (19.99)
7	SML - 668	2.6	2.9	5.5 (13.55)
8	Vaibhav	6.1	10.3	16.4 (23.87)
	S. E. ±			0.39
	CD at 5 %			1.14
	C.V. (%)			4.47

Figures in parentheses indicates arc sin transformed values

Where

F.o. : Fusarium oxysporum

M.p. : Macrophomina phaseolina

Table 3: Effect of seed borne mycoflora on seed germination and seedling vigour index of mung bean (Cv. - Vaibhav)

Sr. No.	Seed borne mycoflora	Seed germination (%)	Reduction in seed germination over Control (%)	Seedling Vigour Index (SVI)	Reduction in seedling vigour index over control (%)
1	*F. oxysporum*	45 (42.12)	47.05	882.0	55.05
2	*M. phaseolina*	38 (38.05)	55.29	741.0	62.24
3	*C. lunata*	54 (47.29)	36.47	1006.5	48.71
4	*B. cinerea*	63 (52.54)	25.88	1218.0	37.93
5	*A. flavus*	50 (44.99)	41.17	985.0	49.80
6	*A. niger*	55 (47.87)	35.29	1171.8	40.28
7	Control	85 (67.33)		1962.4	
	S.E ±	0.90		21.70	
	CD at 5 %	2.67		63.82	
	C.V.(%)	3.73		3.81	

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