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Effect of Seed Mycoflora on Mungbean Seed Health with Respect to Seed Germination and Seedling Vigour

Rameela I. Chaudhari*, Vikram R. Gohel and Tarun K. Mandaviya

Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University,
Anand-388110, Gujarat (India)

*Corresponding author

ABSTRACT

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Mungbean is grown principally for its protein content. Seed borne mycoflora affect the germination and vigour of seeds. 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. Total five cultivars viz., GM 3, GM 4, GAM 5, K 851 and Meha and eight seed mycoflora (fungal species) namely, *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus*, *Macrophomina phaseolina* and *Phoma* sp. were isolated and used for study. Result revealed that the minimum seed germination was observed in the seed treated with *Macrophomina phaseolina* (55.55%). The significantly lowest seedling length was observed in cultivar Meha (4.81 cm). Among all the tested fungus, seedling vigour index (SVI) was highly influenced by *Macrophomina phaseolina* (229.93). The lowest seedling vigour index was found in cultivar Meha (302.48). The highest per cent discolouration intensity was observed by *Macrophomina phaseolina* (85.25) in cultivar Meha.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important and extensively cultivated pulse crop. It is commonly known as “mung”, “green gram” or “mungbean”. It belongs to the family leguminaceae and native to India and Central Asia. The area under mungbean cultivation in India is 3.019 million hectares and production is 1.503 million tonnes (Anon., 2016). It is widely cultivated throughout the Asia including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia and South

China. Major mungbean producing states in India are Odisha, Maharashtra, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajasthan and Bihar. The area under mungbean in Gujarat is 0.129 million hectares and production is 0.061 million tonnes (Anon., 2016). Major mungbean producing districts in Gujarat are Kutchh, Banaskantha, Sabarkantha, Mahesana, Surendranagar and Ahmedabad. However, it is grown in very large areas during summer season in Kheda, Vadodara and Panchmahals districts and with the availability of irrigation water through Narmada project, area under mungbean has

been increase in Central and North Gujarat. Pulse production is very low and become challenging problem against the requirement of increasing population of our country. The pulse availability per capita was 69.9 g in 1951, by decreasing in 1971 it comes to 50 g and in 1982 remained only 40 g, and in 2005 it was 27 g and in 2012 it was 41.7 g. The availability of pulse is very negligible at present as against required 85 g/day/capita for balanced diet. To recover this deficit of production, it is a high time to cultivate pulse crops scientifically with increasing area.

The composition of mungbean seed is approximately 25.0 to 28.0% protein, 1.0 to 1.5% oil, 3.5 to 4.5% fiber, 4.5 to 5.5% ash and 62.0 to 65.0% carbohydrate on a dry weight basis (Singh *et al.*, 1970; Tsou *et al.*, 1979). "Seed health refers primarily to the presence or absence of the microorganisms of various kinds (ISTA, 1985). It has been realized by time and again that seed health, a paramount importance for crop stand and yield returns. Hence, aptly quoted "A healthy seed leads to a healthy harvest".

The infected seed fail to germinate or seedlings and plants developed in the field from infected seeds may escape early infection but may often be infected at the later stage of growth. Besides, pathogens can spread over a long distance and uninfected field may be infected by the seeds which carries different pathogens (Fakir *et al.*, 2001). 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). A large number of mycoflora was reported to be associated with the mungbean seeds. *Alternaria* sp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium equiseti*, *Myrothecium roridum*, *Drechslera* sp., *Aspergillus flavus*, *A. niger* and *Macrophomina phaseolina* were found in germinating seeds and seedlings of mungbean (Bakr and Rahaman, 2001).

Presently, the information the Effect of seed mycoflora on mungbean seed health with respect to seed germination, seedling length, seedling vigour index and per cent discolouration intensity. Keeping this in view, present investigation was envisaged.

Materials and Methods

The present investigation on "Status of seed mycoflora of mungbean [*Vigna radiata* (L.) Wilczek] cultivars and their management" included effect of seed mycoflora on mungbean seed respect to seed germination, seedling length, seedling vigour index and per cent discolouration intensity was under taken at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand during the year 2017-2018.

Effect of seed mycoflora on seed health

Total five mungbean cultivars *viz.*, GM 3, GM 4, GAM 5, K 851 and Meha and eight seed mycoflora (fungal) species namely, *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus*, *Macrophomina phaseolina* and *Phoma* sp. were isolated and used for the study. Following methods were used to study the influence of seed mycoflora on seed health in respect to seed germination and seedling vigour index.

Seed inoculation with seed mycoflora

Mungbean seeds of five cultivars were artificially inoculated with each of the seed mycoflora separately. Seeds moistened by sterilized water were mixed thoroughly with 10 days old respective fungal culture growth obtained on PDA at $25 \pm 2^{\circ}\text{C}$. Such treated seeds were kept in Petri plates for overnight at $25 \pm 2^{\circ}\text{C}$, and then these seeds were used for further study and recording seed germination, seedling vigour index and assessment of seedling abnormalities.

Effect on seed germinability

Effect of seed mycoflora on seed germination was tested by paper towel method. One sheet of germination paper was wetted by distilled water. Seeds (25) of respective mungbean cultivars inoculated with respective seed mycoflora were placed on first sheet evenly in a row. Second sheet of germination paper was placed on first sheet. Second sheet was wetted carefully. Both sheets were rolled along with wax coated paper. The rolled paper were incubated in seed germinator at 25°C for 7 days. At the end of incubation, rolled towel papers were carefully opened. Germinated and ungerminated seeds were counted treatment-wise and variety-wise. Healthy seeds without inoculation of seed mycoflora were kept as control. Four repetitions each of 100 seeds were maintained for each of the treatments. Seedling length of germinated seeds were recorded.

Seedling Vigour Index (SVI)

Seedling vigour index was calculated on the basis of seed germination and seedling length (root length and shoot length) after seven days of incubation (Abdul-Baki and Anderson, 1973).

Vigour Index (VI) = (Mean root length + Mean shoot length) × Seed germination (%)

Assessment of seedling abnormalities by discolouration grade

The symptoms were observed after ten days of incubation of inoculated seeds of respective cultivars by using paper towel method. Each of the developing seedling was observed visually critically and with the help of magnifying hand lens for seedling discolouration due to seed mycoflora infection on developing seedlings. Seedling discolouration was categorized using 0-4

rating scale. Four repetitions each of 25 seeds were evaluated for each of the treatments.

Following formula was used to calculate the percent discoloration intensity (Wheeler, 1969).

$$\text{PDI} = \frac{\text{Sum of total ratings}}{\text{No. of seedlings examined} \times \text{Maximum rating scale}} \times 100$$

Results and Discussion

Effect of seed mycoflora on seed germination

The seed germination was influenced by seed mycoflora. The result revealed that the lowest seed germination was found in cultivar Meha (58.72%) followed by cultivar GM 4 (65.56%) and cultivar GM 3 (73.50%). Significantly highest seed germination was observed in cultivar GAM 5 (78.42%) which was statistically at par with cultivar K 851 (78.11%) (Table 2). All the tested fungus reduced the seed germination on the different mungbean cultivars as evident from reduced germination per cent as compared to control. *Macrophomina phaseolina*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Alternaria alternata*, *Apergillus fumigatus*, *Phoma* sp. and control(untreated) showed average germination per cent 55.55, 56.60, 66.15, 67.90, 72.0, 73.15, 75.75, 81.50 and 89.75, respectively (Plate 1 to 5). Among all the fungus, the minimum seed germination was observed in the seed treated with *Macrophomina phaseolina* (55.55%) which was statistically at par with *Fusarium oxysporum* (56.30%). Maximum germination per cent was observed in *Phoma* sp. (81.50%) as compared to control (89.75%). Seed germination of mungbean seed was decreased

due to infestation of selected dominant seed mycoflora. Similar results were also found by Deshmukh (2012) who reported that maximum seed germination (91.95%) was reduced in the seeds inoculated with mixture of all fungi. *M. phaseolina* also caused comparatively more effect on germination (51.18%). The loss in germination in *A. alternata* (40.77%) and *C. capsici* (36.48%) were medium while it was very less (8.41-21.80%) in rest of the fungal inoculated seeds. Utobo *et al.*, (2011) also reported that *Fusarium* invade the seed coat, endosperm and embryo resulting in failure of germination. Sinha and Prasad (1981) also reported that adverse effect on seed germination due to *Alternaria alternata*, *Bortyodiplodia theobrome*, *Curvularia lunata*, *Fusarium moniliforme* and *Macrophomina phaseolina*.

Effect of seed mycoflora on seedling length

Five different mungbean cultivars were assessed for seedling length influenced by seed mycoflora was carried out by paper towel method. The data are presented in Table 3. Results showed that all test fungi significantly reduced seedling length as compared to control (without inoculation of fungi) (Plate 6). Significantly lowest seedling length was observed in cultivar Meha (4.81 cm) followed by cultivar GM 4 (5.76 cm) and cultivar K 851 (5.86 cm). The highest seedling length was observed in cultivar GAM 5 (6.38 cm) which was statistically at par with cultivar GM 3

(6.21 cm). Eight fungus significantly affected on seedling length during assessment on paper towel method. Among them, lowest seedling length was observed in *Macrophomina phaseolina* (3.95 cm) followed by *Aspergillus flavus* (4.88 cm), *Fusarium oxysporum* (4.96 cm), and *Aspergillus niger* (5.05 cm). The seedling length was least affected by *Phoma* sp. (7.11 cm) as compared to control (8.19 cm). The finding was supported by Sadhu (2014), who studied the effect of six dominant seed-borne fungi on seedling emergence, shoot and root length of green gram and reported that the fungi *Aspergillus niger* and *Drechslera tetramera* affected most adversely the seedling emergence (40% each, control 90%), shoot length (5 cm, control 14 cm), and root length (5 cm, control 10 cm), respectively. Root length was not affected in case of seeds treated with *R. stolonifer* and *A. fumigatus* but more root length (12 cm) was recorded in case of seeds infested with *F. moniliforme* over control.

Effect of seed mycoflora on seedling vigour index

Seedling vigour index (SVI) was influenced by individual seed mycoflora associated with different cultivar of mungbean are presented in Table 4. Results indicated that each of seed mycoflora significantly reduced SVI of all cultivars over the control. The highest seedling vigour index was observed in cultivar GAM 5 (511.44).

Table.1 Discolouration grade for the assessment of seedling abnormalities

Rating Scale	Description
0	Healthy seedling (no visible symptoms)
1	Discolouration of 1-10% part of seedling
2	Discolouration of 11-25% part of seedling
3	Discolouration of 26-50% part of seedling
4	Discolouration of >50% part of seedling

Table.2 Seed germination of mungbean cultivars as influenced by seed mycoflora

Cultivars	Seed germination (%)									Mean (C)
	Fungi									
	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus fumigatus</i>	<i>Macrophomina phaseolina</i>	<i>Phoma sp.</i>	Control	
GM 3	77.50	64.00	70.50	67.75	68.25	76.25	67.25	79.25	90.75	73.50
GM 4	67.75	36.50	62.25	63.50	64.50	66.25	61.50	82.50	85.25	65.56
GAM 5	80.00	69.25	73.50	75.00	75.75	78.25	71.25	87.25	95.50	78.42
K 851	85.50	67.50	75.25	76.50	81.00	84.25	56.25	84.25	92.50	78.11
Meha	55.00	45.75	58.00	48.00	70.50	73.75	21.50	74.25	81.75	58.72
Mean (F)	73.15	56.60	67.90	66.15	72.00	75.75	55.55	81.50	89.75	
	C	F	C X F							
S Em ±	0.64	0.85	1.92							
CD at 5 %	1.78	2.38	5.32							
CV%	5.42									

(Note: **C** = Cultivar, **F** = Fungi)

Table.3 Seedling length of mungbean cultivars as influenced by seed mycoflora

Cultivars	Seedling length (cm)									Mean (C)
	Fungi									
	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus fumigatus</i>	<i>Macrophomina phaseolina</i>	<i>Phoma sp.</i>	Control	
GM 3	7.13	4.75	6.13	4.55	6.48	7.20	4.25	7.48	8.00	6.21
GM 4	5.30	4.80	5.20	5.28	4.75	6.60	4.00	7.25	8.75	5.76
GAM 5	6.33	4.93	5.30	6.50	6.00	7.25	4.00	8.18	9.00	6.38
K 851	5.50	6.30	4.88	3.75	5.95	6.18	4.88	7.13	8.20	5.86
Meha	5.08	4.05	3.75	4.33	5.25	5.68	2.63	5.55	7.00	4.81
Mean (F)	5.86	4.96	5.05	4.88	5.68	6.58	3.95	7.11	8.19	
	C	F	C X F							
S Em ±	0.09	0.12	0.27							
CD at 5%	0.26	0.35	0.77							
CV%	9.53									

(Note: C = Cultivar, F = Fungi)

Table.4 Seedling vigour index of mungbean cultivars as influenced by seed mycoflora

Cultivars	Seedling vigour index									Mean (C)
	Fungi									
	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus fumigatus</i>	<i>Macrophomina phaseolina</i>	<i>Phoma sp.</i>	Control	
GM 3	552.25	305.00	432.13	308.75	441.50	547.90	285.25	591.85	725.90	465.61
GM 4	359.48	175.28	323.50	334.50	307.13	438.38	245.88	598.63	745.83	392.06
GAM 5	506.25	338.00	390.40	486.88	454.78	568.50	285.63	714.08	858.50	511.44
K 851	472.38	425.13	365.50	287.75	480.75	520.78	274.88	601.00	758.75	465.21
Meha	279.13	186.13	217.00	208.50	370.25	418.83	58.00	412.40	572.13	302.48
Mean (F)	433.90	285.91	345.71	325.28	410.88	498.88	229.93	583.59	732.22	
	C	F	C X F							
S Em ±	8.00	10.74	24.02							
CD at 5 %	22.19	29.77	66.58							
CV%	11.24									

(Note: C = Cultivar, F = Fungi)

Table.5 Per cent discoloration intensity of mungbean seedling as influenced by seed mycoflora

Cultivars	Per cent discoloration intensity									Mean (C)
	Fungi									
	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus fumigatus</i>	<i>Macrophomina phaseolina</i>	<i>Phoma sp.</i>	Control	
GM 3	57.50	73.00	67.25	61.75	55.50	52.75	74.00	36.50	4.50	53.64
GM 4	48.75	76.25	68.25	66.75	55.25	44.25	80.75	22.75	6.00	52.11
GAM 5	49.50	68.75	62.50	56.25	58.00	44.00	52.00	8.00	3.25	44.69
K 851	54.50	70.75	66.50	56.00	54.50	43.25	74.50	12.00	4.25	48.47
Meha	60.00	77.50	72.00	64.75	57.50	54.25	85.25	37.00	7.25	57.28
Mean (F)	54.05	73.25	67.30	61.10	56.15	47.70	73.30	23.25	5.05	
	C	F	C X F							
S Em ±	0.57	0.77	1.71							
CD at 5 %	1.58	2.12	4.75							
CV%	6.69									

(Note: **C** = Cultivar, **F** = Fungi)

The lowest seedling vigour index was found in cultivar Meha (302.48) followed by cultivar GM 4 (392.066) and K 851 (465.21). Seed mycoflora and different cultivars were also significantly differed.

The lowest seedling vigour index was by *Macrophomina phaseolina* (58.0) followed by *Fusarium oxysporum* (186.13) in cultivar Meha. The highest seedling vigour index was found by *Phoma* sp. (714.08) in cultivar GAM 5. Among all tested fungus, seedling vigour index was highly influenced by *Macrophomina phaseolina* (229.93) followed by *Fusarium oxysporum* (285.91), *Aspergillus flavus* (325.28) and *Aspergillus niger* (345.71). Seedling vigour index was least affected by *Phoma* sp. (583.59) as compared to control (732.22). Similar result was found by Vasava (2017) who concluded that in respect of average SVI of five cultivars, *Fusarium oxysporum* showed the maximum detrimental effect thereby recorded minimum SVI (314.08) followed by *Aspergillus niger* (373.61) and *Aspergillus flavus* (444.27), while *Aspergillus terreus* (531.01) revealed the lowest adverse effect on SVI among eight seed mycoflora evaluated.

Per cent discolouration intensity of mungbean seedlings

Per cent discolouration intensity (PDI) was influenced by various seed mycoflora are presented in Table 5. Results indicated that per cent discolouration intensity in all mungbean cultivars was significantly differed from each other. The significantly highest per cent discolouration intensity was observed in cultivar Meha (57.28) followed by cultivar GM 3 (53.64) which was statistically at par with cultivar GM 4 (52.11). The lowest per cent discolouration intensity was found in cultivar GAM 5 (44.69). Results revealed that among all tested fungus, the highest per cent discolouration intensity was observed by *Macrophomina phaseolina* (73.30) which was

statistically at par with *Fusarium oxysporum* (73.25). The lowest per cent discolouration intensity was observed by *Phoma* sp. (23.25) followed by *Aspergillus fumigatus* (47.70) and *Alternaria alternata* (54.05). Seed mycoflora and different cultivars was also significantly differ. The highest per cent discolouration intensity was observed by *Macrophomina phaseolina* (85.25) followed by *Fusarium oxysporum* (77.50) in cultivar Meha. The lowest per cent discolouration intensity was found by *Phoma* sp. (8.0) in cultivar GAM 5 as compared to control (3.25). Similar results were obtained by Vasava (2017) who reported that in respect of average PDI showing overall effects on seedling irrespective of cowpea cultivars. *Rhizopus* sp., *Fusarium oxysporum*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata* and *Macrophomina phaseolina* showed PDI in between 51.37 to 69.36. The *Fusarium oxysporum* showed highest PDI in cultivars viz., GC 5 (75.83) and GC 4 (73.20). *Rhizopus* sp. showed significantly lowest per cent discoloration intensity in cultivar GDVC 1 (47.78), followed by AVCP 1 (48.13). Vaghela (2017) also reported that *Aspergillus flavus* showed highest PDI in groundnut cultivars viz., GG 8 (77.75), GG 2 (77.25) and GG 11 (75). *Aspergillus terreus* showed significantly lowest discolouration intensity in cultivar GG 20 (26.25) which was at par with GG 5(26.50). In respect of mean PDI showing overall effects on seedling irrespective of groundnut cultivars, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp., *Aspergillus fumigatus*, *Aspergillus terreus* showed PDI in between 33.42 to 69.55% in descending order.

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