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Study on impact of seed-borne mycoflora on seed germination, seedling growth and vigor of mungbean *Vigna radiata* L. (Wilczek) grown in Madhepura District, Bihar, India

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Abstract- Mungbean also known as green gram *Vigna radiata* L. (Wilczek) is a fabaceous annual crop grown for its high quality protein content in most of the states of India and abroad. Seed samples of Mungbean were collected from nearby villages of Madhepura and stored in different sterilized polythene bags. Collected seeds were used for the isolation of mycoflora associated with seeds. Fungal species were identified by preparing slides. Based on the morphology of conidia and onridiophores/ sporangiophores, different fungal species were identified with the help of standard text. Most common fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizopus stolonifer* were selected for axenic culture. From such culture, spore suspension were obtained and used for treatment of healthy seeds of mungbean. These seeds were germinated and percentage of germination, seedling growth and vigour and reduction in percentage germination, percentage reduction in shoot length and root length etc. were calculated. The Seedling Vigour Index was also calculated.

Key words: Mycoflora, Conidia, Sporangiophore, Axenic Culture, Seedling Vigour Index (SVI)

INTRODUCTION

Mungbean also known as green gram *Vigna radiata* L. (Wilczek) is an annual fabaceous crop. It is a day neutral plant and completes its life-cycle in 85-90 days from date of sowing. Mungbean is known for its nutritional value due to high quality protein, Cultivation of Mungbean has increased not only in Indian states but also in US, China and Australia. [1-3]

From production point of view, India ranks first in its production.[4] India produces about 1.5 – 2.0 million tons of Mungbean annually from about 3-4 million hectares with an average productivity of 0.5 ton/hectare.[5]

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Mungbean seeds are contaminated during storage with different fungal species constituting mycofloral diversity that may affect seed viability degrade their nutritional components thereby reducing seed germination, seedling growth and vigour. There are reports where it has been mentioned that seed borne fungi degrade the seed quality.[6-8] Ghewande and Nagraj (1987)[9] studied adverse effects of *Aspergillus flavus* on seed germination and SVI of ground nut causing yield losses and increase in contamination of mycotoxin which causes plant health problem and ultimate death. Mycoflora of different pulse seeds have been reported by different workers, like – Ashwin and Giri (2014)[10] in Chickpea, Kandher (2014) in Green gram and Arshed and Khan (2019)[11] in different pulse seeds.

MATERIALS & METHODS

Seed of Mungbean *Vigna radiata* L. (Wilczek) were collected from the nearby villages of Madhepura town. Farmers of different villages such as Gamharia, Sabaila, Lalpur and Sahugarh were contacted. Seeds generally stored in earthen pots, earthen storage boxes called "Kothi" and containers made up of *Saccharum munja* and *Saccharum spontaneum* etc. were gathered for testing mycoflora. Storage periods of the seeds were also noted. All categories of seeds were collected separately in air tight and sterilized plastic bags. Proper labeling of all the collected samples containing plastic bags was ensured.

Isolation of Fungal Species from the seeds:

Mycoflora associated with seeds were isolated by:

(a) Moist blotter plate method

(b) Agar plate Method.

Moist Blotter plate Method: Three layers of white blotter papers (8.5 cm diameter) were soaked in sterilized distilled water. These papers were lined in cleaned Petriplates (10 cm diameter). Ten seeds of Mungbean were equidistantly placed in each Petriplates. Petriplates were incubated at room temperature (28°C ± 2°C) and the moisture level was maintained at 98% for 10 days. On the eleventh day seed from each plate were examined under microscope (Zoom stereo microscope). Axenic culture of different species was maintained using PDA medium.

Agar Plate Method: Potato Dextrose Agar medium was also poured in Petriplates, as the medium was solidified, 10 seeds in each plate were placed at equidistance in LAF chamber. Plates were incubated in culture room for 10 days. After then above seeds were examined under Stereomicroscope.

Fungi appearing on seeds were identified on the basis of their morphological features of conidiophores, Sporangiophores, conidia and fruit bodies. For the identification standard texts of Subramanian (1971) and illustrated genera of imperfect Fungi by Burnett (1972) were used.

The identified fungal species were cultured as axenic culture. On the 10th day when maximum Sporulation was seen, 10 ml of sterilized distilled water was added in each plate and rubbed with brush. The water with spores was decanted in separate beaker and spore suspensions of most common fungi were obtained.

Inoculation of Fungal spores in the healthy seeds of Mung bean:

Healthy seeds were washed with distilled water and then treated with 0.1% Hgcl2 solution for few minutes in a flask. Above seeds were taken out and rinsed thrice with sterile distilled water. During this, the flask was shaken vigorously to remove traces of chemical. Ten seeds were treated with the spore suspension of selected fungal species. Similarly, seeds treated with sterile distilled water were used as control.

Both the treated and untreated (control) seeds were placed separately in Petriplates lined with moist filter papers. The moisture was maintained for 10 days. After which, the percentage of germination, shoot length, root length etc. were recorded. Seedling Vigour Index (SVI) was calculated by using the formula given by Vardarajan and Rao (2002)[12]

SVI = (Mean shoot length + Mean root length) x germination percentage

Table 1 (A) & (B) showing isolation of Seed-borne mycoflora in the samples collected from different villages of Madhepura district:

Table 1 (A) Sample-1

Isolated Fungal Species	Blotter Method	Agar Plate Method
Aspergillus niger	+	+
Aspergillus fumigatus	+	+
Aspergillus flavus	+	+
Curvularia lunata,	+	-
Rhizopus stolonifer	+	+
Fusarium oxysporum	+	+
Alternaria alternata,	+	+
Cladosporium spp.	-	+
Penicillium citrinum	+	+
Macrophomina phasiolina	+	+

Table 1 (B) Sample-2

Isolated Fungal Species	Blotter Method	Agar Plate Method
Aspergillus niger	+	+
Aspergillus fumigatus	+	+
Aspergillus flavus	+	+
Curvularia lunata,	+	-
Rhizopus stolonifer	+	-
Fusarium oxysporum	+	+
Alternaria alternata,	+	+
Cladosporium spp.	-	+
Penicillium citrinum	+	+
Macrophomina phasiolina	+	+

Present (+) Absent (-)

Table 2- showing effect of isolated seed-borne fungi on seed germination (%) shoot and root length (in cm) and seedling growth/ vigour Index of Mungbean [*Vigna radiata* L. (Wilczek)] (Mean data were obtained for three replicates)

Seed Mycoflora of Mung bean (Seed-borne fungi)	Seed germination (%)	Reduction (%)	Shootlength (cm)	Reduction (%)	Root length (cm)	Reduction (%)	Seed VigourIndex (SVI)/Reduction %
Aspergillus flavus	28	72	2.16	73	2.80	46.6	138.88 / 90.08
Aspergillus nigar	31	69	2.44	68	3.10	51.6	171.74 / 87.73
Aspergillus fumigatus	44	56	3.68	54	2.88	54	288.64 / 79.38
Alternaria alternate	57	43	4.64	42	3.54	40	466.26 / 66.7
Curvularia lunata	53	47	4.40	45	3.48	42	417.64 / 70.17
Fusarium oxysporum	68	32	5.60	60	4.32	28	674.56 / 51.82
Rhizopus stofonfer	76	24	6.24	22	4.86	19	843.6 / 40
Control	100		8.00	---	6.00	----	1400

RESULT & DISCUSSION

Seed-borne fungi of Mungbean [*Vigna radiata* L. (Wilczek)] were isolated and identified. All together 10 fungal species were isolated, most common fungi were cultured separately and thus axenic cultures were obtained. Spore Culture suspension for selected fungi were prepared, in which healthy seeds of Mungbean were mixed separately. Such seeds were then germinated under laboratory conditions.

Seeds treated with distilled water were used for control. The percentage of seed germination, shoot length and root length of treated seeds were measured. The percentage reduction in treated seeds over control was also measured and means data were obtained which are shown in the Table - 2.

From the Table -2, it was noted that percentage of germination of treated seeds of mungbean ranged from 28% - 76%, where as in control it was 100%. Thus the percentage of reduction was calculated. Those percentages of reduction ranged between 24-72. Seeds treated with spore suspension of *Aspergillus flavus* showed highest reduction (72%) followed by 69% reduction in *Aspergillus niger* spore suspension treatment, minimum reduction in percentage germination spore suspension of *Rhizopus stolonifer*. Present findings are in agreement with the finding of Vasava *et al.* (2018) and Sadhu (2020). Maximum reduction in growth of shoot and root length was observed in seeds treated with spore suspension of *Aspergillus flavus,* may be due to the fact that these species are toxin producing. Through in vitro experiment it has been reported that when seeds of Mungbean were treated with different concentration of pure aflatoxin, there was chemical and physiological changes in the seeds. These factors may be responsible for the reduced growth of shoots and roots of Mungbean.[13] Impact of different fungi associated with seeds of different pulse crops on their germination and seedling growth has been reported.[6-9,14,15]

All the researchers have reported that maximum reduction in seed germination and seedling growth of pulse crops were found when spores of *Aspergillus flavus* were used for treatment. Therefore, present finding corroborate with the findings of the above workers.

From the Table - 2 it may be noted that mean shoot length of controlled seeds was 8.0 cm, while seeds treated with *Aspergillus flavus* spore suspension showed only 2.16 cm which was minimum length and the reduction in length was 73% which was maximum. This was followed by the shoot length of the seeds treated with spore suspension of *Aspergillus niger* (68%). Here the minimum suppression of shoot length was in case of *Rhizopus stolonifer* spore treated seeds that was only 22%. A decreasing trend is noticed in the reduction of shoot length when the seeds were treated with spore suspension of *Aspergillus fumigatus* (54%), *Alternaria alternata* (42%); seeds treated with spore suspension of *Curvularia lunata* however, showed increased percentage of reduction than that of *Alternaria alternata*, while in case of *Fusarium oxysporum* again there was decrease in the percentage reduction (30%). Reduction of roots length of seeds treated with spore suspension of *Aspergillus flavus* was also observed that was 16% in comparison to the control. Here, again this was followed by *Aspergillus niger* treatment which showed 51% reduction. The minimum percentage reduction in root length was found in case of treatment with spore suspension of *Rhizopus stolonifer* (19%) while with *Curvularia lunata*

spore suspension treatment showed an increase root length reduction which corresponded to 42% in respect to treatment with *Alternaria alternata* spore suspension (40%)

The Seedling Vigour Index (SVI) of all the treated seeds of green gram (Mungbean) with different fungal spore suspension was also calculated and placed in the Table -2. For *Aspergillus flavus* treatment, it was 138.88 and reduction in seedling vigour over control was 90.08%. This was followed by *Aspergillus niger* treatment which was 171.74 and percentage of reduction was 87.73%. The minimum reduction in Seedling Vigour Index was found in seedling which was treated with spore suspension of *Rhizopus stolonifer* which showed SVI as 53.91% over control.

CONCLUSION

Different fungal species produce different secondary metabolites. These secondary metabolites in general and aflatoxin and other mycofoxins in particular, have adverse effect on seed physiology. Ultimately, the germination percentage growth and vigour of seedling are reduced in comparison to the control seeds. So, seed-born fungi do act adversely on the seed germination, growth and vigour of the mungbean plants.

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