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Study of seed borne mycoflora of mung bean (*Phaseolus aureus* Roxb.) treated with potassium nitrate during storage

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ABSTRACT

The main purpose of the present study was to enumerate the fungal species and their effect on germination associated with Mung bean seeds. Seeds of the cultivar viz. Pusa Vishal were collected from the Indian Agricultural Research Institute (IARI), Pusa, New Delhi. The seeds were surface sterilized by treating with $HgCl_2$ solution. These seeds were treated with potassium nitrate (KNO_3) and examined for seed mycoflora. Two methods viz. Agar plate and Blotter method were used to isolate the fungi. Total eight fungi were isolated from the test cultivar. Fungus were isolated and identified as Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus candidus, Fusarium semitectum, Penicillium citrinum, Macrophomina phaseolina and Rhizopus stolonifer. During isolation, the Blotter technique yielded higher number of fungi as compared to Agar plate method.

Keywords: Seed Borne mycoflora from Mung bean during storage condition

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INTRODUCTION

Seeds are regarded as highly effective means for transporting plant pathogens over long distances[1]. Seed borne diseases have been found to affect the growth and productivity of crop plants. A seed borne pathogen present externally or internally, may cause seed abortion, seed rot, seed necrosis, reduction in germination capacity as well as seedling damage resulting in development of disease at later stages[2].

Mung bean (*Phaseolus aureus* Roxb.) is an important wide-spreading, herbaceous, self pollinating legume crop under the family Leguminoseae. It is excellent source of protein and minerals. Seed borne mycoflora associated with Mung bean reported recently include *Macrophomina phaseolina*, *Aspergillus flavus*, *Colletotrichum* spp., *Drechslera* spp., *Myrothecium* spp. These fungi affect the germination and vigour of seeds.

Healthy seed is the foundation of healthy plant, a necessary condition for good yield[3]. Among various factors which affect seed health, the most important are seed borne fungi that causes reduction in seed germination and seed vigour. Seed borne pathogens reduces yield upto 15-90% if untreated seeds are grown in the field. The main objective of the present study to see the effect of seed borne mycoflora in Mung bean seeds during storage condition.

MATERIALS AND METHODS

The experiments were conducted from October 2011 to September 2012. In this work, the cultivar viz. Pusa Vishal of Mung bean was used to study the seed mycoflora. Harvested seeds of year 2011 were collected from IARI, Pusa, New Delhi then stored in glass bottles covered with lid under laboratory conditions upto the year at room temperature. The experimental data were recorded from fresh seeds, as well as stored seeds viz. three, six, nine and twelve months.

Seeds were surface sterilized with 0.1% $HgCl_2$ for 1-2 min and washed thoroughly with distilled water and soaked for 16 hrs in KNO_3 solution at 2012^0C. These seeds were dried back again to its original weight and used for further studies. Isolation of seed mycoflora from both untreated and nitrate treated Mung bean seeds was done by Agar plate and Blotter method.

Agar plate method:
Seeds were externally sterilized by 0.1% mercuric chloride solution to 1 to 2 min then washed by sterilized distilled water. The isolated fungi were identified using light microscope after staining the slides with lactophenol[4].

Blotter method:
Blotter method[5] was used to isolate the fungal pathogens with the seeds during storage. The seeds were surface sterilized by 0.1% mercuric chloride, solution to 1-2 min. then washed by sterilized distilled water.

Identification of fungi:
In order to isolate these fungi into pure culture, Potato Dextrose Agar (PDA) was prepared and the fungi were inoculated onto the sterile PDA and incubated for 7 days at the end of which the fungi were identified based in their colour, and mycelia growth using the light microscope[6]. The fungi were identified by various scientist[7, 8].

RESULTS AND DISCUSSION

Total eight fungal species viz. *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Aspergillus candidus, Fusarium semitectum, Penicillium citrinum, Rhizopus stolonifer and Macrophomina phaseolina* were isolated from Mung bean seeds during different period of storage from October 2011 to September 2012.

The dominant field fungi recorded from fresh seeds were *Alternaria alternata, Rhizopus stolonifer, Fusarium semitectum.* Most of these fungi were replaced by storage fungi viz. *Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Macrophomina phaseolina.* It has been observed that the yield decreased along with increase in storage time. It is evident from observation that maximum fungi were recorded in rainy season and summer season and lesser number of species recorded in winter season.

Table-1: Fungi isolated from the fresh & stored seeds of mung bean cv. Pusa Vishal by Agar plate method

Fungal species	Length of storage									
	Fresh seeds		3 Months		6 Months		9 Months		12 Months	
	T	UT	T	UT	T	UT	T	UT	T	UT
Alternaria alternata	-	+	-	-	-	+	+	+	-	+
Aspergillus. niger	+	+	-	+	+	+	+	+	+	+
Aspergillus flavus	+	+	-	+	+	+	+	+	+	+
Aspergillus candidus	-	-	-	-	-	+	-	+	-	+
Fusarium semitectum	-	-	-	-	-	-	-	-	-	-
Penicillium citrinum	+	+	-	+	-	+	+	+	-	+
Macrophomina Phaseolina	+	+	+	+	+	+	+	+	+	+
Rhizopus stolonifer	+	+	-	-	-	+	+	+	-	+

T = Seeds Treated with KNO_3, UT = Untreated Seeds,
+ = Present, - = Absent

Table-2: Fungi isolated from the fresh & stored seeds of mung bean cv. Pusa Vishal by Blotter method

Fungal species	Length of storage									
	Fresh seeds		3 Months		6 Months		9 Months		12 Months	
	T	UT	T	UT	T	UT	T	UT	T	UT
Alternaria alternata	-	+	-	-	-	+	-	+	-	-
Aspergillus. niger	+	+	-	+	+	+	+	+	+	+
Aspergillus flavus	-	-	+	+	-	+	+	+	+	+
Aspergillus candidus	+	-	-	-	+	+	+	+	-	+
Fusarium semitectum	-	+	-	-	-	+	-	+	+	+
Penicillium citrinum	-	+	-	+	+	+	+	+	+	+
Macrophomina Phaseolina	+	+	+	+	+	+	+	+	+	+
Rhizopus stolonifer	-	+	+	+	-	+	-	+	+	+

T = Seeds Treated with KNO_3, UT = Untreated Seeds
+ = Present, - = Absent

As the observation depicted, more number of fungi were isolated by Blotter method as compared to Agar plate Method. This may be due to the reason that some of the slow growing fungi and weak competitors could not grow in the culture plates in compare to the fast growing fungi and the selective nature of the culture medium which might

not have favored the growth of such species. Some fungi such as *Fusarium, semitectum* were observed only by Blotter method.

The fungi isolated from stored seeds were the main cause of deterioration of seeds during storage. Surface sterilization minimize the competition among fungi on the seed[9]. Seed surface sterilization with $HgCl_2$ usually suppresses the growth of saprophytic and other specific growing fungi[10]. It was also observed[11] that surface sterilization of seed with 0.1% $HgCl_2$ significantly decreased *Alterneria alternata* and *Fusarium* sp. of the two methods, used the Blotter method yielded the highest number of fungi as compared to Agar plate method. It is also reported that more fungi were isolated by Blotter technique than Agar plate method.

This may be due to that slow growing fungi and weak competitors could not grow. It has been observed that field fungi decreased along with increase in storage time. It is evident from observation that maximum fungi were recorded in rainy season and summer season and minimum fungi were recorded in winter season. Similar observation were also recorded[12] while studied the succession of fungi on wheat and maize seeds during storage condition. It also has been recorded[13] in Wheat seed during storage[14]. Amongst the species *Aspergillus niger*, *Aspergillus flavus* and *Macrophomina phaseolina* were the most dominant. Decreasing in number of fungal species during storage has been reported[15, 16]. The dominant fungi and fungal growth depend on period of storage and environmental conditions

CONCLUSION

Present study was concluded that Mung bean seeds regularly subjected to deterioration caused by seed borne mycoflora. These fungi implies an irreversible degenerative change in the quality of seed, vigour index and germination capacity.

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