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Seed Mycoflora of French Bean and its Control by Means of Fungicides

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Abstract

Seeds of two varieties of French bean (*Phaseolus vulgaris* L.) Pusa Parvati and Lakshmi were examined after harvesting for external and internal mycoflora using agar plate and blotter methods. Overall 26 species were isolated from all varieties by both techniques. The most common fungi were noted to be *Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Rhizopus nigricans, Aspergillus niger, A. flavus, Penicillium citrinum, Trichoderma viride and Dark sterile mycelium.* The blotter technique proved to be better in comparison to the Agar plate technique. The prominent field fungi recorded were *Alternaria sp., Curvularia lunata, Drechslera avanacea, Cladosporium cladosporioides, Fusurium sp., Aspergillus spp. and Penicillium* spp. during the summer season from fresh seeds. Most of these field fungi were replaced by the storage fungi in winter season e.g. *Aspergillus niger, A. flavus, Trichoderma harzianum* and *Penicillium*. The effect of different concentrations of four common fungicides viz. Dithan M-45, Bavistin, agrasan, GN and PCNB was studied on seed mycoflora and seed germination of two varieties of French bean. All the fungicides used were found to be effective in reducing the seed mycoflora. The percentage of all the treated seeds which germinated was higher than the untreated ones, so the fungicide enhanced the seed germination rate by controlling the seed mycoflora.

1 Introduction

French bean (*Phaseolus vulgaris* L.) is commonly known as Rajmash and was first introduced in India only in the last century from South America. Because of its nutritive value, it now is the most popular bean vegetable and pulse crop in India. The dried seeds are highly nutritious, containing 25 per cent protein, 50 per cent starch, 2.0 per cent fat and 3.0 per cent minerals (CHATFIELD, 1949). Seed infection of crops by mycoflora has its own importance. About 90 per cent of food crops grown on the earth are propagated through seeds. Seeds may carry several destructive pathogens that often take a heavy loss causing several diseases when crops are raised from them. It has been established that up to 5 per cent yield failure is attributable to diseases in Rajmash. Rajmash

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seed borne fungi, in general, cause failure of germination, seedling blight, rot and also diseases in subsequent growth stages. Beside these problems, the affected seeds, when consumed, may cause several diseases in man and domestic animals. Seed infection also reduces the market value of the produce.

Seed borne fungi are chiefly responsible for deterioration of seed in storage (CHRISTENSEN, 1952; LUTEY AND CHRISTENSEN, 1963; CHRISTENSEN, AND KAUFMAN, 1969; SHARMA et al. 1988; PANDEY et al. 1991 and SHAH AND JAIN, 1993) and thus they remarkably reduce the germination potential of stored seeds.

Fungicidal treatments are known to reduce the seed mycoflora and thereby to improve the germination and emergence of seed (RAI AND KANAKMANJARI, 1985(a); PANDEY AND VERMA, 1992; PAUL AND MISHRA, 1992, KLICH et al. 1994; and RAI et al. 1997). Various aspects of seed mycoflora have been reviewed and discussed from time to time by various workers (CHRISTENSEN AND KAUFMAN, 1969; SINHA AND PRASAD, 1989; CHRISTENSEN, 1991; LACEY et al. 1991; MACGEE, 1995 and VISHWANATHAN, 1996) but there is little information on the fungi associated with Rajmash seeds. In view of this, the mycoflora associated with the seeds of Rajmash after harvesting was studied. The effect of fungicides on seed mycoflora and seed germination was also studied to suggest improved control methods.

2 Materials and Methods

Seeds of two varieties of Rajmash (*Phaseolus vulgaris* L.), i.e. Pusa Parvati and Lakshmi, were selected for the present study. Harvested seeds from the current season were collected from Ganeshbag Nursery, Varanasi, India, in March 1997 and stored in glass bottle under laboratory conditions for up to a year. The seeds' mycoflora was isolated by agar Plate (MUSKETT, 1948) and blotter technique (DE TEMPE, 1953) in different seasons, i. e. summer, rainy and winter. Seeds of different varieties were used separately for determining the range of mycoflora. The isolation of external seed mycoflora was sown by plating 100 seeds directly on PDA culture plates. The same number seeds were plated in Petri-dishes on sterilised moist blotting papers. For isolation of endophytically carried mycoflora, surface sterilised seeds (with 0.1 per cent mercuric chloride) were plated in the same way. The plates were incubated at 25°C ± 1°C for 6 and 15 days respectively for the culture plates and blotting paper. Observations were made after seven days. To study the effect of fungicides on seed mycoflora and seed germination, five fungicides viz. Bavistin (2 Methoxy carbamoyl- benzimidazole), Dithane M-45 (75 per cent co-ordination product of zinc ion and manganese ethylene bisdithicarbamate), Agrosan GN (Phenylemercuric acetate and ethyl, mercuric chloride) and P.C. N:B: (Pentachloronitrobenzene) were selected for this study and three concentrations 0.1, 0.2 and 0.3 per cent (prepared in sterilised water) of each were used. Seeds of the listed cultivars were shaken in different concentrations of the fungicides in a flask for 20

minutes on a mechanical shaker and kept stationary for about 18 minutes in the laboratory. Seed treated with sterilised distilled water served as a control group. Two hundred seeds of each cultivars were taken for each concentration and a set of 10 seeds were plated in each Petri-plate on both, agar and medium and sterilised blotter wad, separately. All the plates were than incubated at 25°C ± 1°C and mycoflora were recorded after 7 days seed germination was studied at 25°C ± 1 °C by plating the treated and non-treated control seeds on sterilised moist blotting papers. The rate of germination was recorded after 6 days of incubation.

3 Results and Discussion

Isolated Mycoflora

A total of 26 fungal species were isolated from the two varieties of unsterilised and surface sterilised French bean seeds during summer, rainy and winter season from April 1997 to March 1998 by agar plate, method and blotter paper technique. The maximum number of external mycoflora (24, 25) was isolated from Pusa Parvati and Lakshmi varieties (respectively). The dominent fungal species isolated from all the varieties were *Rhizopus nigricans, Aspergillus niger, A. flavus, Penicillium citrinum, P. rubrum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium sp.* and Dark sterile mycelium. The dominent fungal species isolated from unsterilised seed were *Mucor racemosus, Rhizopus nigricans, Chaetamium globosum Aspergillus niger, A. flavus, Penicillium citrinum, Alternaria alternata, Dreschlera avanaceae, Fusarium oxysporum, Trichoderma, viride,* Dark sterile mycelium, whereas *Alternaria alternata, Dreschlera avanaceae, Curvularia lunata* and white sterile mycelium were recorded from surface sterilised seed by $HgCl_2$.

The prominent field fungi recorded were *Alternaria spp., Curvularia lunata, Dreschlera avanaceae, Cladosporium cladosporioides Fusarium spp., Aspergillus spp., Penicillium spp.* during summer season from fresh seeds. Most of these field fungi were replaced by storage fungi *Aspergillus flavus, A. niger, Trichoderma harzianum* and *Pencillium spp.* It has been observed that the field fungi decreased along with increase in storage time coupled with increase in storage fungi. It is evident from the observations that the maximum number of species of fungi were recorded in rainy and summer season and lesser number of fungal species were recorded in winter season. Summer and rainy seasons showed, mainly, the field fungi along with other seed borne pathogenic fungi. Most of the field fungi were replaced by storage fungi in the winter season. Gupta, et al. (1983) also had earlier observed that there was variation in the percentage incidence of fungi on mung seeds in rainy, summer and winter seasons with maximum average percentage incidence of fungi in rainy season and least in summer. A decline in the number of fungal species during storage has also been reported by other workers (Reddy and Reddy, 1983; Vijay Lakshmi and Rao, 1985 and Paul and Mishra, 1992).

Succession of seed mycoflora may be attributed to their different role in deteriorating the quality of seeds favoured by different seasons as the storage period increased. As the results of the present investigation show, some of the fungal species were encountered in all the seasons of the year - such fungi have wide range of nutrient requirements and have capacity of tolerance for varying environments. Those which were restricted to a particular season reflect greater sensitivity to storage conditions. Fungi of rare occurrence reflect their more demanding nature for nutrients and environmental conditions as suggested by Bilgrami et al. (1979). Besides these variations, most of the fungal species occurred in all the three seasons though their percentage frequency of occurrence varied considerably in different seasons. The continuous occurrence of these species in all the seasons and reduced frequency or absence of some other species may be due to unfavourable storage conditions or lack of competitive ability (CAMPBELL, 1962).

Although a total 26 fungal species were isolated from all the two varieties, only a few species viz., species of *Aspergillus, Fusarium* and *Penicillium* dominated throughout the year. The percentage frequency of occurrence of the storage fungi increased along with the increase in storage time. The percentage frequency of surface-borne field fungi decreased along with storage time and were not recorded during the winter season. A similar observation was also made by Lal and Kapoor (1979) while studying the succession of fungi on wheat and maize seeds during storage. The general decline of the field fungi is due to the development of storage fungi because under the ecological conditions prevailing during storage the latter can thrive better.

Comparison between Agar Plate and Blotter Technique

It was observed that more fungi were isolated by blotter technique in comparison to Agar plate method. This may be due to the reason that some of the slow growing fungi and its weak competitors could not grow in culture plates in competition with fast growing fungi. Similar results has been reported by other workers (RAI AND SINGH, 1976; and UPADHYAY AND SINGH, 1978).

Some fungi viz. *Alternaria solani, Macrophomina* and *Fusarium semitectum* were only observed on the blotter. This may be attributed to the reason that some slow growing fungi could not grow successfully in culture plates in competition with fast growing fungi. Another reason might be the selective nature of the culture medium which might not have favoured the growth of some other fungi.

Effect of Fungicides on Seed Mycoflora

The total fungal species isolated from stored seeds of variety Pusa Parvati and Lakshmi were 16 and 17 respectively (Table 1 and 2). All the used fungicides were found to be effective in reducing mycoflora. The maximum inhibition of fungal species was caused by Dithane M-45 followed by Bavistin, Agrosan GN and PCNB.

Table 1: Effect of fungicides on seed mycoflora of French bean (Variety Pusa Parvati) stored seed

		Bavistin			Diathane m -45			Agrosan gn			Pcnb		
Name of Fungus	Control	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%
Rhizopus nigiricans	+	+	+	-	-	-	-	-	+	-	+	-	-
Mucor racemosus	+	-	-	-	+	-	-	-	-	-	+	+	
Mortierella subtissima	-	-	+	-	-	-	-	-	-	-	-	-	+
Aspergillus niger	+	-	-	+	+	-	-	+	+	-	+	-	-
A. flavus	+	-	+	-	-	+	-	+	-	-	-	-	-
A. luchuensis	+	-	-	-	-	-	-	-	+	+	-	+	+
Penicillium citrinum	+	-	+	-	-	-	+	-	-	+	-	-	-
P. javanicum	-	-	-	-	-	-	-	+	-	-	+	-	+
Alternaria alternata	+	-	-	-	+	-	-	-	-	-	-	-	-
Curvularia lunata	+	-	-	-	-	-	-	-	+	-	-	-	-
Cladosporium cladosporioides	+	-	+	-	-	+	+	-	-	+	-	-	-
Phoma Sp.	+	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium semitectum	+	-	-	-	-	-	-	-	+	-	-	-	-
Trichoderma viride	+	-	+	-	-	+	-	-	-	-	-	+	-
Black sterile mycelium	+	-	-	+	-	-	-	-	+	-	-	+	-
White sterile mycelium	+	-	-	-	-	-	-	-	+	-	+	+	-

Table 2: Effect of fungicides on seed mycoflora of French bean (Variety Lakshmi) stored seed

		Bavistin			Diathane m -45			Agrosan gn			Pcnb		
Name of Fungus	Control	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%
Mucor racemosus	+	-	+	-	-	-	+	+	-	-	+	-	-
Rhizopus nigiricans	+	+	-	-	+	-	-	-	-	-	-	+	-
Chaetomium globosum	+	-	+	-	-	+	-	-	-	-	-	-	+
A. flavus	+	-	-	-	-	-	-	+	+	+	+	-	-
Aspergillus niger	+	-	-	-	-	-	-	+	+	-	-	+	-
A. luchuensis	+	-	-	-	-	-	-	-	-	+	+	-	-
Penicillium citrinum	+	-	+	+	-	+	-	-	-	-	-	-	-
P.rubrum	+	-	-	-	-	-	+	-	-	+	+	-	-
Alternaria alternata	+	+	+	+	-	-	-	-	-	-	-	-	-
Curvularia lunata	+	+	+	+	+	-	-	-	+	-	-	-	-
Dreschelera avaneceae	+	-	+	-	-	-	-	-	-	-	-	-	-
Phoma Sp.	+	+	+	-	+	+	-	+	-	-	+	-	-
Trichoderma viride	+	-	+	-	-	-	-	-	-	+	-	-	+
Fusarium semitectum	+	-	-	+	-	-	-	-	-	-	+	+	-
White sterile mycelium	+	-	+	-	-	-	-	+	-	-	-	-	-
Black sterile mycelium	+	-	-	-	+	-	-	-	+	-	+	+	-
Pink sterile mycelium	+	-	-	-	+	-	+	+	-	-	+	-	+

The total number of fungal species eliminated by Dithane M-45 treatment on the seed of Pusa Parvati and Lakshmi were 7 and 8, respectively. All the species of *Aspergillus*, except *for the seeds A. flavus* and *A. niger, Alternaria alternata, Curvularia lunata, Fusarium* sp. Seed, *Trichoderma viride* and White Sterile mycelium, were eliminated

Table 3: Effect of fungicides on per cent seed germination of stored seeds of French bean

Fungicides	Percentage Germination			
	Variety pusa parvati		Variety lakshmi	
	Blotter method	Ager plate method	Blotter method	Ager plate method
Control	69	66	66	62
Bavistin				
0.1%	78	74	71	69
0.2%	81	83	72	71
0.3%	87	86	78	73
Diathane M-45				
0.1%	78	72	71	69
0.2%	87	87	79	76
0.3%	89	86	85	84
Agrosan GN				
	62	59	63	56
0.1%	61	58	62	59
0.2%	66	63	66	61
0.3%				
PCNB				
0.1%	70	69	67	63
0.2%	72	68	69	66
0.3%	74	69	70	68

from all the treated seeds. Dithane M-45 has little effect on *Mucor racemosus* and *Penicillium* sp.

The number of species removed from Bavistin treated seeds were 7 on Pusa Parvati and 9 on Lakshmi . Species of *Aspergillus* were found to be sensitive to Bavistin even at the lowest concentration - species sensitive at higher concentration were *Rhizopus nigiricans, Mucor racemosus, Phoma hibernica, Trichoderma viride.* Bavistin had no effect on *Alternaria alternata* and *Curvularia lunata.*

The fungal species eliminated from agrosan GN treated seeds were 9 and 8 on variety Pusa Parvati and Lakshmi, respectively. *Aspergillus* species were found to be generally insensitive against agrosan GN, Rhizopus nigiricans, Mortirella subtilissima, Alternaria alternata, Penicillium sp. and Fusarium sp. were recorded as being sensitive to agrosan GN.

11 and 10 species were isolated from the treated seeds of variety Pusa Parvati and Lakshmi, respectively. *Penicillium citrinum, Alternaria alternata, Curvularia lunata* and *Cladosporium cladosporiodes* and *Trichoderma viride* were observed to be sensitive. This fungicide has also little effect on *Mucor racemosus, Penicillium javanicum*, all the species of *Aspergillus, Trichoderma viride* and *Fusarium* sp.

There was a mild effect of fungicidal treatment on some harmful fungi, viz. *Aspergillus flavus, A. niger Cladosporium cladosporioides* and *Penicillium citrinum*. In the case of treated seeds two following points were noted: (a) an increase in population of a few species, (b) appearance of some additional fungal species. This may be attributed to the reason that some antagonistic micro-oganisms, which suppress the growth of a few weaker ones, are eliminated by fungicidal treatments and after their removal from the seeds the suppressed one grows.

The germination of all the treated seed with fungicides was higher than that of the untreated ones except those which were treated with Agrosan GN (Table 3). The improved germination of seed after fungicidal treatments, on the whole, is due to the elimination of fungi because they secrete mycotoxins which are responsible for the reduction of seed germination.

It has been reported that fungicidal seed treatment reduced seed mycoflora and improved seed germination (Shah and Jain 1993 and McGee, 1995). In general, the seeds treated with fungicides of two varieties responded in a better way than the control group with regard to the reduction of seed mycoflora and promotion of seed germination. Amongst all the fungicides, Dithane M-45 proved to be the best one for controlling seed mycoflora and improving seed germinability. Several workers (Paul and Mishra, 1992; Pandey and Verma, 1992) found that Bavistin, Agrosan GN and Dithane M-45 are effective in increasing the rate of seed germination and reducing the incidence of disease.

The present investigation will help to manage the seed mycoflora by treating them with potent fungicides so that their viability will be maintained.

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