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RESEARCH ARTICLE

Study on seed borne mycoflora of soybean, sorghum and groundnut of different zones of Madhya Pradesh

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ABSTRACT

Stored seed-borne mycoflora of soybean, sorghum and groundnut in 18 villages, of different zones *viz.*, Jabalpur, Rewa, Sagar, Damoh, Balaghat, Narshighpur, Seoni, Umariya, Chhaterpur, and Pipparia of Madhya Pradesh were surveyed. A total of 30 seed samples, 10 of each species, were collected during August-September 2009-2012. Data were recorded for seed germination percentage, per cent pathogen frequency and major seed-borne fungi, which were identified and quantified using the blotter method. Seed germination percentages were high in soybean 97.3 per cent followed by sorghum 93.3 per cent and groundnut 91.2%; seven, six and five fungal genera were found in seed samples of seeds crops of soybean and sorghum, groundnut, respectively. Fungi most frequently isolated and identified were species of *Alternaria, Aspergillus, Fusarium, Helminthosprium, Mucor, Penicillium* and *Rhizopus* from sorghum, whereas in soybean above fungal pathogens were identified except *Mucor* and *Penicillium* while in groundnut seed samples *Alternaria, Aspergillus, Fusarium, Helminthosprium, Rhizopus* and *Penicillium* were detected. Per cent pathogen frequency of seed-borne fungi was higher in groundnut 73.0 per cent and minimum in soybean 15.3 per cent.

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INTRODUCTION

Micro-organisms play an important role in affecting the quality of seed, of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigour and weaken the plant at its initial growth stages. Seed-borne diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant. Many diseases of economically important crops are seed-borne of soybean, sorghum, groundnut, loose smut, flag smut, Kernel bunt and ear cockle (Javaid and Anjum, 2006). If seed infected or contaminated by a pathogen that is also soil borne, is sown in non-infested soil, the pathogen may be established in that soil. Seed-borne diseases have been found to affect the growth and productivity of crop plants. A seed-borne pathogen may be present externally or internally or associated with the seed as contaminant and may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection. Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Besides, the mold fungi which grow on the seed substratum, produce mycotoxins which are hazardous to humans and animals (Halt, 1994). Studies were carried out to study the composition of seed-borne mycoflora occurring in soybean, sorghum and groundnut grains which are the main crops grown in Madhya Pradesh. Commercially, discoloured soybean seeds caused by fungi are of poor quality reducing their acceptability and thus, low market value of the produce. Grain mold causes crop loss by reducing seed size and weight, the food value

and keeping quality of grains (Bandyopadhyay, 1986). Seed-borne mycoflora of sorghum reported from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* spp., *Fusarium moniliforme, F. oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora* spp., *Phoma* spp., and *Rhizopus* spp. Fungi are one of the factors in storage seeds which reduce seed viability. Seed-borne fungal diseases are the most limiting factor. Fungi form a major group of pathogens that can be seed-borne or transmitted through seeds.

The significance of sustainable agricultural production is hidden in the use of quality seed. It is the most crucial and vital input for enhancing the productivity. Since seed is the custodian of the genetic potential of the cultivars, the quality of the seed determines the limits of productivity to be realized in a given cropping system. Though seeds are of great economic interest and also contribute a major part of diet, they play a vital role in associating micro-organisms, which prove hazardous for the seed or the new plant created from it, so, any infection agent (bacteria, fungi, nematode, insect, pest, weed etc.) which is associated with seeds having potential of causing a disease in a seedling or plant, is teemed as seed-borne pathogen. Hence, the storage fungi are especially insidious because they invade seeds stored at moisture contents that practical grain men consider safe and often cause serious damage before their presence even suspected. Therefore, with few exceptions, spoilage of stored fungi, which may be introduced during the post harvest handling process. It is well known fact that several fungi are known to cause considerable damage to seeds in storage and produce various activities.

It is in view of this that the current study aimed at detecting seed-borne fungal pathogen on farmer saved or stored soybean, sorghum and groundnut seeds at different zones Madhya Pradesh, India.

MATERIAL AND METHODS

The experiment was conducted in the Mycological Research Laboratory, Department of Biological Sciences, Rani Durgawati University, Jabalpur, Madhya Pradesh. Madhya Pradesh has an average temperature of 35°C and annual rainfall of 388-400 mm level.

Sources of experimental materials :

Thirty seed samples of soybean, sorghum and groundnut were collected for the isolation and identification of seed-borne fungi from Jabalpur, Rewa, Sagar, Damoh, Balaghat, Narshighpur, Seoni, and Umariya, Chhatarpur, Pipparia (Madhya Pradesh) and they survey consisted of 18 villages during August to November 2011. From each seed sample, an amount of 250g seeds were taken and observations made in the laboratory for seed germination, identification of seed-borne fungi and per cent fungal frequency. All materials except seeds, which used in this experiment, were sterilized using 70 per cent ethyl alcohol. Formalin (10%) was used for Petri plate sterilization. Cotton blue and lacto-phenol were used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen (Aneja, 2004).

Plating of the seed component :

Standard blotter method as described by the International Seed Testing Association (ISTA 1976), was used for the isolation of the seed-borne fungi associated with the soybean, sorghum, and groundnut seed samples. The seed samples in their various forms according to their crops were then inoculated on three moistened filter papers (dia. 9.0 cm) in 9.0 cm Oswald Petri-dishes. Twelve seeds were arranged at the periphery of the plate, nine at the middle, and four at the centre in case of soybean and sorghum while in case of groundnut; five seeds were arranged at the periphery of the plate, four at the middle, and two at the centre. A total of ten seed samples per crop, with three replications, were used, and kept in dark place for seed germination.

Examination of incubated seeds :

Sampling for germination was done at 3 days after incubation, while identification of fungi was done at 7^{th} days. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the Light compound microscope.

Slide preparation and identification :

The samples of fungus were taken randomly from each crop seeds. These samples were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi (Bilgrami *et al.*, 1979; Bilgrami *et al.*, 1991; Jamaluddin *et al.*, 2004; Mukerji and Kapoor, 1969; Barnett, 1972; Aneja, 2004; Barnet and Hunter, 1999; Simmons, 1993; Ellis, 1997). The binocular compound microscope was used to determine the type of fungus in each plate. The seed-borne fungi were identified using identification keys and cross-checked for each seed plates to identify the type of fungus growing on each seed. After seven days of incubation, fungal species found growing on the surface of seeds, were identified and their percentage frequency (PF) of occurrence was calculated by applying the following formula :

PF = (No. of seeds on which fungus appears / Total number of seeds) × 100

Total No. of seeds = Frequency of occurrence (%) /No. of seeds on which a fungal species occurs /100

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Seed germination :

The results obtained (Table 1 and Fig. 1) showed that 30 samples, 10 each, of soybean, sorghum and groundnut seeds obtained from 18 villages have average seed germination 76.6, 80.9 and 64.5 per cent, respectively. The results showed that for all the samples, germination of soybean was higher in (S_1 Jabalpur) 93.3 per cent followed by (S_3 Sagar) and (S_8 Chhaterpur) 92.0 per cent each (S_2 Rewa) 90.7 per cent, (S_7 Saliwadha) 89.3 per cent, (S_9 Seoni) 84.0 per cent, (S_{10} Ummaria) 76.0 per cent,(S_4 Damoh) 57.3 per cent, (S_5 Balaghat) 46.6 per cent and (S_6 Nersighpur) 45.3 per cent.

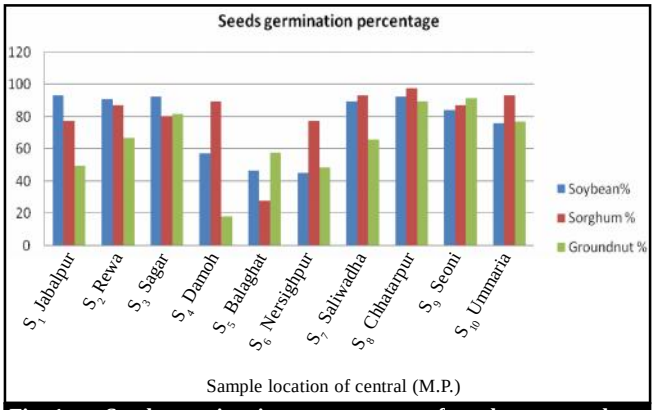

Fig. 1 : Seed germination percentage of soybean, sorghum and groundnut seeds

The results showed that for all the samples germination of sorghum was higher in (S_8 Chhatarpur) 97.3 per cent followed by (S_7 Saliwadha) 93.3 per cent, (S_{10} Ummaria) 93.0 per cent, (S_4 Damoh) 89.3 per cent, (S_2 Rewa) and (S_9 Seoni) 86.7 per cent each (S_3 Sagar)) 80.0 per cent, (S_6 Nersighpur) 77.3 per cent and (S_5 Balaghat) 28.0 per cent. In case of groundnut, seed germination percentage was higher in (S_9 Seoni) 91.2 per cent and minimum in (S_4 Damoh) 18.1 per cent. The results indicated that these seed borne fungi could be the main seed borne pathogens affecting the seed viability. The high frequency of occurrence of mycoflora which affect the seed viability and germination was also observed by Tarp *et al*. (1987).

Frequency of seed borne fungal pathogen :

The results obtained (Table 2 and Fig. 2) showed that per cent frequency of occurrence of the pathogens in sorghum seeds was higher in (S_1 Jabalpur) and (S_9 Seoni) 45.3 per cent followed by (S_3 Sagar) 34.7 per cent, (S_7 Saliwadh) 33.3 per cent, (S_4 Damoh) 33.3 per cent, (S_{10} Ummaria) 24.0 per cent, (S_8 Chhaterpur) 26.7 per cent, (S_2 Rewa) 22.7 per cent and (S_6 Nersinghpur) 21.3 per cent and minimum in (S_5 Balaghat) 15.3 per cent. Frequency of occurrence of the pathogen in soybean (Table 2 and Fig. 2) was higher in (S_9 Seoni) 40.0 per cent followed by (S_4 Damoh)) 37.3 per cent, (S_6 Nersinghpur) 29.0 per cent, (S_{10} Umariya) 28.0 per cent, (S_7 Saliwadh) 27.0 per cent, (S_2 Rewa) 26.7 per cent, (S_1 Jabalpur) 26.7 per cent, (S_8 Chhatarpur) 23.0 per cent, and (S_5 Balagha) 19.3 per cent and minimum in (S_3 Sagar) 18.7 per cent. In case of groundnut, higher frequency of occurrence of the pathogen was recorded in (S_{10} Umariya) 73.0 per cent whereas minimum in (S_8 Chhatarpur) 12.1 per cent. In overall among all three crops average percentage frequency of pathogen in groundnut was 42.1 per cent followed by soybean 30.2 per cent and groundnut 27.6 per cent.

Table 1 : Seed germination percentage of soybean, sorghum and groundnut seeds collected from different zones

Sample location	Soybean %	Sorghum %	Groundnut %
S_1, Jabalpur	93.33	77.3	49.32
S_2, Rewa	90.66	86.66	66.6
S_3, Sagar	92	80	81.6
S_4, Damoh	57.3	89.3	18.1
S_5, Balaghat	46.6	28	57.5
S_6, Nersinghpur	45.3	77.3	48.58
S_7, Saliwadha	89.3	93.3	65.53
S_8, Chhatarpur	92	97.3	89.33
S_9, Seoni	84	86.66	91.22
S_{10}, Umariya	76	93	77.03
Average	76.65	80.88	64.48
C.D. (P=0.05)	1.237	0.796	0.272

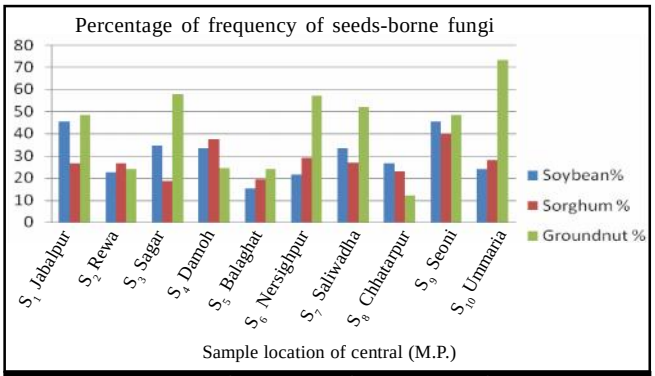

Fig. 2 : Percentage frequency of seed-borne fungi in various seed samples

Results of fungal identification in Table 3 showed that all the seed samples were contaminated with various fungal pathogens. Fungal pathogens identified in soybean included species of *Alternaria*, *Aspergillus*, *Fusarium*, *Helminthosporium*, *Mucor*, *Pencillium* and *Rhizopus*. All the seed samples were found to be infected by *Aspergillus*, whereas five samples with *Fusarium* (S_2 Rewa, S_4 Damoh, S_5 Balaghat, S_8 Chhatarpur and S_9 Seoni samples) each with *Helminthosporium* (S_4 Damoh, S_7 Saliwadha and S_{10} Umariya) and *Rhizopus* (S_2 Rewa, S_5 Balaghat and S_9 Seoni) and two samples with *Alternaria* sp. (S_1 Jabalpur and S_6 Nersinghpur) were detected by blotter method.

All the seed samples of sorghum tested in September to November 2011 were infected by *Aspergillus* and *Alternaria* sp. whereas *Rhizopus* sp. was found in seven samples (S_2 Rewa, S_3 Sagar, S_5 Balaghat, S_6 Nersinghpur, S_7 Saliwadha, S_8 Chhatarpur and S_9 Seoni) *Penicillium* was found in seven samples (S_1 Jabalpur, S_2 Rewa, S_4 Damoh, S_6 Nersinghpur, S_8 Chhatarpur, S_9 Seoni and S_{10} Umariya) while *Fusarium* sp. in four samples (S_1 Jabalpur, S_3 Sagar, S_5 Balaghat and S_6 Nersinghpur) and *Helmithosporium* sp. was also detected from four samples. *Mucor* sp. was encountered only in two samples (S_3 Sagar and S_8 Chhatarpur). The results of this study showed that the associations of groundnut seeds with plant pathogens in different villages of appeared to be a prevalent situation (Table 3, 4 and 5).

The findings of this study are therefore, important as

Table 2 : Percentage frequency of seed-borne fungi in various seed samples collected from different zones

Sample location	Soybean %	Sorghum %	Groundnut %
S_1, Jabalpur	45.33	26.66	48.5
S_2, Rewa	22.66	26.7	24
S_3, Sagar	34.66	18.7	58
S_4, Damoh	33.33	37.3	24.2
S_5, Balaghat	15.32	19.3	24
S_6, Nersinghpur	21.3	29	57
S_7, Saliwadha	33.33	27	52
S_8, Chhaterpur	26.66	23	12.1
S_9, Seoni	45.33	40	48.5
S_{10}, Umariya	24	28	73
Average	30.19	27.57	42.13
C.D. (P=0.05)	0.321	0.135	1.578

Table 3 : Identification of seed-borne fungal pathogens detected in various seed samples of soybean

Sample location	*Alternaria*	*Helminthosporm*	*Fusarium*	*Aspergillus*	*Rhizopus*
S_1, Jabalpur	+	-	-	+	-
S_2, Rewa	-	-	+	+	+
S_3, Sagar	-	-	-	+	-
S_4, Damoh	-	+	+	+	-
S_5, Balaghat	-	-	+	+	+
S_6, Nersinghpur	+	-	-	+	-
S_7, Saliwadha	-	+	-	+	-
S_8, Chhaterpur	-	-	+	+	-
S_9, Seoni	-	-	+	+	+
S_{10}, Umariya	-	+	-	+	-

they highlight the need for effective measures aimed at reducing seed-borne infection of soybean, sorghum and groundnut seed mycoflora: The standard blotter method was used to detect a wide range of fungi which are able to arise easily from seed in presence of humidity. Twenty five seeds from each samples were plates on moisten blotter in Petri dishes and incubated for seven days at room temperature. Examination of incubated seeds of groundnut (Table 5) revealed that *Alternaria* and *Aspergillus* were found in all the seed samples. *Rhizopus* and *Penicillium* were found in three samples. *Fusarium* was detected from two samples (S_5 Balaghate and S_9 Seoni) while *Helmithosporium* from only one sample (S_7 Saliwadha).

Good seed is recognized as an important input in any agricultural production system. One of the important aspects of good seeds besides high germination and purity is the absence of seed-borne pathogen. At least seven fungal genera were encountered in high per cent frequencies of seed-borne fungal pathogen and infection percentage in 30 samples of soybean, sorghum and groundnut collected from own saved seeds from 18 village's zones of Madhya Pradesh (Table 2, 3, 4 and 5).

Alternaria, Aspergillus, Fusarium, Helmithosporium, Penicillium and *Rhizopus* were the main fungi occurring frequently in soybean, sorghum and groundnut seeds. From Madhya Pradesh, *Aspergillus* was detected from all the seed samples. *Aspergillus* sp. is an important mycotoxin producer and produces four major metabolites of aflatoxin B1, B2, G1 and G2 which are heptacarcinogenic (Goldblatt, 1969). There is, therefore, need for reducing the mold growth and mycotoxin production in sorghum, soybean and groundnut seeds by improving the storage condition. The presence of so many pathogenic fungi at high level in farmer saved seeds from various geographical area indicated a clear need for field surveys for these and other pathogens. There is also a great need to increase the public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of seeds.

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Table 4 : Identification of seed-borne fungal pathogens detected in various seed samples of sorghum

Sample location	*Alternaria*	*Helminthosporium*	*Fusarium*	*Mucor*	*Aspergillus*	*Rhizopus*	*Penicillium*
S_1, Jabalpur	+	-	+	-	+	-	+
S_2, Rewa	+	+	-	-	+	+	+
S_3, Sagar	+	-	+	+	+	+	-
S_4, Damoh	+	+	-	-	+	-	+
S_5, Balaghat	+	-	+	-	+	+	-
S_6, Nersinghpur	+	-	+	-	+	+	+
S_7, Saliwadha	+	+	-	-	+	+	-
S_8, Chhaterpur	+	-	-	+	+	+	+
S_9, Seoni	+	-	-	-	+	+	+
S_{10}, Umariya	+	+	-	-	+	-	+

Table 5 : Identification of seed-borne fungal pathogens detected in various seed samples of groundnut

Sample location	*Alternaria*	*Helminthosporim*	*Fusarim*	*Aspergills*	*Rhizops*	*Penicillim*
S_1, Jabalpur	+	-	-	+	+	+
S_2, Rewa	+	-	-	+	-	-
S_3, Sagar	+	-	-	+	-	-
S_4, Damoh	+	-	-	+	+	+
S_5, Balaghat	+	-	+	+	-	-
S_6, Nersinghpur	+	-	-	+	-	-
S_7, Saliwadha	+	+	-	+	+	+
S_8, Chhaterpur	+	-	-	+	+	-
S_9, Seoni	+	-	+	+	-	-
S_{10}, Umariya	+	-	-	+	-	-

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