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Study of seed mycoflora of soybean from north eastern Karnataka*

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Abstract : Soybean is an important oil seed and pulse crop. Mycoflora associated with soybean seeds collected from different region were studied. Among the samples MARS, Raichur sample showed very high percentage of discoloured (30.0%), 21.0 per cent shrivelled, 2.0 per cent broken and very low per cent of healthy (47.0%) seeds. Overall, 11 fungi were isolated by using agar and blotter techniques and six fungi were from seed washing method. Pathogenic fungi frequently isolated were *Macrophomina phaseolina*, *Fusarium oxysporum*, *Aspergillus flavus*, *A. niger*, *Phoma* spp. and *Sclerotinia sclerotiorum*. Less frequently isolated fungi were *F. solani*, *F. moniliformae*, *Rhizophus spp.*, *Botrytis cinerea* and *Cercospora kikuchi*. Highest mycoflora was recorded from storage sample of MARS, Raichur (50.0 – 52.0%) followed by market collected samples (23.5 – 29.0%) and least per cent was recorded from field collected samples (15.5-27.5%). The frequency of *M. phaseolina* (27.0 – 28.0%) and *F. oxysporum* (5.0 - 5.5%) was more in storage sample of MARS, Raichur whereas *A. flavus* (7.0%) and *A. niger* (3.5-4.0%) were predominantly recovered from market collected sample of APMC, Raichur.

Key words: Fungi , Seed mycoflora, Soybean, Survey

Introduction

The soybean (*Glycine max* (L.) Merr.) is an important species of legume native to East Asia. It is an annual plant that has been used in China for 5,000 years to primarily add nitrogen into the soil as part of crop rotation. Fat free (defatted) soybean meal is a primary, relatively low cost, source of protein for animal feeds, soya vegetable oil is another valuable product of processing the soybean crop. The world annual production of soybean is 254 million tons from an area of 102 m ha with an average yield of 2.49 tons per ha during the year 2010. The estimated national production of soybean during 2010-11 was 12.59 million tons from an area of 9.21 m ha with the national productivity of 1065 Kg/ha in India. During 2009-10, around 82,000 tons soybean was produced in Karnataka (Anon., 2009).

Seed - borne diseases of soybean are important aspects which need our attention. More than 40 species of phytopathogenic fungi, bacteria and viruses may infect soybean seed causing various diseases, out of which 15 can result in significant economic losses, reducing yield and deteriorating quality of seed crop. Infected seed can provide primary inoculums for infestation of new crop and seed borne pathogens may be dispersed for long distances with it (Hartman *et al.*, 1999). However, information on seed mycoflora associated with soybean seed in this region is scanty. Therefore, it was thought ideal to investigate on the assessment of seed mycoflora on soybean seeds obtained from different regions.

Material and methods

The present study on seed mycoflora of soybean collected from different sources and locations of north eastern Karnataka was carried out during 2010-11 in the Department of Plant Pathology, College of Agriculture, Raichur. Soybean seed samples were collected from the early (June first week) and late (June 16th) sown crop during *kharif* season (2010) from the farmers' fields of Gulbarga, Bidar and Yadgir districts at the time of harvest. The samples from MARS and APMC of Raichur and those from ARS of Gulbarga, Yadgir and Bidar were also collected for assessing the seed- borne mycoflora. These samples were packed in cloth bags or butter paper bag and stored at room temperature ($27^0 \pm 1^0$C). They were used for dry seed examination and for isolation of mycoflora by using seed washing, agar plate and blotter techniques. In dry seed examination, seeds were examined for impurity, such as plant debris, sclerotia and broken seeds etc. and also for symptoms such as discoloration, malformation and similar indication of infection, including fruiting bodies of fungi, the resting hyphae on the surface of the seed, the spore on the seed as well as mechanical damage were detected from hundred seeds of each sample. Three replications for each sample were maintained.

In Seed washing method, twenty five seeds were placed in a culture tube having 2 ml of sterile distilled water. The seeds were vigorously shaken to dislodge the adhering fungal spores. The washings were collected in separate tubes and placed in a centrifuge that was run at 1500 rpm for 2 min. The supernatant was drained out and remaining pellet was examined for presence of fungal spores (Parasappa Sahabale, 2005). In Agar plate method, fifty seeds were placed on potato dextrose agar, five seeds in each plate and four replications were maintained. Before placing in Petri plate, seeds were surface sterilized with 0.1 per cent mercuric chloride solution for one or two minutes and washed thrice with sterile distilled water to avoid surface contaminants and for the isolation of internal mycoflora, which were placed at equidistance in Petri plates. These plates were incubated for seven days by providing 12 hours light and 12 hours darkness alternatively. The plates were examined for the mycoflora under microscope for mycelial growth, sporulation, colour and pigmentation. The experiment was repeated to detect more number of mycoflora on the seed. In Blotter technique, three layers of blotter paper discs were placed in sterilized Petri plates (9 cm) and moistened with sterilized distilled water. In

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each Petri plate, five seeds were placed for isolation of mycoflora from seeds. Fifty seeds were surface sterilized and plates were incubated as described under agar plate method (Anon., 1996). The fungi were identified based on the morphological characters by using "A Pictorial Guide to the Identification of seed- borne fungi of sorghum, pearl millet, finger millet, chickpea, pigeon pea and groundnut" (Information bulletin No. 34, ICRISAT, 1993), "Illustrated genera of imperfect fungi" (Barnett and Hunter, 1997) and other incidental reference books.

Results and Discussion

Based on the dry seed examination four different categories of seeds were visualized *i.e*. apparently healthy, discolored, shriveled and broken seeds from different locations and sources in north eastern Karnataka (Table 1).

In the collected soybean samples, on an average 79.1 per cent healthy, 11.4 per cent discolored, 6.7 per cent shriveled and 2.8 per cent broken seeds were found.

Table 1. Status of dry seed examination of field, market and storage samples of soybean from different districts of north eastern Karnataka.

Source	Location	Percentage			
		Healthy	Discolored	Shriveled	Broken
Field	Bidar	90	6	2	2
	Basavakalyan	85	7	4	4
	Aurad	89	7	2	2
	Gulbarga	82	8	6	4
	B'gudi	83	7	6	4
Market	APMC, Bidar	88	6	4	2
	APMC, Raichur	80	12	5	3
Storage	ARS, Bidar	87	6	5	2
	MARS, Raichur	47	30	21	2
	ARS, B'Gudi	60	25	12	3
	Mean	79.1	11.4	6.7	2.8
	S.Em±	0.89	0.33	0.16	0.08
	C.D. at 5%	2.64	0.97	0.47	0.23

Hundred seeds were drawn from each sample and three replications were maintained

APMC- Agricultural Produce Marketing committee

ARS- Agricultural Research Station

MARS- Main Agricultural Research Station

Among the samples collected from different locations, Bidar field sample showed the highest percentage of apparently healthy seeds (90.0%) and lowest percentage of discolored (6.0%), shriveled (2.0%) and broken seeds (2.0%), followed by Aurad field sample (89.0% healthy, 7.0% discoloured, 2.0% shriveled and 2.0% broken) which were found to be significantly superior in quality over other samples. Among field samples, Gulbarga sample showed the least number of healthy seeds (82.0%) and the highest values recorded with respect to other parameters *viz.*, discolored (8.0%), shriveled (6.0%) and broken (4.0%) seeds as compared to others. Storage samples recorded the lowest percentage of healthy seeds and highest percentage of other parameters except, ARS, Bidar sample when compared to field harvested samples of different locations followed by market collected samples. Among the storage samples, MARS Raichur sample showed very low percentage of healthy seeds (47.0%), 30.0 per cent discolored, 21.0 per cent shriveled and 2.0 per cent broken seeds, followed by ARS, Bheemarayanagudi sample (60.0, 25.0, 12.0 and 3.0 per cent of healthy, discoloured, shriveled and broken seeds respectively). Among the market collected samples, APMC Raichur sample (80.0%) recorded lowest per cent of healthy seeds, 12.0 per cent of discoloured, 5.0 per cent shriveled and 2 .0 per cent broken seeds. Market (80.0-88.0%) and storage samples (47.0 – 87.0%) recorded the lowest number of healthy seeds because of faulty storage conditions as compared to field harvested samples (82.0 – 90.0%). Kulik (1984) also reported similar type of results on soybean seeds infected with *C. truncatum*. Krishnamurthy *et al*. (2003) made similar observations in green gram, black gram and horse gram. The dry seed examination test helps in diagnosing the fungal seed infection of soybean due to seed mycoflora in seed lots.

In the present studies, seed mycoflora of soybean collected from different locations and other sources were determined by seed washing and results are presented in Table 2.

Six seed mycoflora of soybean were isolated and identified from the seed samples collected from different locations. The results (Table 2) indicated that, the predominant fungi associated with seeds were *Macrophomina phaseolina, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Phoma* spp. and *Sclerotinia sclerotiorum*. The frequency of the occurrence

Table 2. Predominant mycoflora of soybean samples from field, market and storage assayed by seed washing method

Locations	*M. phaseolina*	*F. oxysporum*	*A. niger*	*A. flavus*	*Phoma* spp.	*S. sclerotiarum*
Bidar	+	+	-	+	+	+
Basavakalyan	+	+	-	+	-	-
Auvrad	+	+	-	-	+	-
Gulbarga	+	+	+	+	+	-
B'gudi	+	+	-	+	-	+
APMC, Bidar	+	-	+	+	-	-
APMC, Raichur	+	+	+	+	-	+
ARS, B'Gudi	+	+	+	-	-	-
ARS, Bidar	+	+	-	-	+	+
MARS, Raichur	+	+	+	-	+	-

+ = Present

- = Absent

Table 3. Per cent frequency of mycoflora associated with soybean seed samples from different locations assayed by agar plate method

	Locations	*M. phaseolina*	*F. oxysporum*	*F. moniliforme*	*F. solani*	*A. flavus*	*A. niger*	*Phoma* spp.	*S.* sclerotiarum	*B.* cinerea	*Rhizopus* spp.	*C.* kikuchii	Total mycoflora (%)	Germination (%)
Bidar	Basavakalyan	5.0	4.5	1.0	0.0	3.0	2.5	1.0	1.5	0.5	2.5	1.0	22.5	82.0
	Aurad	14.0	4.0	0.5	0.0	2.0	2.0	2.5	1.0	0.5	0.0	0.0	26.5	78.0
	Bidar	8.0	4.0	0.0	0.0	3.0	2.0	5.5	2.5	1.0	0.0	0.0	26.0	80.0
	APMC, Bidar	5.5	3.5	0.0	1.0	6.5	4.0	3.0	1.5	0.0	2.0	1.0	28.0	75.5
	ARS, Bidar	11.5	5.0	1.5	1.0	0.0	1.0	7.0	6.0	0.0	0.5	0.5	34.0	71.5
Raichur	APMC, Raichur	5.0	3.5	0.5	0.0	9.0	4.0	1.5	3.0	0.5	2.0	0.0	29.0	70.0
	MARS, Raichur	30.0	5.5	1.5	1.5	2.0	3.5	2.0	1.5	0.0	1.0	1.5	50.0	50.0
Yadgir	B'Gudi	6.0	4.0	1.0	0.5	4.0	3.0	4.0	2.0	1.0	1.0	0.5	27.0	74.5
	ARS, B'Gudi	13.0	5.0	2.0	0.5	6.0	4.0	3.0	1.5	1.0	1.0	0.5	37.5	68.0
Gulbarga	Gulbarga	7.0	4.0	0.5	1.0	3.5	3.0	4.5	1.5	1.0	0.5	1.0	27.5	74.0
S.Em±		0.14	0.05	0.01	0.01	0.07	0.06	0.06	0.03	0.01	0.02	0.01	0.38	0.44
C.D. at 1%		0.53	0.18	0.04	0.03	0.27	0.22	0.24	0.12	0.04	0.08	0.04	1.49	1.70

Isolation of fungi was made from 50 seeds of each sample incubated at 27±1^0 C and three replications were maintained

Table 4. Per cent frequency of mycoflora associated with soybean seed samples from different locations assayed by blotter method

	Locations	*M. phaseolina*	*F. oxysporum*	*F. moniliforme*	*F. solani*	*A. flavus*	*A. niger*	*Phoma* spp.	*S. sclerotiarum*	*B. cinerea*	*Rhizopus* spp.	*C. kikuchii*	Total mycoflora (%)	Germination (%)
Bidar	Basavakalyan	5.0	3.5	0.0	0.0	4.0	3.5	3.0	0.5	2.5	1.5	0.0	23.5	80.0
	Aurad	10.5	3.0	0.0	0.5	2.0	0.0	1.5	0.5	1.0	0.5	0.0	19.5	80.5
	Bidar	5.5	3.0	0.0	0.0	2.0	1.0	2.0	1.5	0.5	0.0	0.0	15.5	84.5
	APMC, Bidar	6.5	3.5	0.0	0.0	5.0	3.5	2.5	1.0	1.0	0.5	0.0	23.5	77.5
	ARS, Bidar	9.0	4.5	0.0	1.0	0.0	1.5	3.0	2.0	2.0	0.5	0.5	24.0	75.0
Raichur	APMC, Raichuir	8.0	3.0	1.0	0.5	7.0	3.0	1.0	0.0	1.0	1.5	0.0	26.0	73.5
	MARS, Raichur	27	5.5	1.0	0.5	8.0	1.0	3.0	1.0	4.0	0.0	1.0	52.0	52.0
Yadgir	B'Gudi	5.0	3.0	0.5	0.5	3.5	2.0	3.0	2.0	1.0	1.0	0.5	22.0	78.5
	ARS, B'Gudi	10.0	4.0	1.0	0.5	5.0	4.0	3.0	1.5	1.0	1.0	0.5	31.5	71.5
Gulbarga	Gulbarga	6.0	3.5	0.5	0.5	3.0	2.0	3.5	1.5	1.0	0.5	0.5	22.5	78.0
S.Em±		0.10	0.06	0.01	0.01	0.05	0.04	0.05	0.02	0.02	0.02	0.01	0.41	0.59
C.D. at 1%		0.38	0.22	0.03	0.03	0.18	0.16	0.18	0.07	0.08	0.07	0.03	1.60	2.39

Isolation of fungi was made from 50 seeds of each sample incubated at 27±10 C and three replications were maintained

of these organisms varied from different locations and sources. *Macrophomina phaseolina* was present in all the samples. Similarly, *Fusarium oxysporum* was present in all the samples except APMC, Bidar sample. *A. niger* was present in all the market collected samples and storage samples except ARS, Bidar sample and also present in Gulbarga field sample. *A. flavus* was absent in Aurad field sample and in all the storage samples. *Phoma* spp. was absent in all the market collected samples and Basavakalyan and B'Gudi field samples. *Sclerotinia sclerotiorum* was obtained from field samples of Bidar and B'Gudi, market sample of APMC, Raichur and storage sample of ARS, Bidar. The results are in agreement with Verma and Lohuri (2004) who also reported *Fusarium* spp. predominantly isolated from lentil by seed washing method.

In the present study, different field samples from Bidar, Gulbarga and Yadgir districts and market samples from APMC, Bidar and APMC, Raichur and storage samples from ARS, Bidar, MARS, Raichur and ARS, Bheemarayanagudi (B'Gudi) were used for isolation of mycoflora by agar plate method and observations on both per cent infection of seed and per cent germination were recorded.

Among the fungi isolated, *M. phaseolina, F. oxysporum, A. niger, Phoma* spp. and *S. sclerotiorum* were predominant in all the locations, *A. flavus* was also a predominant in all the locations except ARS, Bidar sample. *F. moniliformea, F. solani, Botryotis ceneria, Cercospora kikuchi* and *Rhizopus* spp. recovered in all most all the locations but their frequency of occurance was low (Table 3).

Frequency of mycoflora was more in storage samples followed by market samples. Among the storage samples, MARS, Raichur sample (50.0%) showed highest per cent of mycoflora and was significantly differed from all other samples followed by ARS, Bheemarayanagudi (37.5%) and ARS, Bidar sample showed the least number of mycoflora (34.0%) as compared to other storage samples. Among market samples, APMC, Raichur showed more number of mycoflora (29.0%). Among the field harvested samples, Gulbarga field showed highest mycoflora (27.5%) which was followed by B' Gudi field sample (27.0%) and Aurad sample (26.5%) with the lowest percentage of germination. The least per cent of mycoflora was isolated from Basavakalyan field sample (22.5%). In case of *M. phaseolina* the frequency was more in storage samples, market and field samples, whereas, *F. oxysporum* was predominantly recovered from storage samples (5.0 -5.5%), followed by field (4.0 - 4.5%) and market samples (3.5%). The highest frequency of *F. oxysporum* was recovered in MARS, Raichur (5.5%) and ARS, B'Gudi (5.0%), followed by Basavakalyan field sample (4.5%). *A. flavus and A. niger* were predominantly recovered from market samples. APMC, Raichur showed the highest frequency (9.0%) of *A. flavus* and *A. niger* predominantly occurred (4.0%) in both the market samples. *Phoma* spp. and *S. sclerotiorum* were recovered predominantly in ARS, Bidar (7.0 and 6.0 % respectively), followed by Bidar field sample (5.5 and 2.5% respectively).

In blotter technique, more than 11 fungi were isolated and identified such as *M. phaseolina, F. oxysporum, A. flavus, A. niger, Phoma* spp., *S. sclerotiorum, B. cinerea, C. kikuchi, F. moniliforme, F. solani* and *Rhizopus* spp. (Table 4). Among all the samples collected from different locations and sources, storage samples recorded the highest mycoflora with lowest percentage of germination which were found to be highly significant with respect to association of mycoflora followed by market samples. Among the storage samples, MARS, Raichur (52.0%) followed by ARS, B'Gudi (31.5%) with least per cent of germination (52.0 and 71.0% respectively). The APMC, Raichur recorded highest per cent (26.0%) of mycoflora with least per cent of germination (75.5%) when compared to other market and field samples. Among the field collected samples, Bsavakalyan field sample showed highest per cent of mycoflora (23.5%) followed by Gulbarga field sample (22.5%) which were at par with each other and the least per cent of mycoflora was recorded in Bidar field sample (15.5%).

M. phaseolina and *F. oxysporum* recorded maximum per cent infection in storage sample followed by market samples. Among the storage samples, MARS, Raichur sample showed the highest per cent of *M. phaseolina* (27.0%) and *F. oxysporum* (5.5 %) and significantly differed from other samples, followed by ARS, B'Gudi sample (10.0%) for *M. phaseolina* and ARS, Bidar sample (4.5%) for *F. oxysporum*. The results agree with the findings of Umechuruba and Nwachukwu (2002) and Goulart (1997) also detected 15-20 species of seed- borne mycoflora which occurred on soybean seeds *viz.*, *Phomopsis* spp., *Colletotrichum truncatum, Cercospora kikuchi, C. sojina, Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium semitectum, Aspergillus* spp. and *Pencillium* spp. Grigaliuniate and Vitkus (1997) isolated *Fusarium* spp., *Thielaviopsis basicola, Botrytis cinerea, Alternaria alternata, Cladosporium clodosoprioides, Diplodiscus* spp., *Trichothecium roseum, Stemphylium botyosum and Pencillium* spp. from soybean seeds. Solanke et al. (1997) isolated *Aspergillus niger, A. flavus, Fusarium moniliformae, Curvularia lunata, Alternaria alternata* and *Pencillium* spp. from soybean cv. PK-472 and MACS-13 seeds by agar plate, blotter paper and moist sand methods. The results were also in agreement with the findings of Agarwal *et al.* (2006) and Shovan *et al.* (2008).

This list indicated that the pathogens causing severe seed borne diseases on soybean are carried through the seed. Most of them are common fungi, some of which get associated in the field and some in storage depending upon the nature of storage practiced.

Blotter and agar plate methods were used for isolation of mycoflora of soybean seeds. The efficacy of blotter and agar plate methods varied with the nature of fungi however, for routine enumeration, blotter could be followed without losing any efficacy, as the method is quicker and cheaper.

Seeds were surface sterilized as per the ISTA recommendation (ISTA, 1966). This was done to remove the fast growing organisms there by providing an opportunity for the slow growing internal seed borne fungi to express themselves. The results indicated that *A. niger, A. flavus, M. phaseolina, F. oxysporum* and *Rhizophus* spp. were recovered even after surface sterilization of seed. This indicated the presence of fungus both inside as well as on the surface of the seed.

The per cent germination of seed is more (52.0 - 84.5%) in blotter technique in comparison with agar plate method (50.0 - 82%). Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. To verify the above aspects, seeds were collected from different locations of north eastern Karnataka Districts *viz,* Bidar, Gulbarga, Raichur and Yadgir.

Results from the present investigation indicated that there was variation in mycoflora from one locality to another. However, *M. phaseolina, F. oxysporum, A. niger, A. flavus, Phoma* spp. and *S. sclerotiorum* were recovered in all four locations with varying degree of infection. The results of the present studies were in conformity with the findings of Dwivedi and Shukla (1990) and Narayanaswamy (1993) reported that the percentage of infection increased, the germinability of seeds decreased. *F. moniliformae, F. solani, B. cinerea, Rhizopus* spp. and *C. kikuchi* were also recovered from the seed samples collected from Bidar, Gulbarga, Raichur, Yadgir, storage and market samples. Storage (34.0-50.0%) and market (28.0-29.0%) recorded the highest mycoflora as compared to field harvested samples (22.5-27.5%). This could be possibly because of faulty storage conditions and moisture conditions, which allow seeds to harbor seed mycoflora. Among field samples, Gulbarga sample showed the highest mycoflora (27.5%) and the least percent of mycoflora was isolated from Basavakalyan field sample (22.5%). *M. phaseolina* and *F. oxysporum* were predominant in storage and field samples with more than 4 per cent infection. *A. flavus* and *A. niger* were predominant in market samples. This was in agreement with findings of Krishnappa *et al.* (2004).

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