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LOCATION OF FUNGI ON DIFFERENT PARTS OF SOYBEAN SEED

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ABSTRACT

Component plating of soybean seed by using ISTA technique showed that greater number of fungi were isolated by blotter method on different parts of seeds followed by agar plate and deep freezing method. Greater number of fungi were observed on seed coat, cotyledon followed by axis. *Fusarium solani* (Mart.)sacc., and *Macrophomina Phaseolina* (Tassi) Goid., were recorded from Cotelydon and axis. Reduced number of fungal species showed that greater number of fungi were located on seed coat. In seedling symptoms test on plain water agar showed that *F.solani* and *M.phaseolina* caused pre and post emergence infection of seedling.

Key words: Soybean seed, Mycoflora, ISTA technique, Seedling symptom

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an oil seed and an important legume crop. It is an important source of plant protein in the human diet (Sastri, 1956). In Pakistan soybean is cultivated on an area of 1320 hectares and the production is about 1898 tones (Anon, 2003). Soybean is grown mainly for food or forage. It is generally regarded as more salt sensitive than other grain legumes (Velagaleti *et al.,* 1990). Soybean is often cultivated as a Pasteur forage or fooder crop and used either as hay or as silage.A number of fungal species are found to be associated with soybean seed which includes *Alternaria alternata, Aspergillus* spp., *Cladosporium* spp., *Fusarium equiseti, Fusrium solani, Macrophomina phaseolina, Rhizoctonia solani, Rhizopus* spp., *Ulocladium botrytis* (Hussain *et al*., 1989, Tariq *et al*., 2005a, 2005b). Prasad and Singh (1983) reported that *A. flavus* and *A. alternata* decreased the oil content and iodine at high relative humidity. Mould fungi are also known to produce mycotoxin (Rodricks, 1976). During prolonged storage of grains, a general decreased in field fungi and a slow increase in storage fungi has been reported (Sinha, 1979). The fungus is localized in xylem vessel (Zazzerini *et al.,* 1985). Infected plant showed stunted growth, blackish stem and undeveloped roots, dark externally and gray to greenish gray internally which either becomes dry and brittle or soft (Sackston, 1958). *M. phaseolina* in sunflower seed reduced the germination and vigour besides producing pre- and post emergence seedling blight and charcoal rot diseases (Fakir *et al.,* 1976). Studies were, therefore, carried out to isolate the fungi from different component of soybean seeds and seedling symptom test was carried out to observe the pre- and post emergence of soybean seeds, the results of which are presented in this paper.

MATERIALS AND METHODS

Four samples of soybean seeds collected from different parts of Pakistan viz., Karachi(1) Sindh (1) Faisalabad (2) were used to study the location of seed borne fungi. Using the method given by Mathur *et al*., (1975), single seed soaked for 4h in sterilized distilled water in test tube was dissected aseptically into seed coat (outer most covering), cotyledon and axis. ISTA technique (Anon., 1976) was used to detect the fungal infection on different parts of seed. Using blotter method, dissected parts of 20 untreated seeds and 20 seeds after treatment with 1% $Ca(OCl)_2$ were placed on three layers of sterilized moistened blotter. For agar plate method, the treated and untreated seed components were placed on potato dextrose agar (PDA). Different components parts of a seed were plated in a petri dish. The dishes were incubated at 24°C for 7 days and fungi growing on different parts were identified after reference to Barnett (1960), Booth (1971), Ellis (1971) and Nelson *et al*., (1983). In seedling symptom test one seed was placed in a test tube containing 10 ml of 1% plain water agar. The tube was closed by loose cotton plug and incubated for 14 days at 20°C under 12h alternating cycle of ADL and darkness. The cotton plug was removed when the seedling reached the mouth of the tube. After 12 to 14 days the seedling showed fungal infection were counted (Khare *et a*l., 1977). Data were subjected to analysis of variance (ANOVA) following the procedure as given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Results obtained by component plating of 4 samples of soybean seed showed that most of the fungi were located on the seed coat (outer covering) followed by cotyledons and axis (Table 1). Of the 4 samples tested, Sindh sample showed highest colonization of pathogenic fungi viz., *F. solani* (Mart) Sacc., and *M. phaseolina* (Tassi) Goid

($p<0.001$) whereas the sample collected from Saddar (Karachi) was infected by storage fungi especially *A. flavus* Link and *A.niger* Van Tieghem. Surface sterilization of seed parts with 1% Ca $(OCl)_2$ reduced the incidence of *Aspergillus* spp. Infection of *M. phaseolina* was recorded in all parts of seed ($p<0.001$) viz.; seed coat, cotyledons and axis. Sadashivaiah *et al.,* (1986) reported that *M. phaseolina* was observed only in pericarp and seed coat. *F. solani* and *M. phaseolina* infection was observed on all parts of seed. Similar observations were made by Dawar and Ghaffar (1990) on sunflower and Rasheed *et al*., (2004) on groundnut.

Table 1. Location of fungi on different parts of soybean seed.

Name of fungi	Seed Coat						Cotelydon						Axis					
	Agar Plate		Blotter method		Deep freezing		Agar plate		Blotter method		Deep freezing		Agar plate		Blotter method		Deep freezing	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	5	3	-	3	-	-	3	3	-	-	-	3	-	-	-	-	-	-
Aspergillus candidus	5	-	5	5	-	-	8	10	-	5	-	15	3	10	-	-	-	-
A.flavus	63	65	40	25	10	30	45	60	58	33	20	35	55	63	30	35	10	23
A. niger	50	68	10	10	10	15	20	23	5	18	5	8	33	25	13	18	13	20
A.sulphureus	-	-	-	-	-	-	8	23	-	-	3	5	8	13	3	-	-	-
A. wentii	-	-	-	-	-	-	-	-	-	13	-	-	3	-	-	3	-	-
Fsariam solani	-	-	-	3	-	-	43	45	5	15	5	8	10	15	-	5	-	5
Macrophomina phaseolina	-	-	3	-	-	-	5	10	3	8	8	13	-	-	-	3	-	-
Rhizopus sp	23	20	8	3	8	5	3	3	3	5	5	-	15	20	5	10	-	-

LSD for fungi 0.05 = 6.735 ; LSD for method 0.05 = 8.249; A = Surface sterilized seed parts ; B = Non-sterilized seed parts.

Table 2. Seedlings symptom test for seed-borne fungi of soybean on plain water agar.

Name of fungi	Pre-emergence Seed rot %		Post emergence rot Dead Seedling %		Healthy seedling %	
	St.	N.St.	St.	N.St.	St.	N.St.
Alternaria alternata	7.5	5	2.5	-	-	-
Aspergillus candidus	-	2.5	-	2.5	-	-
A.flavus	10	17.5	-	5	2.5	15
A.niger	10	17.5	2.5	2.5	10	-
A.sulphureus	20	27.5	2.5	2.5	5	2.5
Fusariam solani	2.5	7.5	-	5	-	-
Macrophomina phaseolina	5	7.5	2.5	2.5	-	-
Penicillium sp	2.5	5	-	-	2.5	-
Rhizoctonia solani	-	2.5	-	2.5	-	-
Rhizopus stolonifer	5	7.5	-	10	-	-

St. = Sterilized ; N.St. = Non-sterilized

In the present study *A. alternata* was found to be present in seed coat and cotyledon but not on the axis. Such similar results have been reported by Bilgrami and Ghaffar (1996) on walnut. Results obtained by seedling symptom test of 4 samples of soybean seed (Table 2) showed pre-emergence and post emergence of soybean. Infection by *M. phaseolina* showed 7.5% pre-emergence death where the seed germinated but the pathogen caused root discoloration and the death of emerging radical where as 2.5% seedling died and showed charcoal rot symptoms on roots. *R. solani* caused 2.5% pre emergence in non sterilized seed. Similar results have been obtained by Rasheed *et al*., (2004) on groundnut Szimai (1941) reported that *R. solani* causes die back of leaves and young stem also root rot diseases in capsicum. *A. alternata* showed 7.5% pre-emergence in surface sterilized seed and 5% in nonsterilized seeds. *F. solani* caused 5% pre-emergence death of seedlings in surface sterilized seeds where as 7.5% in nonsterilized seeds. Dawar (1994) reported that *F. solani* caused pre and post emergence damping off of sunflower seed. Infection of *A. flavus* showed 10% pre-emergence of soybean seed in surface sterilized seed and *A. niger* showed 20% pre-emergence in surface sterilized seed. Gibson (1953a,1953b) observed *A. niger* caused most serious disease of crown rot of peanut where as *A. flavus* is the important mycotoxins producer can cause severe damage to

the liver, kidneys and nervous system of man even in low dosages (Rodricks, 1976). Present result showed that infection of *F. solani* and *M. phaseolina* was observed on embryo of seed as these fungi cause root rot disease and reduced the yield of crop, there is need to control the seed borne fungi and to increase the yield of crop.

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