



SCREENING OF MICROFUNGI FROM SOYBEAN (*GLYCINE MAX L.*) SEEDS

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ABSTRACT

Seed health testing by studying the seed-borne fungi of soybean seeds is an important step in the management of the seed-borne diseases. In the present study seed-borne fungi was studied by using agar plate method. A number of seed-borne fungal species was isolated from the agar plate. These seed-borne fungal sps. were *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Chaetomium globosum*, *Curvularia lunata*, *C. geniculata*, *Nigrospora sphaerica*, *Macrophomina phaseolina*, *Penicillium sp.*, *P. citrinum*, *Rhizopus nigricans*, *Trichoderma sp.*, *Alternaria alternata*, *Dreschlera australiensis*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani* and *Rhizoctonia solani*. Out of these the frequency of *Aspergillus flavus* and *Fusarium oxysporum* was higher.

KEYWORDS: Seed-borne fungi, Soybean, Agar plate method.



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INTRODUCTION

Soybean is the primary source of vegetable oil, protein and fat (1). Soybean dry matter contains 40% protein and 20% fat. Soybean acreage has expanded worldwide with this the number and severity of diseases has also increased. Soybean is cultivated mostly for vegetable oils, food, feed and in industries. Soybean is one of the economical crop and worldwide for soybean pods. The cultivation of soybean was started from Bangladesh. The crop is grown in tropical, sub-tropical and temperate regions. Serious diseases caused in soybean are mostly transmitted due to the infected seeds. Soybean (leguminous grain) is infected by various diseases which are mostly seed-borne. These seed-borne microorganisms adversely affect the Soybean seeds. They reduce seed germination or cause blights, leaf spots and other diseases on mature plants (9). The detailed study of microorganisms associated with Soybean seeds were reported (12). Kilpareik (8) has reported the association of fungi with Soybean seeds. *Circo-spora kikuchii*, *Alternaria* spp. were the most commonly identified Soybean seed-borne fungi. Beside these some saprophytic fungi were also present. Microflora of the seeds differs with locality.

MATERIALS AND METHODS

(i) SEED SAMPLING

The soybean seed (*Glicine max* L.) was collected from Ganpati seed bhandar, Daliganj, Lucknow. The study on seed-borne fungi was conducted in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, Uttar Pradesh, India.

(ii) AGAR PLATE METHOD

The soybean seeds were taken randomly from the sample seeds and were surface sterilized with sodium hypochloride for 2 minutes followed by using rinsing the seeds with sterilized water for 5 to 10 times for the removal of traces of sodium hypochloride. PDA was used as culture

medium. Surface sterilized seeds of Soybean crop were blotted between dried autoclaved Whatman filter paper No.1 before plating onto PDA plates (90mm). Inoculated plates were incubated at $27 \pm 1^{\circ}\text{C}$ under alternating light and dark regimes of 12h intervals.

(iii) DETECTION AND IDENTIFICATION OF FUNGI

The fungi associated with Soybean seeds were isolated and identified under the phase contrast microscope.

RESULTS

A total of 18 different fungi was isolated from the Soybean seeds. The isolated fungi were *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Chaetomium globosum*, *Curvularia lunata*, *C. geniculata*, *Nigrospora sphaerica*, *Macrophomina phaseolina*, *Penicillium* sp., *P. citrinum*, *Rhizopus nigricans*, *Trichoderma* sp., *Alternaria alternata*, *Dreschlera australiensis*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, and *Rhizoctonia solani*. Among these *A. flavus* and *Alternaria alternata* were most common. The highest frequency was recorded by *A. flavus* followed by *Fusarium oxysporum*. It was observed that the overall percent occurrence of the seed-borne fungal species ranges between 13.51 to 2.70%. Out of all fungal species isolated the percent occurrence of *A. flavus* was maximum (13.51%) followed by *Fusarium oxysporum* (10.81%), *F. solani* (6.76%), *Alternaria alternata* (6.76%), *A. niger* (5.4%), *Dreschlera australiensis* (5.41%), *A. fumigatus* (4.05%), *Curvularia lunata* (4.05%), *F. moniliforme* (4.05%), *Trichoderma* sp. (4.05%), *Chaetomium globosum* (4.05%), *Rhizoctonia solani* (4.05%), *C. geniculata* (2.70%), *Penicillium* sp. (2.70%), *P. citrinum* (2.70%), *Rhizopus nigricans* (2.70%), *Nigrospora sphaerica* (2.70%) and *Macrophomina phaseolina* (2.70%) respectively. It was observed that the seed germination in agar plate method ranges between 0 to 20 % (Table 1, Fig 1).

Table 1**Percent occurrence of seed-borne fungal species in each Petriplate of agar plate method**

Fungal species	Replicates/ Percent occurrence (%)					
	R1	R2	R3	R4	R5	R6
<i>Aspergillus niger</i>	11.76	9.09	0.00	0.00	0.00	8.33
<i>Aspergillus flavus</i>	5.88	27.27	15.38	37.50	7.69	0.00
<i>Aspergillus fumigatus</i>	0.00	0.00	7.69	0.00	7.69	8.33
<i>Chaetomium globosum</i>	5.88	9.09	0.00	0.00	0.00	0.00
<i>Curvularia geniculata</i>	5.88	0.00	0.00	0.00	7.69	0.00
<i>Curvularia lunata</i>	5.88	9.09	7.69	0.00	0.00	0.00
<i>Fusarium oxysporum</i>	17.65	18.18	0.00	12.50	7.69	8.33
<i>Fusarium moniliforme</i>	0.00	0.00	0.00	0.00	7.69	16.67
<i>Fusarium solani</i>	5.88	0.00	15.38	12.50	0.00	8.33
<i>Penicillium citrinum</i>	0.00	0.00	7.69	12.50	0.00	0.00
<i>Penicillium sp.</i>	0.00	0.00	7.69	0.00	0.00	8.33
<i>Trichoderma spp.</i>	5.88	0.00	7.69	0.00	7.69	0.00
<i>Alternaria alternata</i>	11.76	9.09	0.00	0.00	15.38	0.00
<i>Rhizopus nigricans</i>	0.00	0.00	7.69	0.00	7.69	0.00
<i>Nigrospora sphaerica</i>	0.00	0.00	0.00	0.00	7.69	8.33
<i>Macrophomina phaseolina</i>	0.00	0.00	7.69	0.00	7.69	0.00
<i>Drechslera australiensis</i>	5.88	9.09	0.00	0.00	7.69	8.33
<i>Rhizoctonia solani</i>	0.00	9.09	0.00	12.50	0.00	8.33
Sterile mycelium	11.76	0.00	7.69	12.50	7.69	8.33

**Figure 1****Petriplate of agar plate method showing seed-borne fungal species of soybean seeds.**

DISCUSSION

Soybean seeds carry various fungi, bacteria and viruses (11). It has been reported that about forty species of phytopathogenic fungi, bacteria and viruses are responsible to cause various diseases. Out of which only 15 have been known to cause the significant economical loss (7). The quantity and quality of the crop are influenced by seed-borne pathogens. Seed-borne diseases of Soybean have been studied by many authors (3, 5). Braccini and Dhingra (5) have reported *Phomopsis* and *Fusarium* as dominant fungi in Soybean seeds in Brazil. The result of the present study showed that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Chaetomium globosum*, *Curvularia lunata*, *C. geniculata*, *Nigrospora sphaerica*, *Macrophomina phaseolina*, *Penicillium sp.*, *P. citrinum*, *Rhizopus nigricans*, *Trichoderma sp.*, *Alternaria alternata*, *Dreschlera australiensis*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, and *Rhizoctonia solani* were observed and isolated from the seeds of the soybean. Out of all these fungal species, *A. flavus* and *Fusarium oxysporum* frequency was higher. Similar result was reported by many other workers. *Aspergillus flavus*, *Fusarium oxysporum*, *A. niger*, *Alternaria alternata*, *C. dematium*, *Penicillium sp.*, *C. globosum*, *C. lunata*, *R. stolonifer* and *M. phaseolina* were isolated as seed-borne fungi (4,6). Ten fungi comprising nine genera namely *Alternaria alternata*,

Aspergillus flavus, *A. niger*, *Chaetomium globosum*, *Colletotrichum dematium*, *Curvularia lunata*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium sp.* and *Rhizopus stolonifer* were found to be associated with Soybean seeds (10). It has been reported that fungi lower the germination percentage of seeds where the prevalence of fungi was higher, germination was low (2) favours our findings and 0 to 20% of germination was recorded. This might be due to the presence of seed-borne fungal species.

CONCLUSION

From the present study it may be concluded that the seed-borne pathogenic fungal diseases are responsible for the economical loss. It was observed that *Aspergillus flavus* and *Fusarium oxysprum* were most frequent. Seed-borne fungal species are responsible to reduce or delay in the seed germination in Soybean seeds.

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