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Effect of leaf extracts of various plants on seed mycoflora and seed germination in some soybean [*Glycine max*(L.)] varieties

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ABSTRACT

Different varieties of Soybean including MACS-13, JS-335 & PK-472 were screened for seed mycoflora. Fourteen fungi were isolated from these varieties. The effect of leaf extracts of various plants were studied for seed mycoflora & seed germination percentage in these varieties of Soybean.

Key words : Seeds of soybean, mycoflora, plant extract.

Introduction :

Soybean (*Glycine max* (L)) is native of eastern Asia. Soybean contains 40-44% protein, 20% oil, 8.77% fats, 27.12% nitrogen and 5.6% fibers and is also adequately rich in both major and minor minerals.

As soybean cultivation expanded throughout the world and number of diseases has also been increased with their severity. More than 700 pathogens including fungi were known to infect soybean of which about 35 are economically important (Sinclair, 1982). Leaf spot, leaf blight, pod spot, seedling rot, pod and collar rot, charcoal rot, downy mildew, root and stem rot and antracnose are some of the common diseases of soybean (Mukharjee and Bhasin, 1986). More than 30 fungi and 3 bacteria are associated with soybean seed (Thapiliyal and Pant, 1995). The seed mycoflora of soybean were


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detected and 16 fungal species were found from soybean seed (Tripathi and Singh, 1993). The pathogenic fungi of soybean associated with seed and seedling were *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Aspergillus* spe. Were isolated from affected seeds (Panth & Mukhopadhyaya, 1998.)

MATERIALS AND METHODS :

The seed samples of soybean [*Glycine max* (L.) Merrill] of varieties MACS-13, JS-335 and PK-472 were collected from soybean research station, Vasantrao Naik Marathwada Agriculture, University Parbhani. Different methods used for detection of seed borne fungi were direct inspection of the seeds, Blotter paper, Agar plate, Moist sand and Rolled towel methods (Muskett 1948, De Tempe 1953, Suryanarayanan and Bhombe 1961, Deshkar and Khare 1975, Paul Neergard 1977, Khare 1996). For the detection of seed mycoflora 400 seeds were selected randomly.

I. Blotter Paper Method:

In this method three layers of blotter paper equivalent to the size of petridish were soaked in distilled water and kept in petridish. Seeds of MACS-13, JS-335 and PK-472 were selected for isolation of seed borne fungi. For the isolation of internal seed born fungi seeds were surface sterilized by dipping in 0.1% mercuric chloride for one to two minutes and washed in three changes of sterile water. Five seeds per plate were placed at equidistant on the three layers of moist blotter paper in petridish. The external seed borne fungi were isolated by placing unsterilized seeds on three layers of moist blotter paper in petridish. All petridishes were incubated at 28 ± 10 C for seven days. After seven days seeds were examined. The percentage of individual seed mycoflora was recorded based on morphological characters (Kamat 1961, Paul neelgard 1977, Hawksorth et. al.1985, Jha 1993 and Mukadam 1997).

II. Agar Plate Method:

Presterilised petridishes were poured with 20 ml of autoclaved PDA medium having PH 5.6. The incubation process and other details were same as described earlier in the blotter paper method. From the seed sample, five seeds were placed in the petridish having equal distance. The seed examination was carried out after seven days and seed mycoflora was recorded.



III. Rolled Towel Method:

For this method from the seed sample of fifty seeds were placed on towel paper, covered with polythene paper and rolled carefully by avoiding disturbance to the seeds. After seven days seeds were observed and recorded the seed mycoflora.

IV. Moist Sand Method:

From the seed sample five seeds were placed in petridish containing sterilized moist sand at equal distance. Isolation method was similar as described in blotter paper method. After seven days the seeds were observed and seed mycoflora was recorded.

For the study of effect of plant extracts seven common plants were selected. Their identification was confirmed using the flora of Marathwada (Naik, 1998). These plants were surface sterilized with 0.1% HgCl₂ and washed repeatedly with sterile distilled water for three times. For this investigation 5% concentration of each plant were used.

RESULTS AND DISCUSSION:

In order to detect the seed mycoflora of Soybean varieties MACS-13, JS-335 & PK-472, blotter paper, Agar plate, Rolled towel and Moist sand method has been used. Among these method blotter paper method was found to be more suitable as it shows higher seed mycoflora as shown in table no. 1

Soybean varieties MACS-13 was associated with 12 fungi i.e. *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Colletotrichum dematium*, *Colletotrichum truncatum*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Nigrospora oryzae*, *Verticillium cinnabarinum*.

In JS-335 variety of Soybean 13 fungi were isolated which are *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Colletotrichum dematium*, *Colletotrichum truncatum*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Verticillium cinnabarinum*.

Soybean variety PK-472 was found to be associated with 13 fungi *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Colletotrichum dematium*, *Colletotrichum truncatum*, *Curvularia*



lunata, *Fusarium moniliforme*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Nigrospora oryzae*, *Sclerotium rolfsii*.

Effect of different plants extracts were observed on seed mycoflora and seed germination. The plant extract of *Allium cepa*, *Azadirachata indica*, *Glossocardia bovisalla*, *Eucalyptus cidriodora*, *Ocimum sanctum*, *Polyathia longifolia*, *Zingiber officinale* were used during the study. It was noted that the plant extracts of *Allium cepa*, *Ocimum sanctum* and *Azadirachata indica* was more effective as compare to other plant extracts to reduce seed mycoflora and increase in seed germination as shown in table no. 2.

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Table No.1 : Seed mycoflora on different varieties of Soybean [Glycine max (L.) Merrill]

Sr.	Fungi Isolated	MACS-13				JS-335				PK-472			
		Blotter Paper	Agar Plate	Rolled Towel	Moist Sand	Blotter Paper	Agar Plate	Rolled Towel	Moist Sand	Blotter Paper	Agar Plate	Rolled Towel	Moist Sand
01	<i>Alternaria alternata</i> (Fr.)	+	+	+	-	+	+	+	+	+	+	+	+
02	<i>Alternaria tenuissima</i> (Nees.)	+	+	-	+	+	+	-	-	+	+	+	-
03	<i>Aspergillus flavus</i> (Link.)	+	+	-	-	+	+	+	+	-	+	-	-
04	<i>Cladosporium cladosporioides</i> (Fres.)	+	-	+	+	+	-	-	+	+	-	+	+
05	<i>Cladosporium herbarum</i> (Pers.)	-	-	-	-	+	+	+	+	+	+	-	+
06	<i>Colletotrichum dematium</i> (Pers:Fr)	+	-	-	-	+	+	-	-	+	-	+	+
07	<i>Colletotrichum truncatum</i> (Schw.)	+	+	+	-	+	-	-	+	-	+	+	+
08	<i>Curvularia lunata</i> (Walkkar.) Boedign	+	+	+	+	+	+	+	+	+	+	+	+
09	<i>Fusarium moniliforme</i> (Sheldon.)	+	-	-	+	+	+	+	+	+	+	-	-
10	<i>Fusarium oxysporum</i> (Schlecht.)	+	+	+	-	+	-	-	+	+	-	-	+
11	<i>Macrophomina phaseolina</i> (Tassi.) Goid	+	+	+	-	-	+	+	-	+	+	+	+
12	<i>Nigrospora oryzae</i> (Bark & Broome.)	+	-	+	+	-	-	-	-	-	+	+	-
13	<i>Sclerotium rolfsii</i> (Saccardo.)	-	-	-	-	+	+	+	+	+	+	-	+
14	<i>Verticillium annabarinum</i> (Nees.)	+	+	+	+	+	+	+	+	-	-	-	-



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Table No. 2 Effect of leaf extracts of various plants on seed mycoflora and seed germination in some varieties of Soybean [*Glycine max* (L.) Merrill]

Sr.	Name of the Plants Concentration (5%)	Seed mycoflora (%)			Seed germination (%)		
		MACS-13	JS-335	PK-472	MACS-13	JS-335	PK-472
01	Allium cepa	7.60	8.15	8.00	98.60	90.00	92.30
02	Azadirachata indica	10.13	10.00	10.50	90.00	91.00	92.00
03	Glassocardia bovsalla	13.50	12.00	12.80	88.00	85.00	87.00
04	Eucalyptus citriodora	13.80	14.00	13.00	88.40	80.50	83.80
05	Ocimum sanctum	8.50	8.90	8.00	92.30	91.80	95.00
06	Polyathia longifolia	12.50	11.80	12.00	89.30	87.50	88.20
07	Zingiber officinale	13.00	12.50	13.60	89.00	90.80	88.50


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