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Studies on Seed Borne Mycoflora of Soybean Seeds by Incubation Methods

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted to detect and identify seed-borne pathogenic fungi in some soybean varieties and their effect on seed germination. Six varieties of soybean seed samples *viz*.JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and local variety were selected for the experiment. Twelve seed-borne mycoflora *viz*. *Aspergillus flavus, Aspergillus niger, Alternaria* spp*., Cladosporium* spp*., Curvularia* spp*., Rhizopus* spp*., Trichoderma* spp*., Rhizoctonia* spp*., Nigrospora* spp., *Macrophomina* spp*., Fusarium* spp. and *Penicillium* spp. were detected and identified from the seeds of six soybean varieties by using the standard blotter method, agar plate method, 2,4-D blotter method, roll paper towel method and deep freeze method. The seed sample of local variety recorded the highest percentage of mycoflora with the lowest germination percentage, whereas CG Soya-1 variety found as the least percentage of mycoflora with the highest germination percentage compared to other varieties of soybean taken in the study. Among all of the incubation methods, the agar plate was found as the most effective in detecting seed-related mycoflora, on soybean seeds.

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Keywords: Soybean; seed borne mycoflora; agar plate method; seed germination.

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most valuable crops in the world. It is also known as the "Golden Bean" of the 20th century. "Soybean seeds have a greater nutritional value as it is a major source of protein and vegetable oil. It contains 40-42% proteins, 20-22% oil, 21% starch, vitamins- A, B, C, D & K besides essential amino acids like lysine (5%) and a small amount of calcium, phosphorous, magnesium and iron" [1]. "The quality of seeds is affected by seed-borne mycoflora. The attack of plant pathogens is one of the reasons for the low productivity of soybean. Most of the economically important plant pathogen is transported from one region to another through seeds or propagating materials. Seed-borne diseases are commonly occurring during storage periods if the seeds are stored in a moist dark place" [2]. "The pathogens can be found in seeds after or before the germination of seeds. The seed-borne disease can be spread through wind, water, insect, agricultural equipment and transportation. Germination and seedling vigour are reduced by seed-borne mycoflora of soybean and they can destroy or affect grains during storage and become not suitable for human consumption. Some seed-associated fungi can affect the seedling or plant resulting in decrease in productivity capacity" [3]. Some fungal pathogens are associated with the testa (Seed coat) and cotyledons of seeds infected form of conidia (spores) and mycelium, after seed germination the infection moves to hypocotyls and the base of the stem. The seeds of fungal flora plays a significant role in determining seed quality and longevity. Among all mycoflora of soybean *Aspergillus niger, Aspergillus flavus, Rhizopus* spp*., Nigrospora* spp*., Fusarium* spp*., Curvularia* spp*., Macrophomina* spp*.* and *Cladosporium* spp*.* reduces the germination and seedling vigour to a greater extent compared to others and they can spoil the quality of grain during storage. The aim of the study on seed-borne mycoflora of soybean seeds using incubation methods is to investigate and identify the fungal species present in soybean seeds. This type of study is conducted to assess the quality and safety of soybean seeds used for cultivation.

2. MATERIALS AND METHODS

The present investigation was conducted in the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G) during the year 2020-21. Six varieties of soybean seed samples *viz*. JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and local variety were selected for the experiment. Unless and otherwise mentioned for each experiment, 200 seeds were used. In general, the Petri dish with seeds was incubated at 25±1˚C under a 12-hour light and dark cycle with NUV light for 7 days. Seven days after sowing, observations were made to determine the type of mycoflora present and its frequency [2]. The microorganisms were observed on the seeds using a stereo binocular microscope to determine their morphology and characteristics.

The associated mycoflora was identified with the help of standard literature like illustrated Genera of Imperfect Fungi [4], a pictorial guide to the Identification of Seed borne Fungi of Pigeon pea, Chickpea, Groundnut, Sorghum, Pearl Millet and Finger Millet [5].

Several seed testing methods have evolved for the identification of mycoflora associated with the seeds. Some of the most suitable techniques have been suggested by the International Seed Testing Association (ISTA) [6]. The methods to recognize microorganisms in or on the seed differ quite markedly depending on the location of the microorganism and the mode of seed transmission [7] and the specific group to which the microorganism belongs.

The following standard incubation methods recommended were used in the present investigation for studies on seed-borne mycoflora of soybean seeds.

1. Standard blotter method [8]
2. Agar plate method [9]
3. 2,4-D blotter method [8]
4. Roll paper towel method [10]
5. Deep freeze method [11]

2.1 Standard Blotter Method

"Four hundred seeds of each testing sample were kept equidistantly and aseptically on sterilized plastic Petri dishes, 3 good quality sterilized blotter paper of the same diameter was kept moist with sterilized distilled water. In each plate, 10 seeds were placed on moist blotter paper in such a way that 9 seeds formed the periphery of the Petri plate and 1 seed at the

center of the Petri plate. For each seed sample, 40 replicated plates were maintained. The seeded plates were incubated at 25±1˚C for 7 days in alternating cycles of 12 hours of darkness and 12 hours of light in NUV. Observations were taken as explained earlier. All seeds of the periphery of the plate were examined first, then finally seeds in the center of the Petri plate and expressed in percentage of seed-borne mycoflora, individually" [12] (Fig. 1).

The frequency of the mycoflora was calculated by the following formula:

$$\frac{\text{No.of seeds containing a particular fungus}}{\text{Total seeds examined}} \times 100$$

2.2 Agar Plate Method

Four hundred seeds of each testing sample were kept equidistantly, aseptically on each sterilized petri plate containing 15-20 ml of potato dextrose agar (PDA) medium. A small amount of Streptomycin sulfate was added to the medium at the time of pouring to prevent bacterial contamination. Each variety's seeds were surface-sterilized for 30 seconds in a 1.0 percent NaOCl solution. The seed was placed on the previously poured potato dextrose agar medium in a Petri plate in such a manner that 9 seeds were in the periphery and 1 seed at the center of the Petri plate. For each seed sample, 40 replicated plates were maintained and incubated at 25±1˚C under alternate cycles of 12 hours dark and 12 hours light in NUV. Observations were taken as described earlier (Fig. 2).

2.3 2,4-D blotter Method

In this method, the blotter paper was soaked in 0.2 percent 2,4-D suspension and then placed in plates. Four hundred seeds were incubated for 7 days as in the blotter method. The plated seeds were then incubated at 25± 1°C, under the alternate cycle of 12hr light and 12hr darkness for 7 days. After 7 days of incubation, seeds were examined under a stereoscopic microscope.

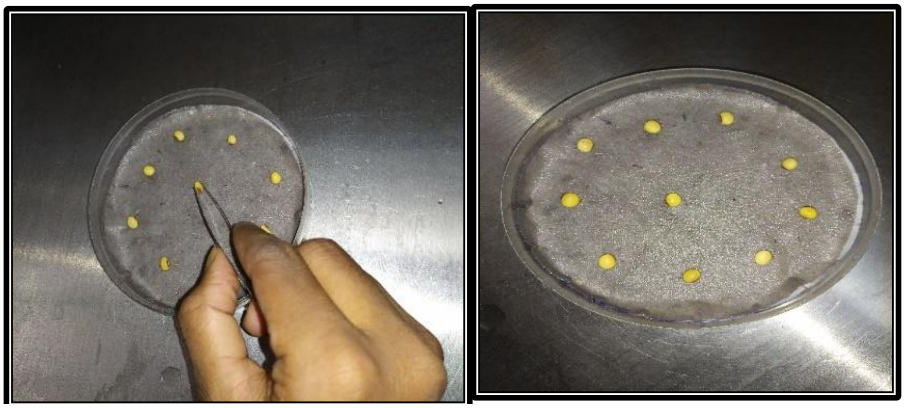


Fig. 1.

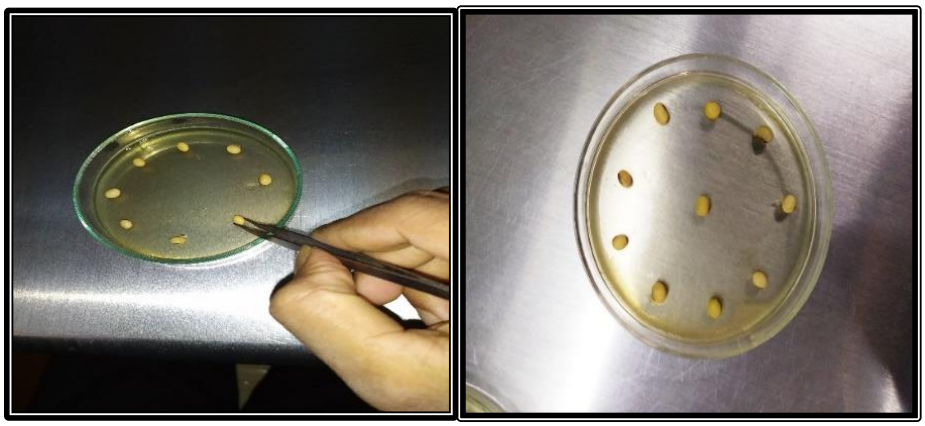


Fig. 2.

Fig. 3.

2.4 Roll Paper Towel Method

The 50 seeds were placed on a moist paper towel at equidistance and covered with another moist paper towel and rolled carefully without disturbing already arranged seeds. With the help of a rubber band tied the towel on both sides ends. To avoid water loss, used wax-coated paper or polythene for wrapping the rolled paper towels containing seeds. Incubated for 4 to 5 days at room temperature. Examined the abnormal and normal seedlings and ungerminated seeds with the naked eyes and the presence of different mycoflora by stereoscopic microscope (Fig. 3).

A. **Normal seedlings –**Seedlings showed the potential for continued growth & development into satisfactory plants when grown in good-quality soil and under favorable conditions of temperature, light and moisture.
B. **Abnormal seedlings –**seedlings do not show the potential to develop into a plant when grown in good quality soil and under the favorable condition of moisture, light and temperature.
C. **Ungerminated seeds –** Seeds did not germinate at the end of the test period.

2.5 Deep Freeze Method

This method is a modification of the standard blotter paper method used for the experiment in the present research. In this method, 400 hundred seeds were kept equidistantly and aseptically. Seeds were placed on moistened blotters with a solution containing 0.2% Streptopenicillin (to avoid bacterial contamination) and incubated for 24 hours. under normal conditions in a growth chamber. Plates were further incubated at 10±1˚C for 3 days and then transferred to the deep freezer (-20˚ C) under complete darkness for 24 hours. Plates were again incubated at 25 ±1˚C for 5 days. Observations were recorded as described earlier.

3. RESULTS AND DISCUSSION

3.1 Standard Blotter Method

Seed lots of different varieties of soybean were evaluated for the seed-associated mycoflora by using the standard blotter method and data presented in Table 1. The data indicated that a total of 619.87 mycoflora were found associated with 6 varieties of soybean. The seed lot of the local variety had a maximum frequency of mycoflora (126.64) with a minimum germination percentage (73.33%). Mycoflora were detected as *A. niger* (36.66%), *Fusarium* spp. (33.33%), *A. flavus* (26.66%), *Cladosporium* spp. (16.66%), and *Alternaria* spp. (13.33%). This was followed by JS 95-60 variety (106.65) with germination percentage (80.00%) and detected mycoflora were *Fusarium* spp. (43.33%), *Nigrospora* spp. (23.33%), *A. flavus* (16.66%), *A. niger* (13.33%) and *Trichoderma* spp. (10%).

In the seed lot of varieties RSC 10-46, RSC 10-71, JS 97-52 and CG Soya-1, the frequency of associated mycoflora were (106.64), (99.98), (96.65) and (83.31), respectively. In CG Soya-1 variety, the minimum frequency of mycoflora (83.31) includes *Trichoderma* spp. (26.66%), *Aspergillus niger* and *Curvularia* spp. (16.66%),

Aspergillus flavus (13.33%) and *Fusarium* spp. (10%) with maximum germination percentage (100%). In variety RSC 10-46, the frequency of associated mycoflora namely *Fusarium* spp. (36.66%), *Aspergillus niger* (36.66%), *Cladosporium* spp. (26.66) and *A. flavus* (6.66%) with germination percentage (90.00%) were recorded. In variety RSC 10-71, the frequency of associated seed-borne mycoflora was recorded as *Cladosporium* spp. (26.66%) *Aspergillus flavus* (23.33%), *Penicillium* spp. (16.66%), *Trichoderma* spp. (13.33%) and *A. niger* (10%) with germination percentage (93.33%). In variety, JS 97-52, frequency of mycoflora (96.65) includes *Fusarium* spp. (33.33%), *Aspergillus niger* (26.66%), *Curvularia* spp. (13.33%), *Aspergillus flavus* and *Trichoderma* spp. (10%) and *Rhizopus* spp. (3.33%) with germination percentage (96.66%) recorded.

Different mycoflora were detected from soybean varieties by this method, in which *Fusarium* spp. (156.65), *A. niger* (139.97) was recorded maximum. This was followed by *A. flavus* (96.64), *Cladosporium* spp. (79.98), *Trichoderma* spp. (59.99), *Curvularia* spp. (29.99), *Nigrospora* spp. (23.33), *Penicillium* spp. (16.66), *Alternaria* spp. (13.33%) and *Rhizopus* spp. (3.33).

In this method, the local variety showed a maximum frequency (126.64) of mycoflora with a minimum germination percentage (73.33%) while the lowest frequency (83.31) of mycoflora was observed in CG Soya-1 variety among all the varieties of soybean taken in the study. Soybean varieties recorded *Fusarium* spp. (156.65) in highest frequency and *Rhizopus* spp. (3.33) in the lowest frequency.

Present findings are in agreement with the findings of Soesanto [13] studied 8 soybean varieties by using the blotter test methods A total of 8 fungi namely *A flavus*, *A. niger*, *C. oxysporum*, *C. dematium*, *Curvularia pallescens*, *Fusarium solani, Melanospora zamiae* and *Nigrospora* spp. Sahu [14] tested the different varieties of lentil by using the blotter paper method. In this method, the local variety showed the highest frequency (129.99%) of mycoflora while the least frequency (99.99%) of mycoflora was observed in the JL-3 variety. Singh et al. [15] studied which also supports the finding of the present study.

3.2 Agar Plate Method

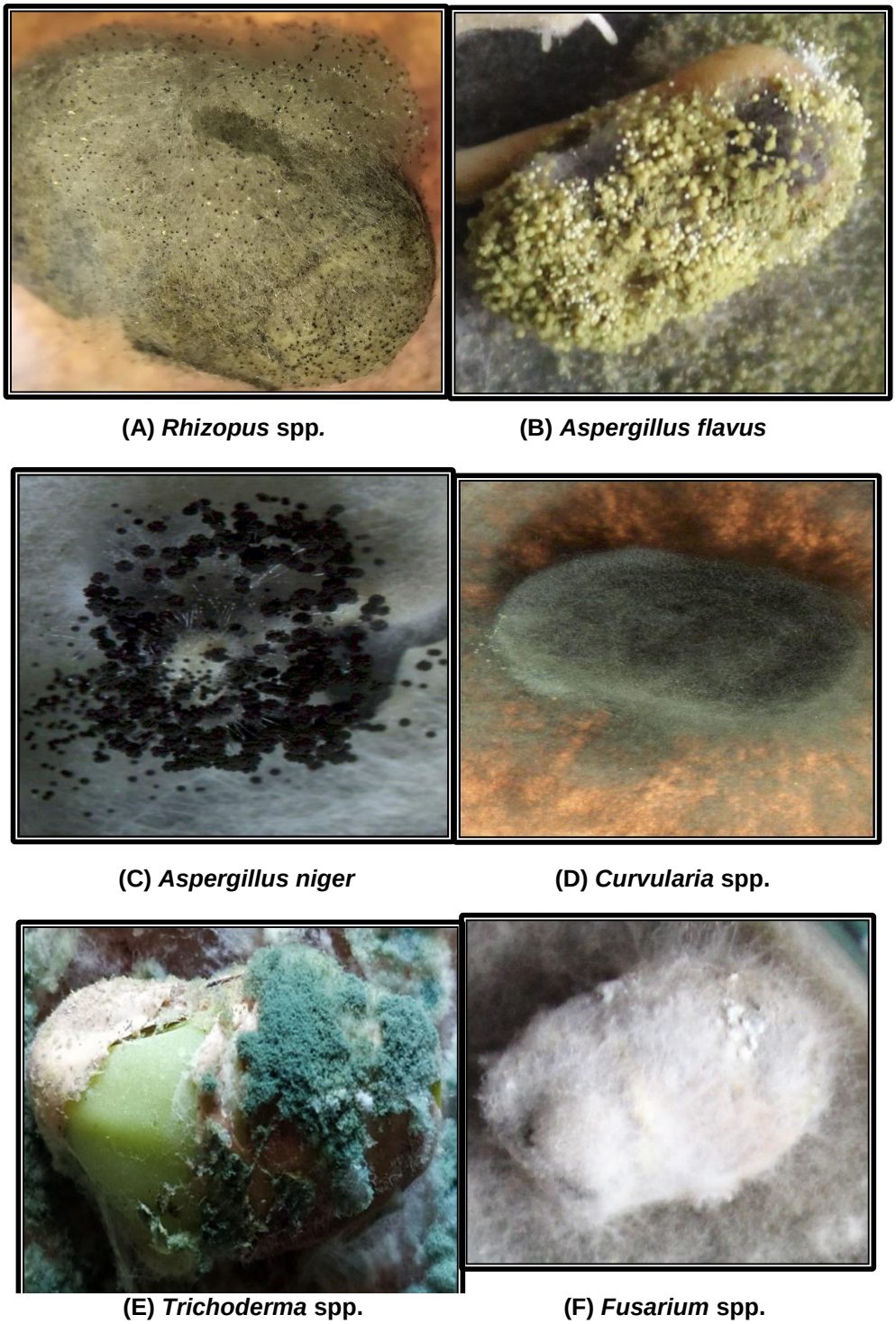
Seeds of 6 varieties of soybean were evaluated for the associated seed-borne mycoflora by the agar plate method and data are presented in Table 2. The data indicated that a total of 713.17 mycoflora were found associated with 6 varieties of soybean. The maximum frequency of mycoflora was recorded from seeds of local variety (146.63) which include *A. flavus* (33.33%), *Fusarium* spp. (26.66%), *Rhizopus* spp. (26.66%), *Cladosporium* spp. (23.33%), *A. niger* (16.66%), *Alternaria* spp. (16.66%) and *Curvularia* spp. (3.33%) with minimum germination percentage (73.33%) followed by JS 95-60 frequency of mycoflora was recorded (133.31) which includes *Fusarium* spp. (63.33%), *Cladosporium* spp. (23.33%), *Macrophomina* spp. (16.66%), *Rhizopus* spp. and *A. niger* (13.33%) and *Alternaria* spp. (3.33%) with germination percentage (80.00. Frequencies of mycoflora recorded from the seeds of other varieties of soybean RSC 10-46 (116.64), JS 97-52 (109.97), RSC 10-71 (106.64) and the least in CG Soya-1 (99.98) with varying germination percentage as 83.33, 83.33, 86.66 and 90.00, respectively.

In variety RSC 10-46, the frequency of associated mycoflora noticed namely A. *flavus* (26.66%), *A. niger* (23.33%), *Fusarium* spp. (33.33%), *Macrophomina* spp. (16.66%), *Colletotrichum* spp. (10.00%) and *Trichoderma* spp. (6.66%). In variety JS 97-52, the frequency of associated mycoflora was noticed namely *A. niger* (56.66%), *A. flavus* and *Trichoderma* spp. (16.66%), *Fusarium* spp. (33.33%) and *Rhizopus* spp. (6.66%). In variety, RSC 10-71, the frequency of associated mycoflora was noticed namely *Fusarium* spp. (36.66%), *A. niger* (33.33%), *A. flavus* (26.66%), *Trichoderma* spp. (6.66%) and *Curvularia* spp. (3.33%). In variety CG Soya-1, the frequency of associated mycoflora was observed namely *Macrophomina* spp. (63.33%), *Fusarium* spp. (26.66%), *A. niger* (6.66%) and *A. flavus* (3.33%).

Different mycoflora were detected from soybean varieties by this method, in which *Fusarium spp.* (199.97), *A. niger* (149.97) was recorded maximum. This was followed by *A. flavus* (106.64), *Macrophomina* spp. (96.65), *Cladosporium* spp. (46.66), *Rhizopus* spp. (46.65), *Trichoderma* spp. (29.98), *Alternaria* spp. (19.99), *Colletotrichum* spp. (10.00) and *Curvularia* spp. (6.66).

The present findings were in line with the work of Dhawan et al. [16] to detect the associated seed mycoflora in soybean by agar plate methods and modified PDA methods. Across the two methods

adopted, a total of 6 fungal genera including *Fusarium verticillioides, A. niger, A. flavus, M. phaseolina, A. alternata,* and *R. stolonifer* with the seeds of soybean were detected. Similarly, Parashar et al. [17] also studied the Agar plate method for the isolation of seed-borne mycoflora. Zanjare et al. [18] observed the seed-borne fungal flora of cowpea by using the agar plate method. Fungal flora was detected as *Fusarium oxysporum* (16%), *Alternaria alternata* (4%) and *Penicillium* spp. (1%). The findings of the present study are in corroboration with the findings of the above researchers as seed-borne mycoflora is a concern.



(A) *Rhizopus* spp. **(B) *Aspergillus flavus***

(C) *Aspergillus niger* **(D) *Curvularia* spp.**

(E) *Trichoderma* spp. **(F) *Fusarium* spp.**

Fig. 4. Morphology characters of various mycoflora associated with soybean seeds

Table 1. Efficacy of standard blotter method to detect the mycoflora associated with soybean seeds

S.N.	Varieties	Germination (%)	Frequency of mycoflora associated (%)										Total frequency
			A. niger	*A. flavus*	*Trichoderma* spp.	*Cladosporium* spp.	*Curvularia* spp.	*Alternaria* spp.	*Nigrospora* spp.	*Penicillium* spp.	*Fusarium* spp.	*Rhizopus* spp.	
1.	**RSC 10-46**	75.00	36.66	6.66	-	26.66	-	-	-	-	36.66	-	106.64
2.	**RSC 10-71**	93.33	10	23.33	13.33	36.66	-	-	-	16.66	-	-	99.98
3.	**CG Soya-1**	100	16.66	13.33	26.66	-	16.66	-	-	-	10	-	83.31
4.	**JS 97-52**	96.66	26.66	10.00	10.00	-	13.33	-	-	-	33.33	3.33	96.65
5.	**JS 95-60**	80.00	13.33	16.66	10.00	-	-	-	23.33	-	43.33	-	106.65
6.	**Local variety**	73.33	36.66	26.66	-	16.66	-	13.33	-	-	33.33	-	126.64
Total Mycoflora			139.97	96.64	59.99	79.98	29.99	13.33	23.33	16.66	156.65	3.33	**619.87**

Table 2. Efficacy of agar plate method to detect the mycoflora associated with soybean seeds

S.N.	Varieties	Germination (%)	Frequency of mycoflora associated (%)										Total frequency
			A. niger	*A. flavus*	*Trichoderma* spp.	*Cladosporium* spp.	*Curvularia* spp.	*Alternaria* spp.	*Colletotrichum* spp.	*Macrophomina* spp.	*Fusarium* spp.	*Rhizopus* spp.	
1.	**RSC 10-46**	83.33	23.33	26.66	6.66	-	-	-	10.00	16.66	33.33	-	116.64
2.	**RSC 10-71**	86.66	33.33	26.66	6.66	-	3.33	-	-	-	36.66	-	106.64
3.	**CG Soya-1**	90.00	6.66	3.33	-	-	-	-	-	63.33	26.66	-	99.98
4.	**JS 97-52**	83.33	56.66	16.66	16.66	-	-	-	-	-	13.33	6.66	109.97
5.	**JS 95-60**	80.00	13.33	-	-	23.33	-	3.33	-	16.66	63.33	13.33	133.31
6.	**Local variety**	73.33	16.66	33.33	-	23.33	3.33	16.66	-	-	26.66	26.66	146.63
Total mycoflora			149.97	106.64	29.98	46.66	6.66	19.99	10.00	96.65	199.97	46.65	**713.17**

Table 3. Efficacy of 2,4-D blotter method to detect the mycoflora associated with soybean seeds

S.N.	Varieties	Frequency of mycoflora associated (%)									Total frequency
		A. niger	*A. flavus*	*Cladosporium* spp.	*Fusarium* spp.	*Trichoderma* spp.	*Rhizopus* spp.	*Alternaria* spp.	*Curvularia* spp.	*Rhizoctonia* spp.	
1.	**RSC 10-46**	3.33	10..	6.66	23.33	23.33	-	-	-	-	66.65
2.	**RSC 10-71**	6.66	23.33	13.33	10.00	3.33	-	-	-	6.66	63.31
3.	**CG Soya-1**	6.66	26.66	-	10.00	6.66	6.66	-	3.33	-	59.97
4.	**JS 97-52**	6.66	26.66	-	13.33	13.33	6.66	-	-	-	66.64
5.	**JS 95-60**	-	33.33	-	13.33	16.66	3.33	-	3.33	-	69.98
6.	**Local variety**	10.00	36.66	-	13.33	3.33	-	6.66	-	10.00	79.98
Total mycoflora		35.98	156.64	19.99	83.32	66.64	16.65	6.66	6.66	16.66	**406.53**

Table 4. Efficacy of roll paper towel method to detect the mycoflora associated with soybean seeds

S.N.	Varieties	Frequency of mycoflora associated (%)										Total frequency
		Germination (%)	*A. niger*	*A. flavus*	*Cladosporium* spp.	*Fusarium* spp.	*Trichoderma* spp.	*Rhizopus* spp.	*Alternaria* spp.	*Curvularia* spp.	*Penicillium* spp.	
1.	**RSC 10-46**	86.00	16.00	18.00	12.00	8.00	14.00	12.00	10.00	-	6.00	96.00
2.	**RSC 10-71**	86.00	6.00	24.00	8.00	26.00	-	12.00	8.00	4.00	10.00	98.00
3.	**CG Soya-1**	94.00	8.00	16.00	-	18.00	6.00	10.00	-	6.00	12.00	76.00
4.	**JS 97-52**	90.00	16.00	16.00	6.00	18.00	8.00	16.00	8.00	4.00	-	92.00
5.	**JS 95-60**	82.00	14.00	20.00	18.00	20.00	-	16.00	14.00	-	6.00	108.00
6.	**Local variety**	80.00	8.00	26.00	10.00	24.00	6.00	20.00	12.00	6.00	-	112.00
Toral mycoflora			68.00	120.00	54.00	114.00	34.00	86.00	52.00	20.00	34.00	**582.00**

Table 5. Efficacy of deep freeze method to detect the mycoflora associated with soybean seeds

S.N.	Varieties	Frequency of mycoflora associated (%)						Total frequency
		A. niger	*A. flavus*	*Fusarium* spp.	*Curvularia* spp.	*Rhizopus* spp.	*Alternaria* spp.	
1.	**RSC 10-46**	-	20.00	16.66	13.33	23.33	-	73.32
2.	**RSC 10-71**	13.33	26.66	10.00	6.66	3.33	16.66	76.64
3.	**CG Soya-1**	10.00	16.66	13.33	-	16.66	3.33	59.98
4.	**JS 97-52**	23.33	13.33	20.00	-	10.00	-	66.66
5.	**JS 95-60**	20.00	23.33	16.66	3.33	13.33	-	76.65
6.	**Local variety**	16.66	26.66	20.00	13.33	23.33	6.66	106.64
Total mycoflora		83.32	126.64	96.65	36.65	89.98	26.65	**459.89**

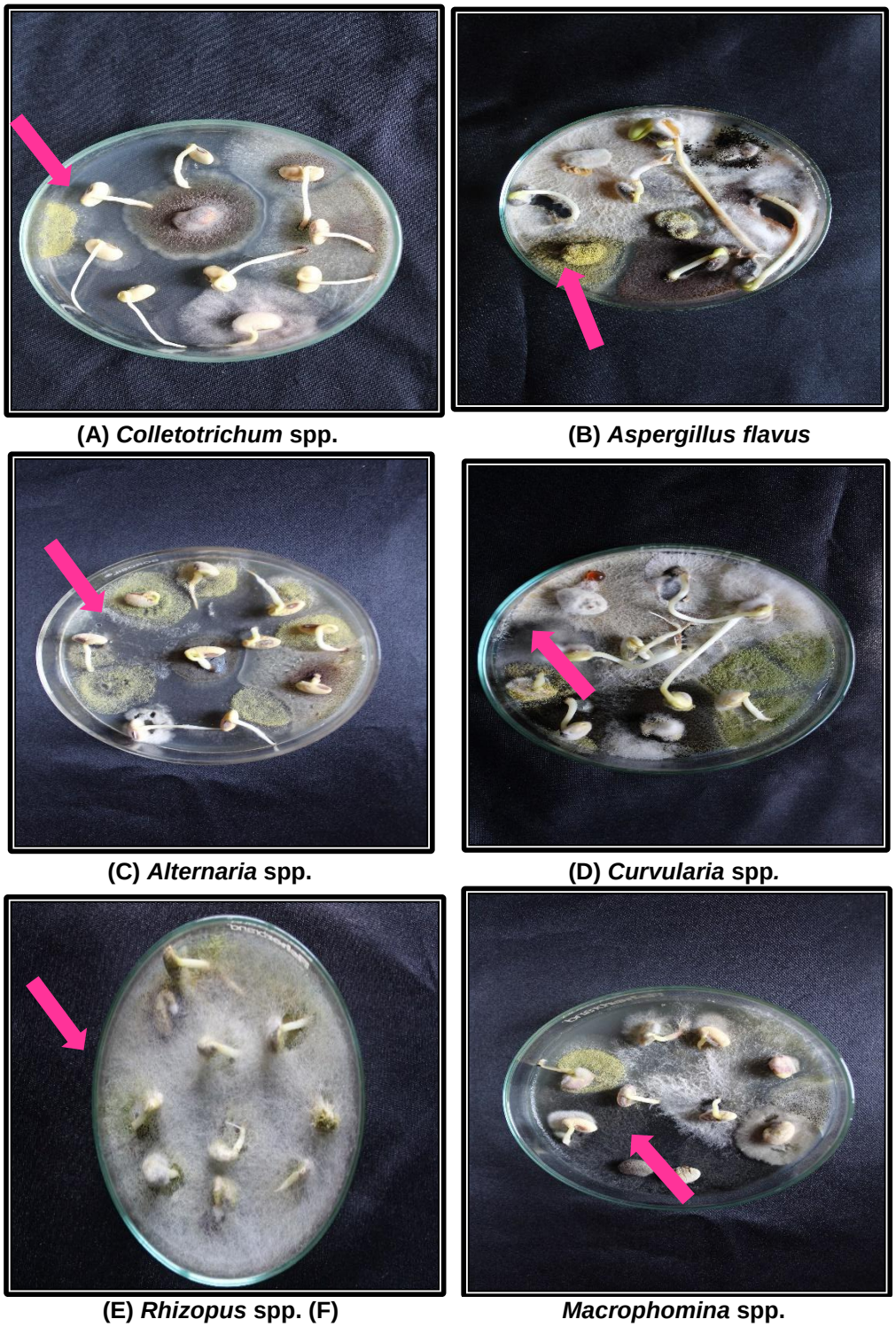


Fig. 5. Detection of mycoflora associated with seeds of soybean by agar plate method

3.3 2,4-D Blotter Method

Seed lots of different varieties of soybean were evaluated for the associated mycoflora by using the 2,4-D blotter method and data are presented in Table 3. The data indicate that a total of 406.53 mycoflora frequency were found associated with 6 varieties of soybean, and the seed lot of local variety was found with a maximum frequency of mycoflora (79.98). The mycoflora were detected as *A. flavus* (36.66%), *Fusarium* spp. (13.33%), *A. niger* (10.00%),

Rhizoctonia spp. (10.00%), *Alternaria* spp. (6.66%) and *Trichoderma* spp. (3.33%). This was followed by JS 95-60 variety (69.98) and detected mycoflora were *Aspergillus flavus* (33.33%), *Trichoderma spp.* (16.66%) *Fusarium* spp. (13.33%), *Rhizopus* spp. and *Curvularia* spp. (3.33%).

In the seed lot of varieties RSC 10-46, JS 97-52, RSC 10-71 and CG Soya-1, the frequency of associated mycoflora were (66.65), (66.64), (63.31) and (59.97), respectively. In CG Soya-1 variety, the minimum frequency of mycoflora (59.97) includes *Aspergillus flavus* (26.66%), *Fusarium* spp. (10.00%) *Trichoderma* spp. *Aspergillus niger* and *Rhizopus* spp. (6.66%) and *Curvularia* spp. (3.33%) were noticed. In variety RSC 10-46, the frequency of associated mycoflora namely *Fusarium* spp. (23.33%), *Trichoderma* spp. (23.33%), *A. flavus* (10.00%), *Cladosporium* spp. (6.66) and *A. niger* (3.33%) were recorded. In variety RSC 10-71, the frequency of associated seed-borne mycoflora was recorded as *Aspergillus flavus* (23.33%), *Cladosporium* spp. (13.33%), *Fusarium* spp. (10.00%), *Rhizoctonia* spp. (6.66%), *A. niger* (6.66%) and *Trichoderma* spp. (3.33%). In variety JS 97-52, the frequency of mycoflora (66.64%) includes *Aspergillus flavus* (26.66%), *Fusarium* spp. (13.33%), *Trichoderma spp.* (13.33%),*Aspergillus niger* (6.66%) and *Rhizopus* spp. (6.66%) were recorded.

In this method, the local variety showed a maximum frequency (79.98) of mycoflora while the lowest frequency (59.97) of mycoflora was observed in the CG Soya-1 variety among all the varieties of soybean taken in the study. Soybean varieties recorded *Aspergillus flavus* (156.64) in the highest frequency and *Alternaria* spp. (6.66) in the lowest frequency.

Rao et al. [1] also tested the 2,4-D blotter paper method that has been employed for the detection of internal and external seed-borne mycoflora of soybean. Similarly, Amule et al. [19] detect seed-borne mycoflora associated with chickpea seeds by the 2,4- D blotter method. A total of 8 genera of mycoflora recorded in different varieties of chickpea was supported the findings in the present study.

3.4 Roll Paper Towel Method

Seed lots of 6 varieties of soybean were examined for associated seed-borne mycoflora in varying frequency with a normal seedling, abnormal seedling and ungerminated seeds by rolled paper towel method and the data presented in Table 4. It was observed that the presence of mycoflora may be the cause of abnormalities and failure in seed germination. In this method, mycoflora were detected associated with seeds and seedlings of different soybean varieties. The data indicated that a total of 582.00 mycoflora were found associated with 6 varieties of soybean. The maximum frequency of mycoflora was observed in the local variety (112.00) and mycoflora detected were *Aspergillus flavus* (26.00%), *Fusarium* spp. (24.00%), *Rhizopus* spp.(20.00%), *Alternaria* spp.(12.00%), *Cladosporium spp.* (10.00%), *Aspergillus niger* (8.00%) and *Curvularia* spp.(6.00%), with minimum germination percentage (80.00%) followed by frequency of mycoflora in varieties, JS 95-60 (108.00), RSC 10-71 (98.00), RSC 10-46 (96.00), JS 97-52 (92.00) and CG Soya-1 (76.00%) and germination percentage recorded in all these different soybean varieties were 82.00, 86.00, 86.00, 90.00 and 94.00, respectively.

In seeds of JS 95-60 variety, mycoflora were recorded as *Aspergillus flavus* and *Fusarium* spp. (20.00%), *Cladosporium spp.* (18.00%), *Rhizopus* spp. (16.00%), *Aspergillus niger* (14.00%), *Alternaria* spp. (14.00%) and *Penicillium* spp. (6.00%). In RSC 10-71 variety, the associated mycoflora were *Fusarium* spp. (26.00%), *Aspergillus flavus* (24.00%), *Rhizopus* spp. (12.00%), *Penicillium* spp. (10.00%), *Cladosporium* spp. and *Alternaria* spp. (8.00%), *Aspergillus niger* (6.00%) and *Curvularia* spp. (4.00%). Mycoflora detected in RSC 10-46 variety were *Aspergillus flavus* (18.00%), *Aspergillus niger* (16.00%), *Trichoderma* spp. (14.00%), *Rhizopus* spp. and *Cladosporium spp.* (12.00%), *Alternaria* spp. (10.00%), *Fusarium spp.* (8.00%) and *Penicillium* spp. (6.00%). In JS 97-52 variety, mycoflora detected were *Fusarium* spp. (18.00%), *Aspergillus flavus* (16.00%), *A. niger* (16.00%), *Rhizopus* spp. (16.00%), *Alternaria* spp. and *Trichoderma spp.* (8.00%), *Cladosporium spp.* (6.00%) and *Curvularia* spp. (4.00%). In CG Soya-1 variety, showed the lowest frequency of mycoflora including *Fusarium* spp. (18.00%), *Aspergillus flavus* (16.00%), *Penicillium* spp. (12.00%), *Rhizopus* spp. (10.00%), *A. niger* (8.00%), *Trichoderma* spp. (6.00%) and *Curvularia* spp. (6.00%).

Different mycoflora were detected from soybean varieties by this method, in which *A. flavus* (120.00), *Fusarium spp.* (114.00) was recorded

maximum. This was followed by *A. niger* (68.00), *Rhizopus* spp. (86.00), *Cladosporium* spp. (54.00), *Alternaria* spp. (52.00), *Trichoderma* spp. (34.00), *Colletotrichum* spp. (10) and *Curvularia* spp. (20.00).

Similarly, Pawar et al. [20] determined the seed-borne fungi associated with soybean and their effect on germination by using Rolled towel paper method and fungi detected from the seeds viz., *A. flavus, A. alternata, Colletotrichum truncatum, F. oxysporum, Helminthosporium* spp. and *R. stolonifera*. Kesharwani et al. [21] also recorded "seed-associated fungal pathogens in different pea varieties as *A. niger, A. flavus, A. fumigatus*, *Trichoderma* spp., *Curvularia* spp., *Alternaria* spp., *Chaetomium* spp. and *Rhizopus* spp. by roll paper towel method". Pradhan and Lakpale [22] recorded "different varieties of Indian bean seed and observed different seed-borne fungal flora by using the roll paper towel method as *A. niger, A. fumigatus, A. terreus, Curvularia* spp., *Alternaria* spp. and *Fusarium* spp". The findings of the present investigation are in agreement with the findings of earlier researchers that most of the common fungi in varying frequencies and their impact on germination were recorded.

(A) Germinated seeds with abnormality and infection

(B) Ungerminated infected seeds (C) Ungerminated uninfected seeds

Fig. 6. Categorization of germinated and ungerminated seeds of Soybean varieties in rolled paper towel method

3.5 Deep Freeze Method

The seed lot of soybean varieties were tested for the associated seed-borne mycoflora using the deep freeze method and data presented in Table 5. The data indicated that a total of 459.89 mycoflora were found associated with 6 varieties of soybean. The frequency of mycoflora associated was maximum in local variety seed (106.64) and mycoflora detected were *Aspergillus flavus* (26.66%), *Rhizopus* spp. (23.33%) *Fusarium* spp. (20.00%), *A. niger* (16.66%), *Curvularia* spp. (13.33%) and *Alternaria* spp. (6.66%) recorded. It was followed by JS 95-60 variety (76.65), RSC 10-71 (76.64), RSC 10-46 (73.32), JS 97-52 (66.66) and CG Soya-1 (59.98) respectively.

Mycoflora observed in seed lot of JS 95-60 were *Aspergillus flavus* (23.33%), *A. niger* (20.00%), *Fusarium* spp. (16.66%), *Rhizopus* spp. (13.33%) and *Curvularia* spp. (3.33%). In RSC 10-71 variety, seed-borne mycoflora were *Aspergillus flavus* (26.66%), *Alternaria* spp. (16.66%), *A. niger* (13.33%), *Fusarium* spp. (10.00%), *Curvularia* spp. (6.66%) and *Rhizopus* spp. (3.33%). Mycoflora observed in RSC 10-46 variety were *Rhizopus* spp. (23.33%), *A. flavus* (20.00%), *Fusarium* spp. (16.66%) and *Curvularia* spp. (13.33%). In variety, JS 97-52, recorded mycoflora were *A. niger* (23.33%), *Fusarium* spp. (20.00%), *A. flavus* (13.33%) and *Rhizopus* spp. (10.00%). In variety, CG Soya-1, recorded mycoflora were *Aspergillus flavus* (16.66%), *Rhizopus* spp. (16.66%), *Fusarium* spp. (13.33%), *A. niger* (10.00%) and *Alternaria* spp. (3.33%).

In this method, *Aspergillus flavus* (126.64) was found the highest frequency of mycoflora while *Alternaria* spp. (26.65) was found as the lowest frequency among all the associated mycoflora in different varieties of soybean.

Similarly, findings were reported by Amule et al. [19] that recorded seed-associated mycoflora in chickpea seeds by deep freeze method as *F. oxysporum, A. flavus, A. niger, R. bataticola, A. alternata, C. luanata, Rhizopus* spp. and *Penicillium* spp. Pradhan [23] reported in Indian bean varieties by deep freeze method and detected associated seed mycoflora as *A. atlternata, A. flavus, A. niger, A. fumigatus, C. lunata* and *Fusarium spp.* Sewedy et al. [24] detected Thirteen genera *i,e*. *Alternaria alternata, Aspergillus niger, A. ochraceous, A. flavus, Botryodiplodia* spp., *Cladosporium* spp., *C. lindemuthianum*, *C. dematium, Fusarium solani, F. moniliforme, F. oxysporum, M. phaseolina, Myrothecium* spp., *Penicillium* spp., *R. solani, Stemphylium* spp., *Trichoderma* spp. and *Trichothecium* spp. by deep freeze method supports the findings of above the study due to the occurrence of common seed-borne mycoflora in various legumes.

3.6 Comparative Efficacy of Different Incubation Methods in Detecting Seed-Borne Mycoflora

Comparative efficacy of five incubation methods *viz.* standard blotter, agar plate, 2,4-D blotter, rolled paper towel and deep freeze methods in detecting seed-borne mycoflora in six varieties of soybean. Among them, the agar plate method was found to be the best for routine seed health evaluation as it could detect (118.86%) mean frequency of mycoflora as compared to (103.31%) in the standard blotter paper method, (97.66%) in the roll paper towel method (76.64%) in deep freeze method and (67.75%) in 2,4-D blotter method.

Table 6. Comparative efficacy of different incubation methods to detect the seed-borne mycoflora of soybean seeds

Varieties	Detection method					Mean Frequency (%)
	Frequency of mycoflora (%)					
	Standard blotter method	Agar plate method	2,4-D blotter method	Deep freeze method	Rolled paper towel	
RSC 10-46	106.64	116.64	66.65	73.32	96.00	91.85
RSC 10-71	99.98	106.64	63.31	76.64	98.00	88.91
CG Soya-1	83.31	99.98	59.97	59.98	80.00	76.64
JS 97-52	96.65	109.97	66.64	66.66	92.00	86.38
JS 95-60	106.65	133.31	69.98	76.65	108.00	98.91
Local variety	126.64	146.63	79.98	106.64	112.00	114.37
Total Mycoflora	619.87	713.17	406.53	459.89	586	
Mean	103.31	118.86	67.75	76.64	97.66	

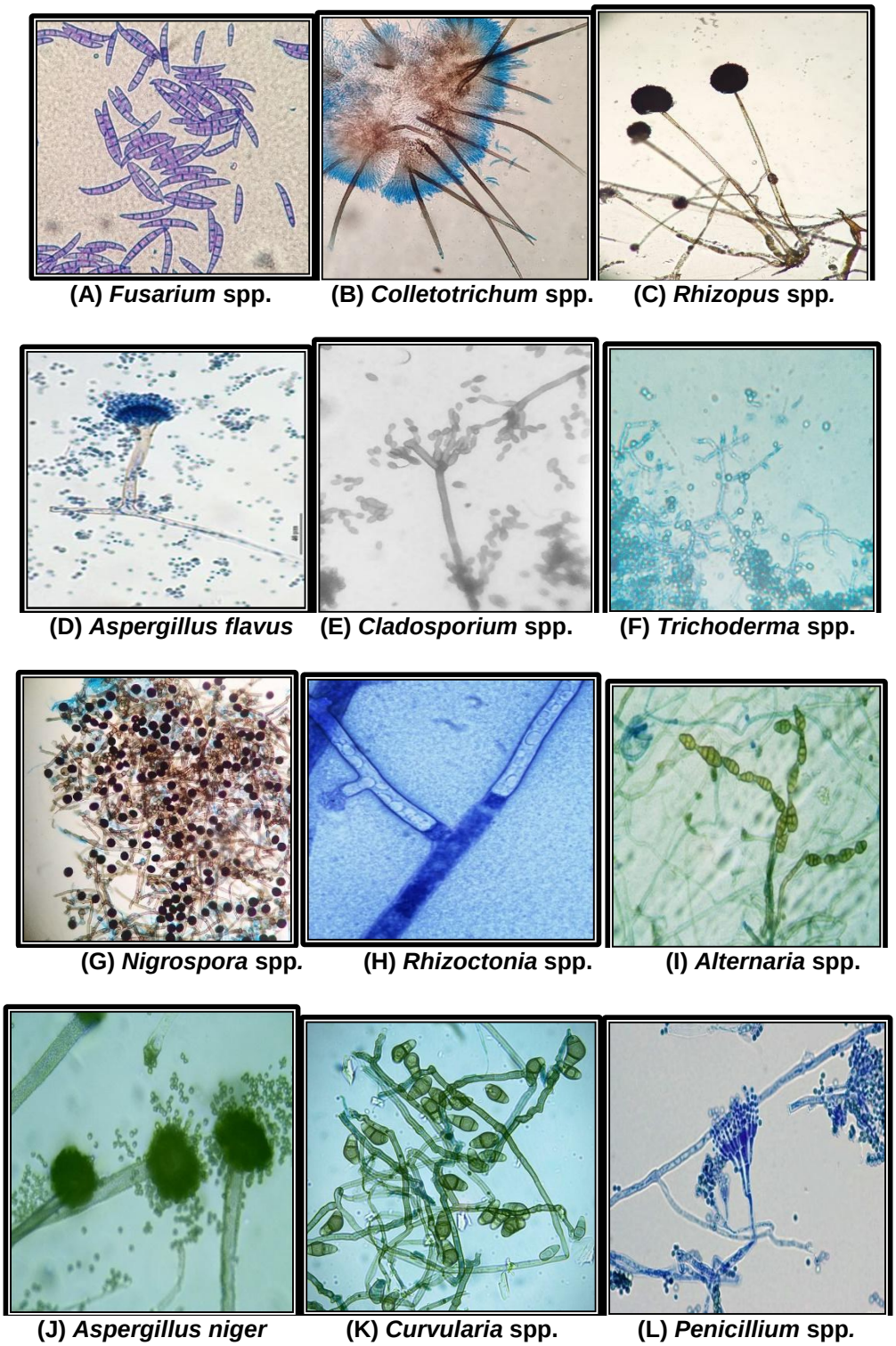


Fig. 7. Microphotographs of mycoflora detected from soybean seeds in various incubation methods

4. SUMMARY

The studies on seed-borne mycoflora of soybean seeds using incubation methods provide critical insights into disease management, seed quality assessment, pathogen identification, seed treatment development, and risk assessment. These benefits contribute to the improvement of

soybean production practices, seed health, and overall crop productivity.

5. CONCLUSION

Among the five different incubation methods employed for the detection of seed-borne fungal infections of soybean. The agar plate method was found most effective in detecting seed-borne mycoflora. Ten fungi species *viz. Aspergillus flavus, Aspergillus niger, Alternaria* spp*., Cladosporium* spp*., Curvularia* spp*., Rhizopus* spp*., Trichoderma* spp*., Rhizoctonia* spp*.*, *Macrophomina* spp*.* and *Fusarium* spp. were reported.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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