

## Original Research Article

### Studies on Seed Borne Mycoflora of Soybean Seeds by Incubation Methods

#### ABSTRACT

The present investigation was conducted in the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G) during the year 2020-2021. 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 was 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 as 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.

**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 "Golden Bean" of the 20<sup>th</sup> 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 beside essential amino acids like lysine (5%) and a small amount of calcium, phosphorous, magnesium and iron (References). The quality of seeds is affected by seed borne mycoflora. The attack of plant pathogen 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 (References). 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 affected grains during storage them unfit and become not suitable for human consumption. Some seed associated fungi can affect the seedling or plant resulting in decrease in productivity capacity reduced (Rahman *et al.* 1999). Some fungal pathogens are associated with the testa and cotyledons of seeds infected form of conidia (spores) and mycelium, after seed germination the infection move to

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hypocotyls and base of the stem. The Seed of fungal flora plays a significant role in determining seed quality and longevity. Among all mycoflora of soybean *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp., *Nigrosporaspp.*, *Fusarium* spp., *Curvulariaspp.*, *Macrophominaspp.* 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. Please stated the aim of the study/research..

## 2. MATERIALS AND METHODS

Unless and otherwise mentioned for each experiment, 200 seeds were used. In general, the Ppetri dish with seeds was incubated at  $25\pm 1^{\circ}\text{C}$  under a 12 hours 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. The microorganisms were seen observed on the seeds using a stereo binocular microscope to determine their habit morphology and characteristics.

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The associated mycoflora was identified with the help of standard literature like illustrated Genera of Imperfect Fungi (Ellis, 1976). a pictorial guide to the Identification of Seed borne Fungi of Pigeon pea, Chickpea, Groundnut, Sorghum, Pearl Millet and Finger Millet (ICRISAT,1978).

Several seed testing methods have been 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) (Anon, 1966). 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 (Neergaard,1977) 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:-

- 2.1. Standard blotter method (ISTA, 1976)
- 2.2. Agar plate method (Muskett and Malone, 1941)
- 2.3. 2,4-D blotter method (ISTA 1966)
- 2.4. Roll paper towel method (Yaklich, 1985)
- 2.5. Deep freeze method (Limonard, 1968)

### 2.1. Standard blotter method

Four hundred seeds of each testing sample were kept equidistantly and aseptically on sterilized plastic Petri dishesplates, 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 Ppetri plate and 1seed at the center of the Ppetri plate. For each seed sample, 40 replicated plates were maintained. The seeded plates were incubated at  $25\pm 1^{\circ}\text{C}$  for 7 days in alternating cycles of 12 hours darkness and 12 hours light in NUV. Observations were taken as explained earlier. All seeds of the periphery of the plate were examined first, then finally seed in the center of petri plate and expressed in percentage of seed borne mycoflora, individually.

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**The frequency of the mycoflora was calculated by the following formula: (Reference?)**

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

## 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 sulphate 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 petri plate in such a manner that 9 seeds in the periphery and 1 seed at the center of 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.

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## 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. Please include the picture/figure of the method or illustration

## 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 help of a rubber band tied the towel at 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 by naked eyes and presence of different mycoflora by stereoscopic microscope.

**A. Normal seedlings** –Seedlings showed the potential for continued growth & development Please include the picture/figure of the method or illustration 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.

**D. Please include the picture/figure of the method or illustration**

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## 2.5. Deep freeze method

This method is the 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% Strepto-penicillin (to avoid bacterial contamination) and incubated for 24 hours. under

normal conditions in a growth chamber. Plates were further incubated at  $10 \pm 1^\circ\text{C}$  for 3 days and then transferred to the deep freezer ( $-20^\circ\text{C}$ ) under complete darkness for 24 hours. Plates were again incubated at  $25 \pm 1^\circ\text{C}$  for 5 days. Observations were recorded as described earlier. [Please include the picture/figure of the method or illustration](#)

### 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, 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, 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%) were 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, local variety showed maximum frequency (126.64) of mycoflora with minimum germination percentage (73.33%) while 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 lowest frequency.

Present findings are in agreement with the findings of Soesanto (2020) studied 8 soybean varieties by using blotter test method. A total of 8 fungi namely *A. flavus*, *A. niger*, *C. oxysporum*, *C. dematium*, *Curvularia pallescens*, *Fusarium solanii*, *Melanospora zamiae* and

*Nigrospora* spp. Sahu (2020) tested the different varieties of lentil by using the blotter paper method. In this method, local variety showed the highest frequency (129.99%) of mycoflora while least frequency (99.99%) of mycoflora was observed in JL-3 variety which also supports ~~to~~the finding of the present study.



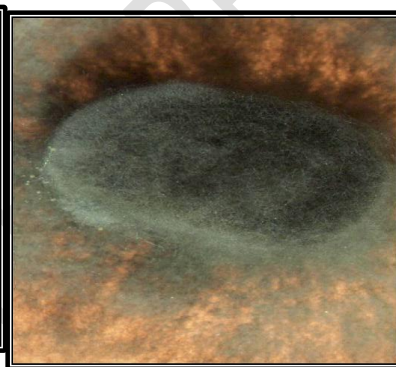
(A) *Rhizopus* spp.



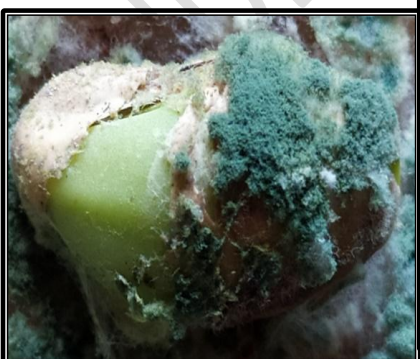
(B) *Aspergillus flavus*



(C) *Aspergillus niger*



(D) *Curvularia* spp.



(E) *Trichoderma* spp.



(F) *Fusarium* spp.

Fig . 1 Habit-Morphology characters of various mycoflora associated with Soybean seeds

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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
			<i>A. niger</i>	<i>A. flavus</i>	<i>Trichoderma</i> spp.	<i>Cladosporium</i> spp.	<i>Curvularia</i> spp.	<i>Alternaria</i> spp.	<i>Nigrosporaspp.</i>	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.	<i>Rhizopus</i> 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
<b>Total Mycoflora</b>			139.97	96.64	59.99	79.98	29.99	13.33	23.33	16.66	156.65	3.33	<b>619.87</b>

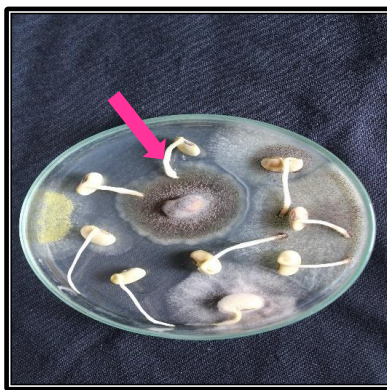
### 1. Agar plate method

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 include *Fusarium* spp. (63.33%), *Cladosporium* spp.(23.33%), *Macrophomina*spp. (16.66%),*Rhizopus* spp.aAnd*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 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 observed 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 noticed 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).

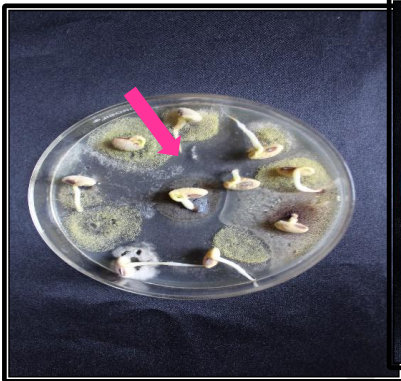
The present findings were in line with the work of Dhawan *et al.* (2019) to detect the associated seed mycoflora in soybean by agar plate methods and modified PDA methods. Across the two methods adopted, a total of 6 fungal genera including *Fusarium verticillioides*, *A. niger*, *A. flavus*, *M.phaseolina*, *A.alternata*, and *R.stolonifer* with the seeds of soybean were detected. Similarly, Zanjareet *al.* (2020) observed 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) *Colletotrichum* spp.



(B) *Aspergillus flavus*



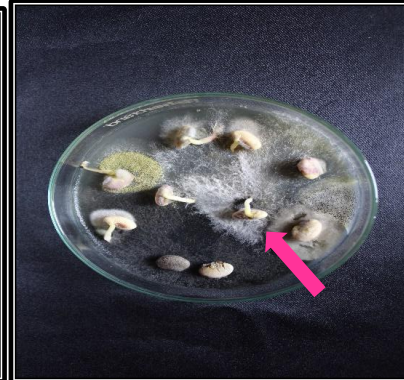
(C) *Alternaria* spp.



(D) *Curvularia* spp.



(E) *Rhizopus* spp.



(F) *Macrophomina* spp.

Fig .2 Detection of mycoflora associated with seeds of soybean by agar plate method

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
			<i>A. niger</i>	<i>A. flavus</i>	<i>Trichoderma</i> spp.	<i>Cladosporium</i> spp.	<i>Curvulariaspp.</i>	<i>Alternaria</i> spp.	<i>Colletotrichum</i> spp.	<i>Macrophominaspp.</i>	<i>Fusarium</i> spp.	<i>Rhizopus</i> 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
<b>Total mycoflora</b>			149.97	106.64	29.98	46.66	6.66	19.99	10.00	96.65	199.97	46.65	<b>713.17</b>

## 2. 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%), *Rhizoctoniaspp.* (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 *Curvulariaspp.* (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 *Curvulariaspp.* (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, frequency of associated seed borne mycoflora was recorded as *Aspergillus flavus* (23.33%), *Cladosporium* spp. (13.33%), *Fusarium*spp. (10.00%), *Rhizoctoniaspp.* (6.66%), *A. niger* (6.66%) and *Trichoderma* spp. (3.33%). In variety JS 97-52, 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, local variety showed maximum frequency (79.98) of mycoflora while the lowest frequency (59.97) of mycoflora was observed in 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 *Alternariaspp.* (6.66) in the lowest frequency.

Rao *et al.* (2015) also tested the 2,4-D blotter paper method that have been employed for the detection of internal and external seed-borne mycoflora of soybean. Similarly, Amuleet *al.* (2019) detect seed-borne mycoflora associated with chickpea seeds by the 2,4- D blotter method. Total 8 genera of mycoflora recorded in different varieties of chickpea was supported the finding in the present study.

**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
		<i>A. niger</i>	<i>A. flavus</i>	<i>Cladosporium</i> spp.	<i>Fusarium</i> spp.	<i>Trichoderma</i> spp.	<i>Rhizopus</i> spp.	<i>Alternaria</i> spp.	<i>Curvularia</i> spp.	<i>Rhizoctonia</i> spp.	
1.	<b>RSC 10-46</b>	3.33	10..	6.66	23.33	23.33	-	-	-	-	66.65
2.	<b>RSC 10-71</b>	6.66	23.33	13.33	10.00	3.33	-	-	-	6.66	63.31
3.	<b>CG Soya-1</b>	6.66	26.66	-	10.00	6.66	6.66	-	3.33	-	59.97
4.	<b>JS 97-52</b>	6.66	26.66	-	13.33	13.33	6.66	-	-	-	66.64
5.	<b>JS 95-60</b>	-	33.33	-	13.33	16.66	3.33	-	3.33	-	69.98
6.	<b>Local variety</b>	10.00	36.66	-	13.33	3.33	-	6.66	-	10.00	79.98
<b>Total mycoflora</b>		35.98	156.64	19.99	83.32	66.64	16.65	6.66	6.66	16.66	<b>406.53</b>

### 3. Roll paper towel method

Seeds lot 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 seed and seedling 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 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 *Curvulariaspp.*(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 *Curvulariaspp.* (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 *Curvulariaspp.* (4.00%). In CG Soya-1 variety, showed lowest frequency of mycoflora includes *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 *Curvulariaspp.* (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, Kesharwani *et al.* (2018) also recorded seed associated fungal pathogen in different pea varieties as *A. niger*, *A. flavus*, *A. fumigatus*, *Trichoderma* spp., *Curvulariaspp.*, *Alternaria* spp., *Chaetomium* spp. and *Rhizopus* spp. by roll paper towel method. Pradhan and Lakpale (2020) recorded in different varieties of Indian bean seed observed different seed borne fungal flora by using 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.

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

S.N.	Varieties	Germination (%)	Frequency of mycoflora associated (%)								Total frequency	
			<i>A. niger</i>	<i>A. flavus</i>	<i>Cladosporium</i> spp.	<i>Fusarium</i> spp.	<i>Trichoderma</i> spp.	<i>Rhizopus</i> spp.	<i>Alternaria</i> spp.	<i>Curvulariaspp.</i>		<i>Penicillium</i> 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
<b>Total mycoflora</b>			68.00	120.00	54.00	114.00	34.00	86.00	52.00	20.00	34.00	<b>582.00</b>

#### 4. 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 Amuleet *al.* (2019) that recorded seed associated mycoflora in chick pea seed by deep freeze method as *F.oxysporum*, *A. flavus*, *A. niger*, *R. bataticola*, *A. alternata*, *C. lunata*, *Rhizopus* spp. and *Penicillium* spp. Pradhan (2019) reported in Indian bean varieties by deep freeze method and detected associated seed mycoflora as *A. alternata*, *A. flavus*, *A. niger*, *A. fumigatus*, *C. lunata* and *Fusarium* spp. supports the findings of above the study due to the occurrence of common seed borne mycoflora in various legumes.

Please bring here more general discussion about comparison results among all the methods and varieties (commercial vs local). And also what is the benefit of this study for soybean producers..

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

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

Conclusion (???)

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