Studies on association of seed borne mycoflora in different varieties of black gram [*Vigna mungo* (L.) Hepper] by incubation methods

ABSTRACT

The present study was conducted to detect and identify seed-borne pathogenic fungi in some different varieties of black gram and their effect on seed germination.Six varieties of black gram (Indira urd-1, Pratap-1, KU-96, TPU-4, TIU-22) and local variety collected from village for experiment. The Eleven seed associated mycoflora recorded were *Aspergillus flavus Aspergillus niger, Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Penicillium* spp., *Curvularia* spp., *Cladosporium* spp., *Colletotrichum* spp., *Chaetomium* spp. and *Trichoderma* spp. were detected and identified from the seeds of different varieties of black gram by using the standard blotter method, agar plate method, 2,4-D blotter method, roll paper towel method and deep freeze method. In all these methods, poor germination percentage was recorded in local variety, may be due to the presence of seed associated mycoflora with maximum frequency, whereas KU-96 variety has most extreme germination percentage due to the minimum frequency of seed associated mycoflora as compared to other varieties of black gram taken in the examination.

Key words :(Black gram, seed borne mycoflora, Standard Blotter method, Agar Plate Method Seed germination)

INTRODUCTION

Black gram is one of the most important pulse grown in both Kharif and Rabi seasons.In India, black gram is an essential food legume that is commonly consumed. It also contributes to sustainable agriculture by improving soil fertility through biological nitrogen fixation. It is a common short-duration pulse crop grown throughout the India. This crop is cultivated as a mixed crop, cash crop, and sequential crop in cropping systems, as well as growing as a single crop after rice harvest and before and after the harvest of other summer crops in semi-irrigated and dry land conditions.

Black gram is known for its high phosphoric acid content. It contains 25g protein/100g of seed and carbohydrates 58.99g/100g of seeds indicate that it is nutritious pulse. It also has good amount of phosphorus (3.85mg/100g), iron (10.2 mg/100g), thiamin (0.42%/100g), riboflavin (0.20mg/100g), niacin (2mg/100g) and vitamin C (3mg /100g) (Shakuntala Manay and M.Shadaksharaswamy,1987).

Among all mycoflora of black gram *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Fusarium oxysporum*, *Rhizopus stoloniferus,Alternaria alternaria*, *Chaetomium* sp., *Colletotrichum* sp., *Macrophomina phaseolina* are various seed borne fungi which affect Black gram leading to loss in quality and quantity of the seed and reduce the productive capacity and also kill the seedling as well as plants. In view of above, present study was conducted to know the effect of seed borne mycoflora on germination and identify the pathogen present in black gram seed This type of study is conducted to assess the quality and safety of black gram seeds used for cultivation .

MATERIAL AND METHODS

The current study was conducted in the Department of Plant Pathology, Collage of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, between 2020-2021. The different varieties of black gram with a view to assess seed borne mycoflora associated with six varieties of black gram (Indira urd-1, Pratap-1, KU-96, TPU-4, TIU-22) and local variety collected from village for experiment

For each type of study 400 seeds were drawn from the sample and were tested for seed health. 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. Seeds were observed for the presence of seed borne mycoflora after 7 days of incubation. The mycoflora present on the seeds were examined under the wide field Stereobinocular microscope and their habit features were confirmed under the compound microscope.

The associated seed mycoflora were identified with the standard manual i.e. Illustrated Genera of Imperfect fungi (Barnett, 1962), More *Dematiaceous Hypomcetes* (Ellis, 1976) and An Illustrated Manual for Identification (Paul *et al*.) and a Pictorial Guide to the Identification of seed borne fungi of sorghum, Pearl Millet, Chickpea, Pigeon pea and Groundnut (ICRISAT,1978).

Detection of seed borne mycoflora

Several methods have been developed to detect seed borne mycoflora by International Seed Testing Association (ISTA). The following method were used to assess the seed borne mycoflora of black gram varieties.

1. Standard blotter paper method (ISTA, 1976)
2. Agar plate method(Muskett and Malone, 1941)
3. Roll paper towel method (Yaklich, 1985)
4. Deep freeze method(Limonard, 1968)
5. 2, 4 D blotter method

1. Blotter paper method

Good quality round blotter papers of the same size of plastic petri-dishes diameter were cut and moist with sterilized distilled water. There-after moistened blotter papers were kept in surface sterilized petri-

dishes. Then 25 seeds were placed on blotter paper in such a way that 16 seeds formed the outer circle 8 in middle and 1 seed formed the center of petri plate. A total of 40 replicated plates were kept for each seed lot (total of 400 seeds tested for each seed sample).The seeded plates were incubated in NUV at 25±1˚C for 7 days in alternating cycles of 12 hours darkness and 12 hours light. After 7 days of incubation, seeds were examined under a stereoscopic microscope. All seeds of the outer ring were examined first then seed in the center of the petri plate and seed mycoflora association are expressed as a percentage of the total seed mycoflora, individually.

The following formula was used to calculate the fungus frequency :

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

2. Agar plate method

For this method, first of all PDA medium was prepared. The sterilized medium (15-20ml) was poured in sterilized petri plates. Thereafter, plates were kept for solidification of medium, after solidification 400 seeds of each variety were arranged on medium. Prior to arrange the seeds were surface sterilized for 30 seconds with a 1.0 percent NaOCl solution and immediately thoroughly washed the with sterile distilled water 3 times to remove NaOCl traces of solution. In each petri plate, 25 seeds were placed each plate had 16 seeds in outer ring 8 in middle periphery and 1 seed in the centre of the plates. Sixteen replicated plates were maintained for each variety and incubated at 25±1˚C under alternate cycle of 12 hours dark and 12 hours light in NUV. The plate seeds were examined after 7 days of incubation for the associated mycoflora.

3. Roll paper towel method

In this procedure, seeds were placed on moist paper towels (80x45cm) at equal distance and 50 seeds were kept on each towel. Thereafter these seeds were covered with another towel. Then they were carefully rolled without disturbing the seeds already placed. After rolling the towels both side ends of towel were tied with rubber band. To prevent the water loss, polythene or wax coated paper wrapping rolled paper towels were used. There seeded towels 50 seeds were sown on each towel at equal distance. Incubate 4 to 5 days at 25±1^{o}C. After incubation seeds were analysed for normal and abnormal seedlings, cause of abnormalities, failure in germination and non-germinated seeds and as well as the presence of mycoflora by naked eye and using a Stereobinocular microscope. The observations were recorded for the following purposes:

Categories:

1. Normal seedling

2. Abnormal seedling

3. Ungerminated seeds

4. Deep freeze method

In this method 400 seeds of each variety were taken and tested. Seeds of each variety were arranged on moistened blotter paper which were dipped in 0.2% streptomycin sulphate prior to kept in plastic petriplate. There seeded plates were kept at 25±1˚C for 24 hrs in growth chamber after 24 hrs than plates were transferred at -20^{o}C and kept for 24 hrs thereafter these plates were again transferred in growth chamber and incubated for 5 days and observations were recorded for associated mycoflora. Sixteen replications were maintained for each variety and each plates had 25 seeds of black gram.

5. 2,4 D blotter method

The blotters were soaked in 0.2% solution of 2,4-D (Dichlorophenoxy acetic acid) instead of plane dipped in water. Four hundred seeds of each variety were tested by placing seeds on 2-4, D differed blotter papers. Thereafter, plates was incubated in growth chamber where 25±1°C and 90% RH was maintained with cycles of 12 hours light and 12 hours darkness. After 7 days of incubation the each seed was examined for the association mycoflora using stereoscopic-binocular microscope.

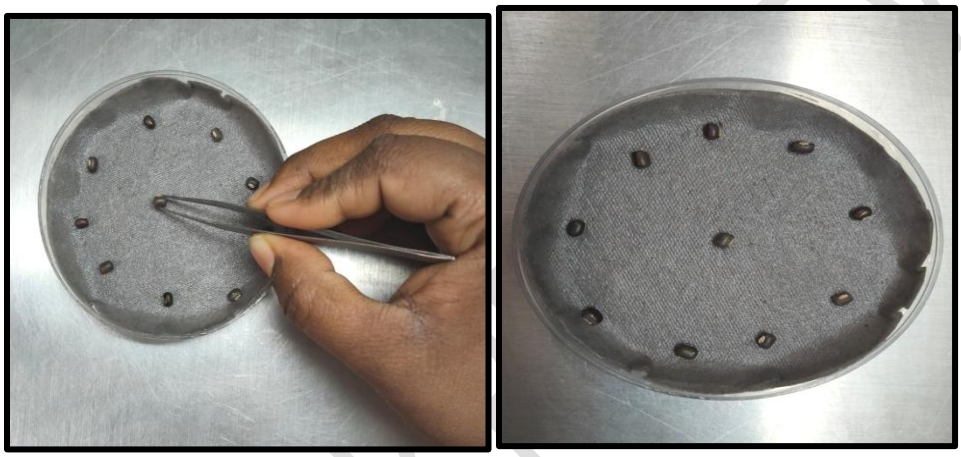

Fig.1 Blotter paper method

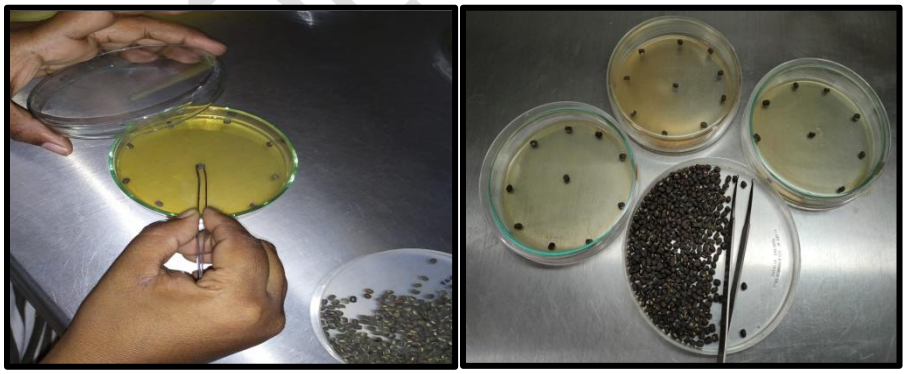

Fig 2- Agar plate method

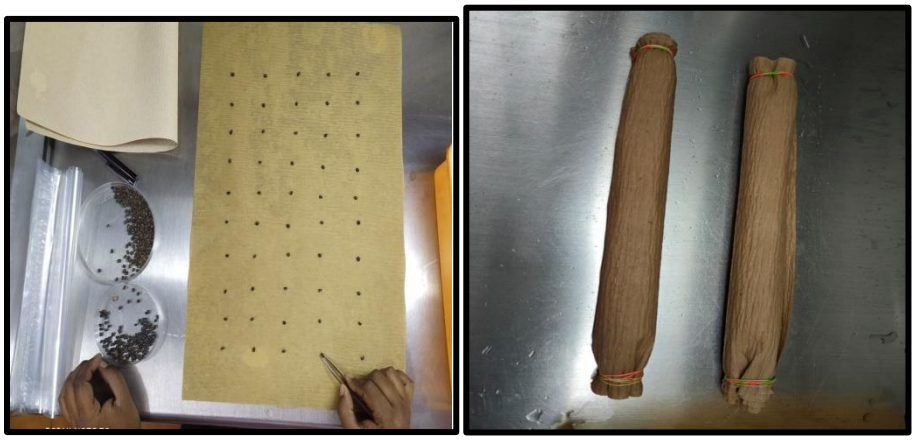

Fig.3. Roll paper towel method

RESULTS AND DISCUSSIONS

Blotter paper method

Seed sample of different black gram varieties were evaluated for the associated seed borne mycoflora by using standard blotter method and data presented in table 1 indicates that maximum frequency of mycoflora were recorded from the seed sample of local variety were recorded maximum frequency of mycoflora (139.97%) with lowest germination percentage (80%) and mycoflora detected were *A. flavus*(26.66 %), *Fusarium* sp. (36.66%), *A. niger* (23.33%), *Rhizopus* sp. (16.66%) and *A. alternata* and *T. viride* (13.33%). and *Curvularia* sp. (10.00%). This was followed by Indira urd-1 seed lot (123.31%) with germination percentage (90.00%) and mycoflora were identified as *A. niger* (26.66%), *A. flavus* and *Fusarium* sp. (23.33%), *Rhizopus* sp. (20.00%) *A. alternata* (13.33%),*Penicillium* sp.(10,00%) and *Chaetomium* sp. (6.66%).

Also, in seed lot TIU-22 mycoflora frequency (123.30%) with germination percentage (86.66%) and mycoflora were identified as *A. flavus*(36.66 %), *A. niger* (26.66%), *Rhizopus* sp. (23.33%), *T. viride* (16.66%), *Fusarium* spp. (13.33%), and *Penicillium* sp. (6.66%). In variety TPU-4, frequency of associated mycoflora namely *A. flavus* (26.66%), *A. niger* and *Alternaria* spp.(23.33%), *Fusarium* spp. (20.00%), *Rhizopus* spp. (16.66%) and *Chaetomium* spp.(10.00%), with germination percentage (86.66%) were recorded. In variety Pratap-1, frequency of mycoflora (109.97%) with germination percentage (93.33%) includes *A. niger* (26.66%), *Rhizopus* spp. (23.33%), *A. flavus* (20.00%), *Fusarium* spp. (16.66%), *Curvularia* sp.(13.33%) and *T. viride* (6.66%). In KU-96 variety, minimum frequency of mycoflora (99.98%) includes *A. flavus* (23.33%), *A. niger* (20.00%), *Chaetomium* spp. and *Penicillium*

spp.(16.66%), *Rhizopus* spp.(13.33) *Fusarium* sp. (10.00%), and with maximum germination percentage (96.66%).

The relative abundance of mycoflora *Aspergillus flavus* (156.64%) was found maximum from seed lot of black gram. Other predominated mycoflora were *A. niger* (146.64%), *Fusarium* spp. (119.98%), *Rhizopus stolonifer* (113.31%), *Alternaria* spp.(53.32%) *Trichoderma* spp. (36.65%), *Chaetomium* spp.(33.32%), *Penicillium* spp. (33.32%), and *Curvularia* spp. (23.33%).

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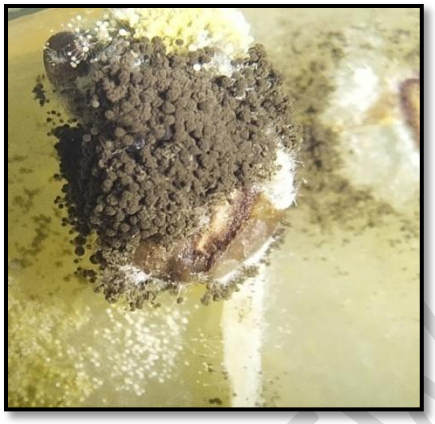
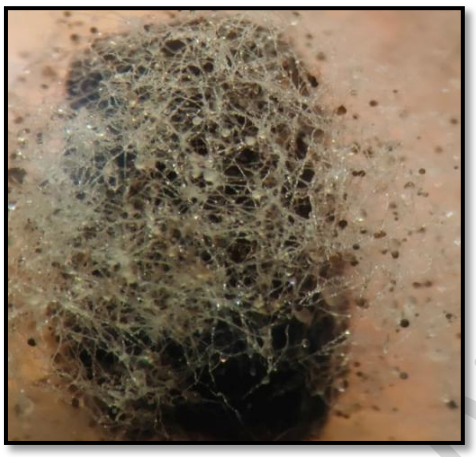
Table 1. **Frequency percentage of mycoflora**

S. No.	Varieties	Germination (%)	Frequency of mycoflora %									Total frequency %
			A. flavus	*A. niger*	*Fusarium* spp.	*Rhizopus* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Culvularia* spp.	*Penicillium* spp.	*Trichoderma* sp.	
1	**Indira urd-1**	90.00	23.33	26.66	23.33	20.00	13.33	6.66	**-**	10.00	-	**123.31**
2	**Pratap-1**	93.33	20.00	26.66	16.66	23.33	3.33	**-**	13.33	**-**	6.66	**109.97**
3	**KU-96**	96.66	23.33	20.00	10.00	13.33	**-**	16.66	**-**	16.66	-	**99.98**
4	**TPU-4**	86.66	26.66	23.33	20.00	16.66	23.33	10.00	**-**	**-**	-	**119.98**
5	**TIU-22**	86.66	36.66	26.66	13.33	23.33	**-**	**-**	**-**	6.66	16.66	**123.30**
6	**Local variety**	80.00	26.66	23.33	36.66	16.66	13.33	**-**	10.00	**-**	13.33	**139.97**
Total mycoflora			**156.64**	**146.64**	**119.98**	**113.31**	**53.32**	**33.32**	**23.33**	**33.32**	**36.65**	



Aspergillus flavus ***Aspergillus niger***



***Rhizopus* spp.** ***Fusarium* spp.**

***Alternaria* spp.**

Fig-4 Habit characters of various mycoflora associated with black gram seed

Agar plate method

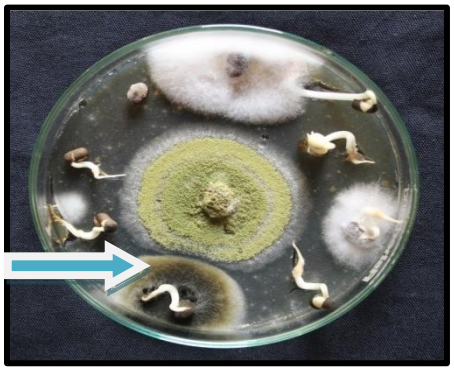
Seeds of 6 varieties of black gram were evaluated for the associated seed borne mycoflora by agar plate method and the data presented in table 2. shows that the maximum frequency of mycoflora were recorded from seeds of local variety (149.98%) which include *Rhizopus* spp.(26.66%), *Fusarium* spp. (33.33%), *Alternaria* spp.(23.33%), *A. niger* and *A. flavus* (20.00%), *Trichoderma* spp. (16.66%) and *Curvularia* spp. (10.00%) with minimum germination percentage (70%) followed by TIU-22 seed lot mycoflora (129.97%) with germination percentage (76.66%) and mycoflora were identified as *A. flavus*(33.33 %), *A. niger* and *Fusarium* spp. (26.66%), *Rhizopus* sp. (23.33%), *T. viride* (16.66%) and *Penicillium* sp. and *Cladosporium* spp.(10.00%). Frequencies of mycoflora recorded from the seeds of other varieties of black gram Indira urd-1(126.64%), Pratap-1 (119.98%), TPU-4 (116.61%) and least in KU-96 (106.64%) with varying germination percentage as 80.00, 83.33, 83.33 and 86.66, respectively.

In Indira urd -1 variety frequency of associated mycoflora namely *A. flavus* (26.66%), *Alternaria* spp.(30.00%), *Fusarium* spp. (23.33%), *A. niger* (16.66%),*Colletotrichum* spp. (13.33%), *Curvularia* spp. (10.00%) and *Penicillium* spp. (6.66%) with germination percentage (80.00%) were recorded. In variety Pratap-1 frequency of associated seed borne mycoflora recorded as *A. niger* and*Rhizopus* spp. (33.33%),*A. flavus* (20.00%), *Fusarium* spp. (16.66%), *Penicillium* spp. (13.33%) and *Curvularia* spp. (3.33%) with germination percentage (83.33%) were recorded. In variety TPU-4 frequency of mycoflora (116.64%) with germination percentage (83.33%) includes *Fusarium* spp. (33.33%), *Colletotrichum* spp. (20.00%), *A. flavus* (16.66%), *Cladosporium* spp. and *Rhizopus* spp. (13.33%), *A. niger* (10.00%), *Trichoderma* spp.(6.66%) and *Penicillium* spp. (3.33%). In KU-96 variety, minimum frequency of mycoflora (106.64%) includes *A. niger* (36.66%), *A. flavus* (23.33%), *Rhizopus* spp. (16.66%), *Fusarium* spp. and *Alternaria* spp.(13.33%),*Colletotrichum* spp. (3.33%).

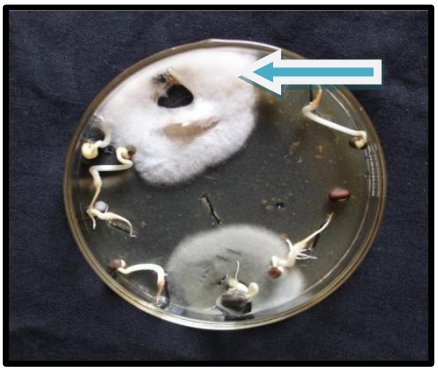
Different mycoflora were detected from black gram varieties by this method, in which *Fusarium* sp.(146.64%) was recorded maximum and it was most frequent in local variety and TPU-4 (33.33%). This was followed by *A. niger* (143.31%) *A. flavus* (139.98%)*Rhizopus* spp. (89.98%), *Alternaria* spp.(66.66%) *Colletotrichum* spp. (59.99%), *Penicillium* spp. (29.99%) *Cladosporium* spp.and *Curvularia* spp.(23.33%),and *Trichoderma* spp.(23.32%) was found least in black gramvarieties.

Table 2 Agar plate method to detect the mycoflora associated with different varieties of black gram

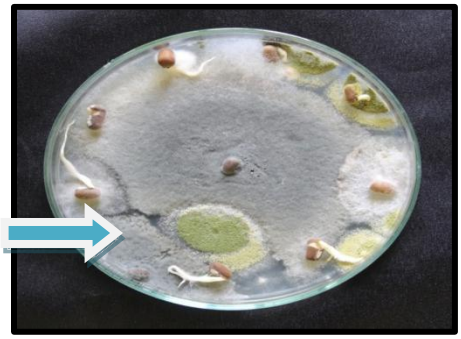
S. No.	Varieties	Germination (%)	Frequency of mycoflora associated (%)										
			A. flavus	*A. niger*	*Fusarium* spp.	*Alternaria* spp.	*Rhizopus* spp.	*Colletotrichum* spp.	*Curvularia* spp.	*Penicillium* spp.	*Cladosporium* spp.	*Trichoderma* spp.	Total frequency (%)
1	**Indira urd-1**	80.00	26.66	16.66	23.33	30.00	-	13.33	10.00	6.66	-	-	**126. 64**
2	**Pratap-1**	83.33	20.00	33.33	16.66	-	33.33	-	3.33	13.33	-	-	**119.98**
3	**KU-96**	86.66	23.33	36.66	13.33	13.33	16.66	3.33	-	-	-	-	**106.64**
4	**TPU-4**	83.33	16.66	10.00	33.33	-	13.33	20.00	-	3.33	13.33	6.66	**116.64**
5	**TIU-22**	76.66	33.33	26.66	26.66	-	-	23.33	-	10.00	10.00	-	**129.98**
6	**Local variety**	70.00	20.00	20.00	33.33	23.33	26.66	-	10.00	-	-	16.66	**149.98**



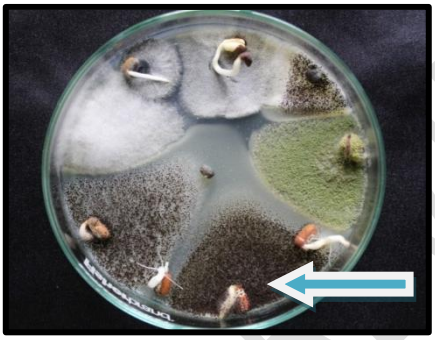
***Alternaria* spp.**



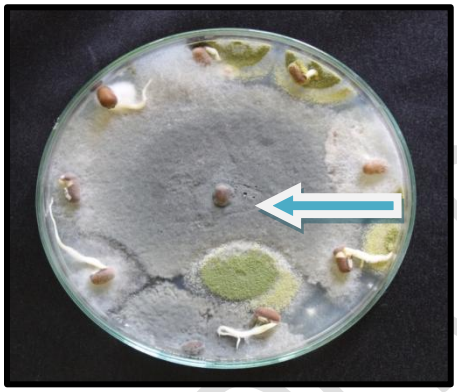
***Fusarium* spp.**



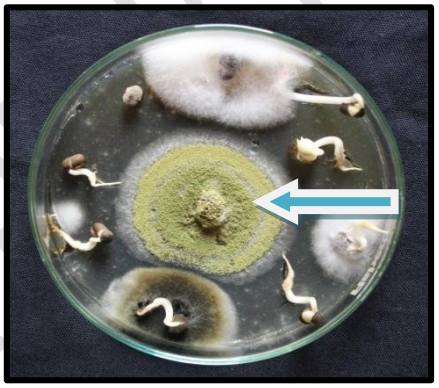
***Curvularia* spp.**



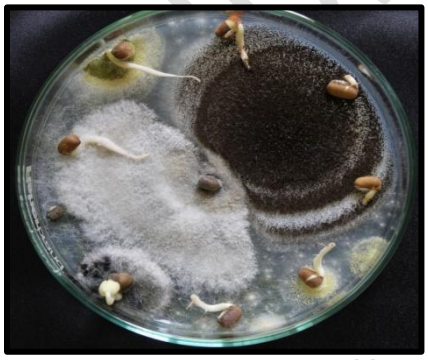
Aspergillus niger



***Macrophomina* spp.**



Aspergillus flavus



***Rhizopus* spp.**

Fig 5- Detection of mycoflora associated with seeds of black gram by agar plate method

3. Roll paper towel method

Seed lot of 6 varieties of black gram were examined for associated seed borne mycoflora in varying frequency with normal seedling, abnormal seedling and ungerminated seeds by rolled paper towel method. It was observed that 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 black gram varieties. Maximum frequency of mycoflora was observed in local variety (99.98%) and mycoflora detected were *A. niger* (33.33%), *A. flavus* (26.66%), *Fusarium* spp. (23.33%), *Rhizopus* spp. (13.33%), and *T. viride* (3.33%), with minimum germination percentage (70.00%) followed by frequency of mycoflora in varieties TPU-4 (96.63%), Pratap-1(93.31%), TIU-22 (89.98%), Indira urd-1 (89.97%) and KU-96 (86.65%) and germination percentage recorded in all these different black gram varieties were 76.66, 83.33, 83.33, 86.66 and 90.00, respectively.

In seeds of TPU-4 variety, mycoflora were recorded as *Fusarium* spp. (26.66%),*Curvularia* spp. (23.33%), *A. niger* and*Rhizopus* spp. (16.66%), *Alternaria* spp.(6.66%), and *Penicillium* spp. (6.66%). Pratap-1 variety, associated mycoflora were *A. flavus* (26.66%), *Fusarium* spp. (23.33 %), *A. niger* (16.66%) *Trichoderma* spp.and *Alternaria* spp.(10.00%) and *Rhizopus* spp.(6.66%). Mycoflora detected in TIU-22 variety were *A. niger* (26.66%), *A. flavus* (23.33%), *Trichoderma* spp.(20.00%), *Fusarium* spp. (13.33%) and *Curvularia*spp.(6.66%). Indira urd-1variety, mycoflora detected were *A. flavus* and *Rhizopus* spp. (23.33), *A. niger* (16.66%), *Fusarium* spp. (13.33%), *Trichoderma* spp.(6.66 %), *Penicillium* spp. and *Alternaria* spp.(3.33%). In KU-96 variety, showed least frequency of mycoflora (86.65%) includes *A. flavus* (36.66%), *A. niger* (23.33%), *Fusarium* spp. and *Rhizopus* spp.(13.33%).

Relative abundance of mycoflora were *Aspergillus flavus* (136.64%) followed by *Aspergillus niger* (133.3%), *Fusarium* spp. (113.31%), *Rhizopus stolonifer* (73.31%), *Trichoderma viride* (39.99%), *Curvularia* spp. (29.99 %), *Alternaria* spp. (19.99%) and *Penicillium* spp. (9.99%)

Table 3: Paper towel method to detect the mycoflora associated with different varieties of black gram

S. No.	Varieties	Germination (%)	Frequency of mycoflora associated (%)								
			A. flavus	*A. niger*	*Fusarium* spp.	*Alternaria* spp.	*Rhizopus* spp.	*Trichoderma* spp.	*Curvularia* spp.	*Penicillium* spp.	Total frequency (%)
1	**Indira urd-1**	**86.66**	23.33	16.66	13.33	3.33	23.33	6.66	**-**	3.33	**89.97**
2	**Pratap-1**	**83.33**	26.66	16.66	23.33	10.00	6.66	10.00	**-**	**-**	**93.31**
3	**KU-96**	**90.00**	36.66	23.33	13.33	**-**	13.33	**-**	**-**	**-**	**86.65**
4	**TPU-4**	**76.66**	-	16.66	26.66	6.66	16.66	**-**	23.33	6.66	**96.63**
5	**TIU-22**	**83.33**	23.33	26.66	13.33	**-**	**-**	20.00	6.66	**-**	**89. 98**
6	**Local variety**	**70.00**	26.66	33.33	23.33	-	13.33	3.33	**-**	**-**	**99.98**
Total mycoflora			**136.64**	**133.3**	**113.31**	**19.99**	**73.31**	**39.99**	**29.99**	**9.99**	

(A) germinated seeds with abnormal seeds

(B) Ungerminated with infected seeds

Fig 6. Categories of germination and ungerminated seeds of black gram varieties in rolled paper towel method

4. Deep freezing method

Seed lot of black gram varieties were tested for the associated seed borne mycoflora using deep freeze method and data are presented in table 4. Frequency of mycoflora associated were maximum in local variety (83.31%) and detected were *A. flavus* (26.66%), *A. niger* (16.66%), *Fusarium* spp. (13.33) and *Rhizopus* spp.(20.00%) with minimum germination (56.66%) and next were Indira urd-1 (79.98), Pratap-1 (69.98%),TIU-22 (59.99%), TPU-4 (56.66%) and KU-96 (43.32%) and 60.00%, 60.00%,70.00%,73.33% and 76.66%, germination respectively.

Mycoflora observed in seed lot of Indira urd-1were *A. flavus* (26.66%), *Rhizopus* spp.(23.33%), *A. niger*(16.66%), *Fusarium* spp. (13.33%), and *Alternaria* spp.(6.66%). In Pratap-1 variety, seed borne mycoflora were *Rhizopus* spp. (26.66%), *Fusarium* spp. (23.33%), *A. niger* (13.33%) and *Chaetomium* spp. (6.66%). Mycofloraobserved in TIU-22 variety were *Aspergillus flavus* (23.33%), *Chaetomium* spp. (20.00%) and *Rhizopus* spp. (16.66%).In variety TPU-4,recorded mycoflora were *Fusarium* spp. (20.00%), *Aspergillus niger*(16.66%), *A. flavus* (10.00%), and *Rhizopus* spp. (10.00%). In variety KU-96, recorded mycoflora were *A. flavus* (23.33%), *Rhizopus* spp.(16.66%) and *Fusarium* spp. (3.33%).

In this method, relative abundance of different mycoflora such as *Rhizopus stolonifer* (113.31%), *Aspergillus flavus* (109.98%), *Fusarium* sp.(73.32%), *Aspergillus niger* (63.31%), *Chaetomium globosum* (26.66%), and *Alternaria alternata* (6.66%) were recorded. In this method mycoflora associated with seeds of black gram varieties were observed maximum in local variety with lower germination percentage, while minimum frequency of mycoflora were observed from seeds of KU-96 variety with higher germination than the other varieties taken in the study.

Table 4: Deep freeze method to detect the mycoflora associated with different varieties of black gram

S. No.	Varieties	Frequency of mycoflora associated (%)						
		A. flavus	*A. niger*	*Fusarium* spp.	*Alternaria* spp.	*Rhizopus* spp.	*Chaetomimum* spp.	Total frequency (%)
1	**Indira urd-1**	26.66	16.66	13.33	6.66	23.33	-	**79.98**
2	**Pratap-1**	**-**	13.33	23.33	**-**	26.66	6.66	**69.98**
3	**KU-96**	23.33	-	3.33	-	16.66	-	**43.32**
4	**TPU-4**	10.00	16.66	20.00	-	10.00	**-**	**56.66**
5	**TIU-22**	23.33	-		-	16.66	20.00	**59.99**
6	**Local variety**	26.66	16.66	13.33	**-**	20.00	**-**	**83.31**
Total mycoflora		**109.98**	**63.31**	**73.32**	**6.66**	**113.31**	**26.66**	

5.2,4 D blotter method

Seed lot of black gram varieties were tested for the associated seed borne mycoflora using 2,4 D blotter method and data presented in table 4. Frequency of mycoflora associated were maximum in local variety seed lot (79.99%) and mycoflora detected were *A. flavus* and *Rhizopus* spp.(23.33%), *Fusarium* spp. (20.00) and *A. niger* (13.33%), and with minimum germination (60.00%) recorded. It was followed by Pratap-1 (73.32%) variety and Indira urd-1 (63.31%),TPU-4 (49.99%), TIU-22 (43.32%) and KU-96 (36.65%) and with the germination percentage of the black gram varieties were 70.00%,73.33%,76.66%, 83.33 and 86.66% respectively.

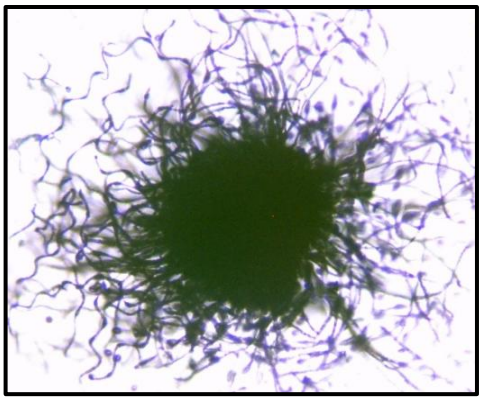
Mycoflora detected in seed lot of Pratap-1 as *Rhizopus*spp. (36.66%), *A. flavus* (23.33%) and *A. niger* (13.33%). Indira urd-1 variety consists of mycoflora as *A. flavus* (26.66%), *A. niger* (16.66%), *Fusarium* spp. (13.33%) and *Chaetomimum* spp. (6.66%). Mycoflora detected in seed lot of TPU-4 variety as *A. flavus* (23.33%), *Rhizopus* spp.(16.66%). and *Fusarium* spp. (10.00%). TIU-22 variety consists of mycoflora as *Chaetomimum* spp. (16.66%), *Fusarium* spp. (13.33%), *A. flavus* (10.00%) and *A. niger* (3.33%). Least frequency of mycoflora recorded in KU-96 variety were *Chaetomimum* sp. (16.66%), *A. niger* (13.33%) *Fusarium* spp. (6.66%). Highest germination (86.66%) was also recorded in KU-96 variety.

In this method, relative abundance of *Aspergillus flavus* (106.65%) was recorded, followed by *Rhizopus* sp. (76.65%), *Fusarium* sp. (63.32%), *Aspergillus niger* (59.98%), and *Chaetomium* sp.(39.98%). Frequency of *Aspergillus flavus* were found maximum in Indira urd-1 variety (26.66%), followed by Pratap-1, TPU-4 and local variety (23.33%) and TIU-22 (10.00%). Frequency of *Chaetomium* sp. were found least in Indira urd-1 variety (6.66%), followed by KU-96 and TIU-22 (16.66%).

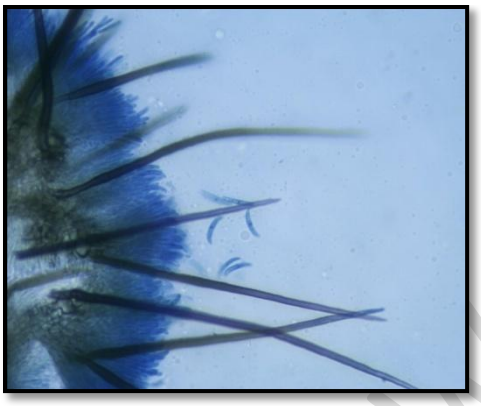
In this method mycoflora associated with seeds of black gram varieties were observed maximum in local variety with lower germination percentage, while minimum frequency of mycoflora were observed from seeds of KU-96 variety with higher germination than the other varieties taken in the study.

Table 5: 2,4 D blotter method to detect the mycoflora associated with different varieties of black gram

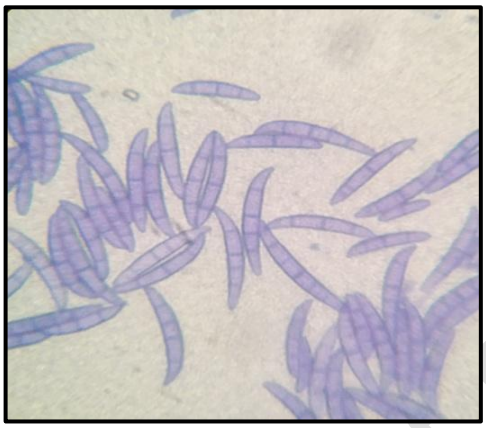
S. No	Varieties	Frequency of mycoflora associated (%)					Total frequency (%)
		A. flavus	*A. niger*	*Fusarium* spp.	*Rhizopus* spp.	*Chaetomium* spp.	
1	**Indira urd-1**	26.66	16.66	13.33	-	6.66	**63.31**
2	**Pratap-1**	23.33	13.33	-	36.66	-	**73.32**
3	**KU-96**	-	13.33	6.66	-	16.66	**36.65**
4	**TPU-4**	23.33	-	10.00	16.66	-	**49.99**
5	**TIU-22**	10.00	3.33	13.33	-	16.66	**43.32**
6	**Local varieties**	23.33	13.33	20.00	23.33	-	**79.99**
Total mycoflora		**106.65**	**59.98**	**63.32**	**76.65**	**39.98**	



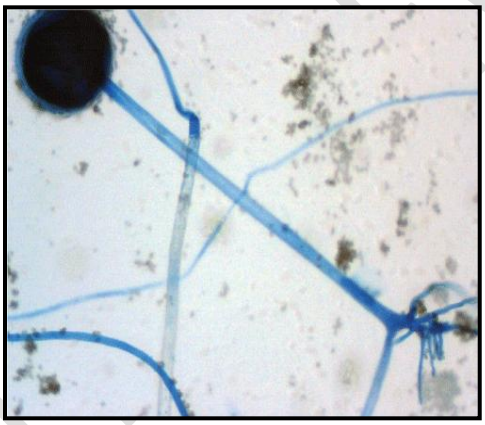
(A) *Chaetomium* spp.



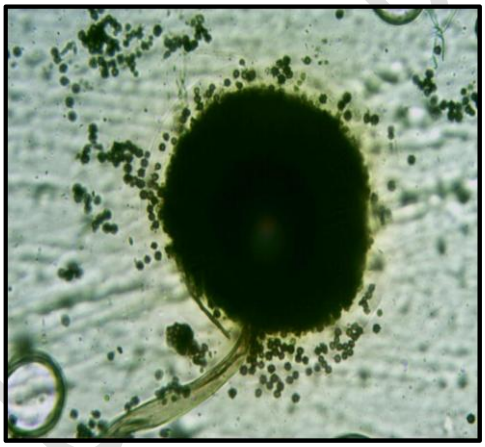
(B)*Colletotrichum* spp.



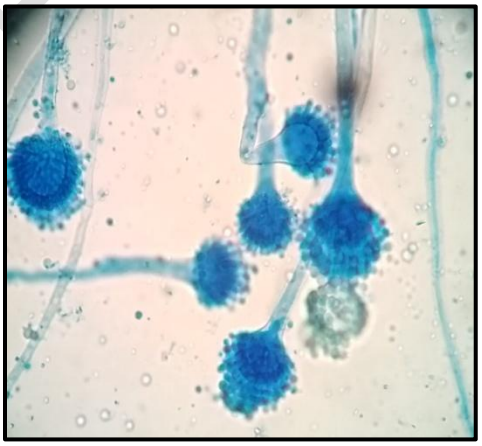
(C) *Fusarium* spp.



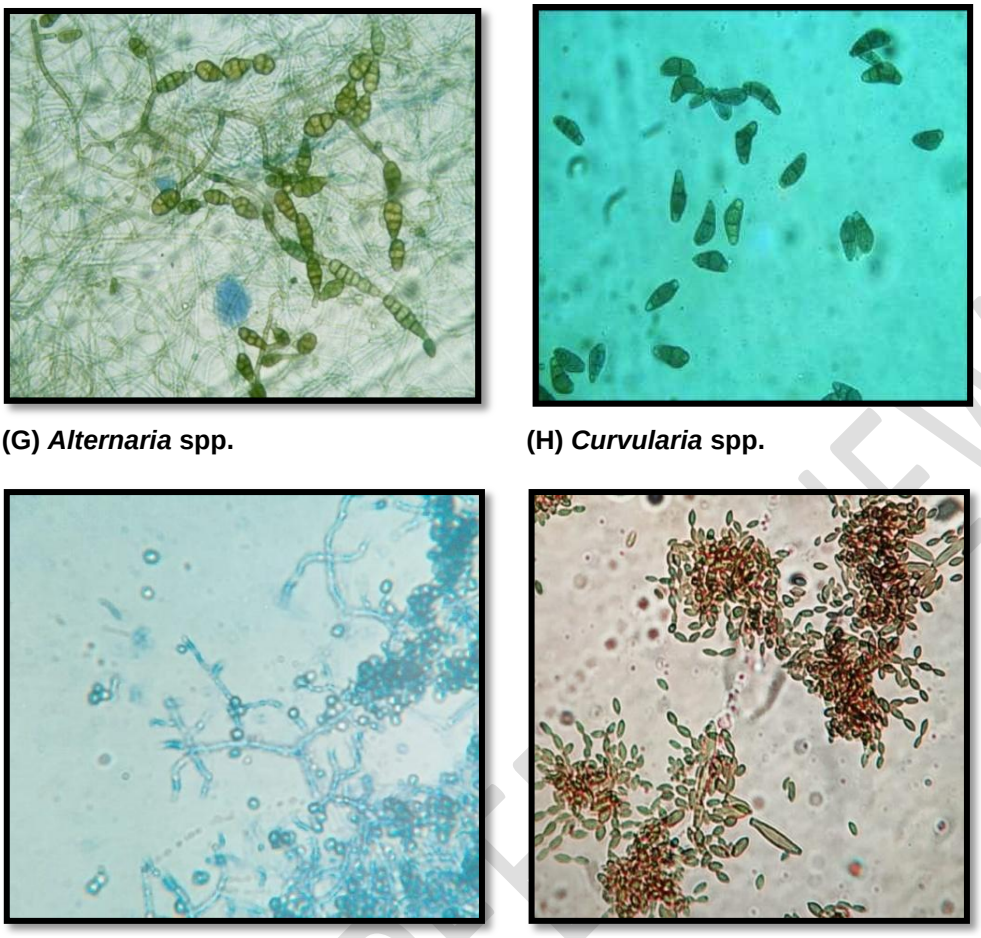
(D) *Rhizopus* spp.



(E) *Aspergillus niger*



(F) *Aspergillus flavus*



(G) *Alternaria* spp. **(H) *Curvularia* spp.**

(I) *Trichoderma* spp. **(J)*Cladosporium* spp.**

Fig 7- Microphotographs of mycoflora detected from Black gram seeds in various incubation methods

Comparative efficacy of different incubation methods to detecting seed borne mycoflora of black gram varieties

Comparative efficacy of 5 incubation methods viz. standard blotter, agar plate, rolled paper towel, deep freeze and 2,4 D blotter methods in detecting seed borne mycoflora in 6 varieties of black gram were presented in table 5 (a). Among them, agar plate method was found to the best for routine seed health evaluation as it could detect (124.97%) mean frequency of mycoflora as compared to (119.41%) in standard blotter paper method, (92.75%) in roll paper towel method (65.54%) in deep freeze method and (57.76%) in 2,4 D method.

Per cent efficiency wise, agar plate method recorded maximum efficiency (116.36%) over 2,4 D blotter method followed by (90.67%) over deep freeze method (34.73%) over roll paper towel method, (7.39%) over standard blotter paper method.

SUMMARY

The studies on seed-borne mycoflora of black gram 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 black gram production practices, seed health, and overall crop productivity

CONCLUSION

These seed samples when subjected to incubation methods, varying frequency and type of mycoflora associated with black gram seeds were recorded. The seed associated mycoflora recorded were *Aspergillus flavus Aspergillus niger, Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Penicillium* spp., *Curvularia* spp., *Cladosporium* spp., *Colletotrichum* spp., *Chaetomium* spp. and *Trichoderma* spp. In all these methods, poor germination percentage was recorded in local variety, may be due to the presence of seed associated mycoflora with maximum frequency, whereas KU-96 variety has most extreme germination percentage dueto the minimum frequency of seed associated mycoflora as compared to other varieties of black gram taken in the examination.

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