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 recorded 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 roll method and deep freeze method. In all these methods, poor germination percentage was recorded in local variety, which may be due to the presence of seed associated mycoflora with maximum frequency, ~~whereas~~ whileKU-96 variety has the 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.

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

INTRODUCTION

Black gram is one of the most important pulses grown in both Kharif and Rabi seasons.In India, black gram is an essential ~~food~~ legume that is ~~commonly~~ often 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~~ grown 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 content of phosphoric acid ~~content~~. ~~It c~~Contains 25g protein/100g of seed and carbohydrates 58.99g/100g of seeds indicate that it is nutritious ~~pulse~~legume. It also has good amount of phosphorus (3.85mg/100g), iron (10.2mg/100g), thiamin (0.42%/100g), riboflavin (0.20mg/100g), niacin (2mg/100g) and vitamin C (3mg/100g) (Shakuntala Manay and M.Shadaksharaswamy,1987~~),~~ not cited in the References!

Comment [SS1]:

Among the black gram all mycoflora~~of black gram~~ *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Fusarium oxysporum*, *Rhizopus stoloniferus,Alternaria alternaria*, *Chaetomium* sp., *Colletotrichum* sp., *Macrophominaphaseolina* are various seed borne fungi ~~which~~ thataffect ~~Black~~ black gram leading to loss in quality and quantity of the seeds and reduce the productive capacity and also kill ~~the~~ seedling as well as plants. In view of above, present study was ~~conducted~~ carried outto ~~know~~ find outthe 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~~the aim of ~~to~~ assessing 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~~ research,400 seeds were ~~drawn~~ extractedfrom 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~~ a wide-field ~~Stereobinocular~~ stereobinocularmicroscope, and their habit features were confirmed under ~~the~~ acompound microscope.

~~The~~ aAssociated seed mycoflora~~were~~ wasidentified ~~with the~~using standard manuals 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

The International Seed Testing Association (ISTA) has developed s~~S~~everal methods ~~have been developed to~~ for detection of seed-borne mycoflora ~~by International Seed Testing Association (ISTA)~~. The following methods 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~~ as the diameter of the plastic ~~petri~~Petri-dishes ~~diameter~~ were cut and moisenedt with sterilized distilled water. The~~re after~~ moistened blotter papers were then kept in surface-sterilized ~~petri~~Petri ~~-~~dishes. Then 25 seeds were placed on blotter paper in such a way that 16 seeds formed the outer circle of 8 in middle and 1 seed formed the center of ~~petri~~ Petri plate. A total of 40 replicate~~d~~ 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 of light. After 7 days of incubation, the seeds were examined under a stereoscopic microscope. All seeds of the outer ring were examined first, then seed in the center of the ~~petri~~ Petri plate and seed mycoflora association ~~are~~ wasexpressed as a percentage of the total seed mycoflora, individually.

The following formula was used to calculate the ~~fungus~~ frequency of the fungi:

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

2. Agar plate method

For this method, ~~first of all~~ PDA medium was first prepared. ~~The s~~Sterilized medium (15-20ml) was poured into sterilized ~~petri~~ Petri ~~plates~~dishes. ~~Thereafter~~After that, the plates were ~~kept~~ left~~for to~~~~solidification~~ solidifyof medium, after solidification 400 seeds of each variety were arranged on the 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 traces of NaOCl~~traces of~~ solution. In each ~~petri~~ Petri plate, 25 seeds were placed. E~~-~~each plate had 16 seeds in outer ring 8 in middle periphery and 1 seed in the centre of the plates. Sixteen replicate~~d~~ plates were maintained for each variety and incubated at 25±1˚C ~~under~~ in analternate 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~~ After that,the~~o~~se seeds ~~were~~ arecovered with another towel. Then they were carefully rolled without disturbing the seeds already placed. After rolling up the towels, both ~~side~~ ends of towel ~~were~~ aretied with rubber band. To prevent ~~the~~ water loss, polyethylene or wax coated paper was used to wrap~~ping~~ 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, the seeds were ~~analysed~~ analyzed for normal and abnormal seedlings, the cause of abnormalities, failure ~~in to~~germination germinateand non-germinated seeds, ~~and~~ as well as the presence of mycoflora~~by~~ with

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thenaked eye and using a ~~S~~stereobinocular microscope. The observations were recorded for the following purposesby category: normal seedling, abnormal seedling orungerminated seeds.~~:~~

~~**Categories:**~~

~~**1. Normal seedling**~~

~~**2. Abnormal seedling**~~

~~**3. Ungerminated seeds**~~

4. Deep freeze method

~~In~~Using this method, 400 seeds of each variety were taken and tested. Seeds of each variety were ~~arranged~~ spreadon moistened blotter paper ~~which were~~ dipped in 0.2% streptomycin sulphate ~~prior to~~before being kept in plastic ~~petriplate~~Petriplate. The~~reseeded~~ plantedplates were kept at 25±1˚C for 24 hrs in the growth chamber after 24 hrs,~~than~~ then the plates were transferred ~~at~~ to-20^{o}C and kept for 24 hrs. After that,~~thereafter~~ these plates were again transferred ~~in~~ to thegrowth chamber and incubated for 5 days and the observations were recorded for the associated mycoflora. Sixteen ~~replications~~ replicateswere maintained for each variety and each plate~~s~~ had 25 seeds of black gram.

5. 2,4 D blotter method

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

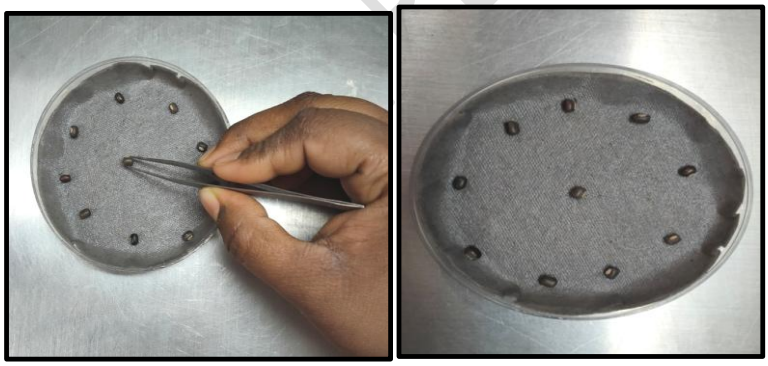

Fig.1. Blotter paper method

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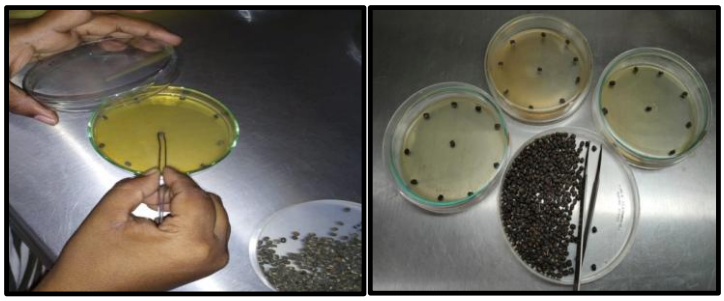

Fig. 2– Agar plate method

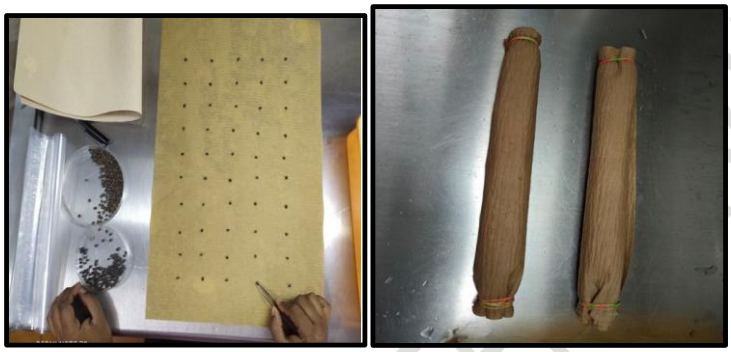

Fig.3. Roll paper towel method

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RESULTS AND DISCUSSIONS

Blotter paper method

Seed sample of different black gram varieties were evaluated for the associated seed borne mycoflora by using the standard blotter method and the data presented in ~~table~~ Table 1 indicates that ~~maximum~~ the highestfrequency of mycoflora~~were~~ wasrecorded from the seed sample of local variety were recorded maximum frequency of mycoflora (139.97%) with lowest germination percentage (80%) and detected 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 the seed lot of Indira urd-1 ~~seed lot~~ (123.31%) with germination percentage (90.00%) and themycoflora~~were~~ wasidentified 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 the ~~seed lot~~ TIU-22 seed lot mycoflora, the frequency was (123.30%) with germination percentage (86.66%), and the 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 the TPU-4variety ~~TPU-4~~, the 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 thePratap-1variety ~~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 the 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~~ determined as themaximum from the series ~~seed lot~~ of black gram seeds. Other ~~predominated~~ dominantmycoflora 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 black gram mycoflorausing standard blotter method

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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

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Agar plate method

The sSeeds of 6 varieties of black gram were evaluated for the associated seed-borne mycoflora by agar plate method and the data presented in table Table 2. shows that the maximum highestfrequency of mycoflora were recorded from the seeds of local variety (149.98%) which includes*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 mycoflora of TIU-22 seed lot seriesmycoflora (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 variety Indira urd -1,variety frequency of associated mycofloranamely wasA. *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 was 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 variety 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%).

Using this method,Ddifferent mycoflorawere wasdetected from black gram varieties by this method, in which *Fusarium* sp.(146.64%) was recorded maximum the most,and it the highestwas most frequent in local variety and TPU-4 (33.33%). This was fFollowed 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 were the leastfound least in black gramvarieties.

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

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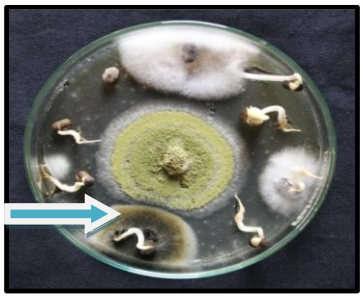
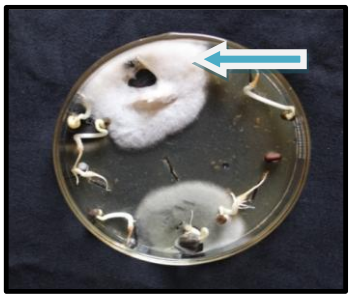
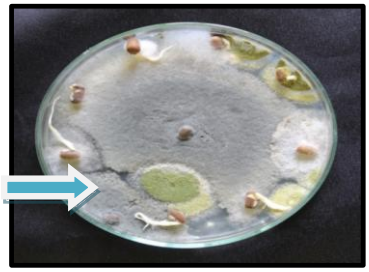
S. No.	Varieties	Germination (%)	Frequency of mycoflora associated (%)										
			A. flavus	*A. niger*	*Fusarium* spp.	*Alternaria* spp.	*Rhizopus* spp.	*Colletotrichum* spp.	*Curvularia*spp.	*Penicillium* spp.	*Cladosporium* spp.	*Trichoderma* spp.	Total frequency (%)
1.	**Indira urd-1**	80.00	26.66	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**



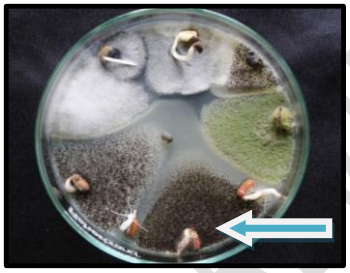
***Alternaria* spp.**



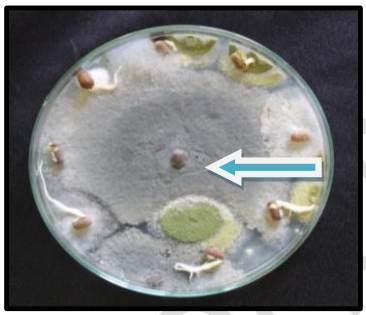
***Fusarium* spp.**



***Curvularia*spp.**

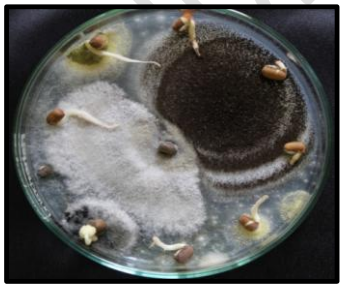


Aspergillus niger



***Macrophomina*spp.**

Aspergillus flavus



***Rhizopus* spp.**

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

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3. Roll paper towel method

A s~~S~~eed lot of 6 varieties of black gram ~~were~~ was~~examined~~ tested for associated seed-borne mycoflora~~in varying~~at different~~frequency~~ frequencies with normal seedlings, abnormal seedlings and ~~ungerminated~~ non-germinated seeds ~~by~~ using rolled paper towel method. It ~~was~~ has been observed that the presence of mycoflora~~may~~ can be the cause of ~~abnormalities~~ abnormality and failure ~~in~~ of seed germination. ~~In~~ Using this method, the mycoflora~~were~~ was detected associated with seed and seedlings of different black gram varieties. ~~Maximum~~ The highest frequency of mycoflora was observed in local variety (99.98%) and detected 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~~ was 76.66, 83.33, 83.33, 86.66 and 90.00, respectively.

In the seeds of TPU-4 variety, mycoflora~~were~~ was 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%). In Pratap-1 variety, the associated mycoflora~~were~~ was*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~~the lowest frequency of mycoflora (86.65%) includes *A. flavus* (36.66%), *A. niger*(23.33%), *Fusarium* spp. and *Rhizopus* spp.(13.33%).

The r~~R~~elative abundance of mycoflora~~were~~ was*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 forte detection of the mycoflora associated with different varieties of black gram

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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) Ungerminatedwith infected seeds

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

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4. Deep freezing method

The sSeed lot of black gram varieties were tested for the associated seed-borne mycoflora using deep freeze method and data are presented in table Table 4. The Ffrequency of associatedmycofloraassociated were was the highestmaximum in the 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 minimalgermination (56.66%) and next werefollowed by 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, ofgermination.

The mMycoflora observed in the seed lot of Indira urd-1were wasA. *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 mycoflorawere wasRhizopus spp. (26.66%), *Fusarium* spp. (23.33%), *A. niger*(13.33%) and *Chaetomium* spp. (6.66%).Mycofloraobserved in TIU-22 variety were wasAspergillus *flavus* (23.33%), *Chaetomium* spp. (20.00%) and *Rhizopus* spp. (16.66%).In variety TPU-4,recorded mycoflorawere wasFusarium spp. (20.00%), *Aspergillus niger*(16.66%), *A. flavus* (10.00%), and *Rhizopus* spp. (10.00%). In variety KU-96, recorded mycoflorawerewasA. *flavus* (23.33%), *Rhizopus* spp.(16.66%) and *Fusarium* spp. (3.33%).

In this method, the 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 wasrecorded. In this method, themycoflora associated with the seeds of black gram varieties were wasobserved maximum the mostin the local variety with a lower germination percentage, while minimum frequency of mycoflorawere wasobserved from in theseeds of the KU-96 variety with a higher germination than the other varieties taken in the study.

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Table.4~~:~~. Deep freeze method ~~to~~ for detection of the mycoflora associated with different varieties of black gram

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S. No.	Varieties	Frequency of mycoflora associated (%)						
		A. flavus	*A. niger*	*Fusarium* spp.	*Alternaria* spp.	*Rhizopus* spp.	*Chaetomimums* pp.	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 lots of black gram varieties were tested for the ~~associated~~ seed-borne associatedmycoflora using the 2,4 D blotter method and data presented in ~~table~~ Table 4. The f~~F~~requency of associatedmycoflora~~associated were~~ was the highest~~maximum~~ in the local variety seed lot (79.99%) and ~~mycoflora~~ detected mycoflora~~were~~ was*A. flavus* and *Rhizopus* spp.(23.33%), *Fusarium* spp. (20.00) and *A. niger*(13.33%), and ~~with minimum~~ minimalgermination (60.00%) recorded. ~~It was~~Ffollowed 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~~ was70.00%,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~~ The lowestfrequency of mycoflora was recorded in KU-96 variety ~~were :~~*Chaetomimum*sp. (16.66%), *A. niger* (13.33%) *Fusarium* spp*.* (6.66%). The h~~H~~ighest germination rate (86.66%) was also recorded in KU-96 variety.

~~In t~~This method~~,~~ recorded 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%). The f~~F~~requency of *Aspergillus flavus* ~~were found maximum~~was highest 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. w~~ere found~~as the~~least~~ lowestin Indira urd-1 variety (6.66%), followed by KU-96 and TIU-22 (16.66%).

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

Table 5.- 2,4 D blotter method to fordetection of 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**	

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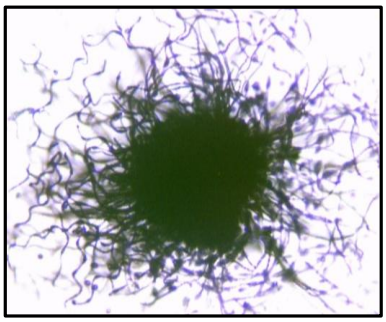
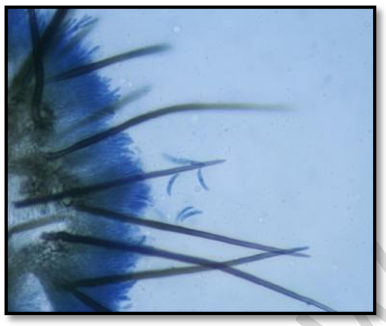
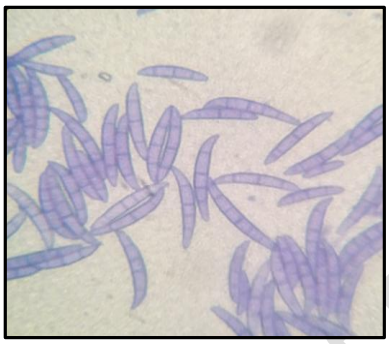


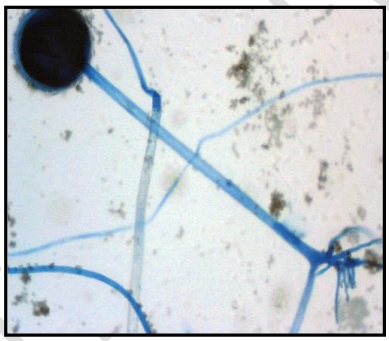
(A) *Chaetomium* spp.



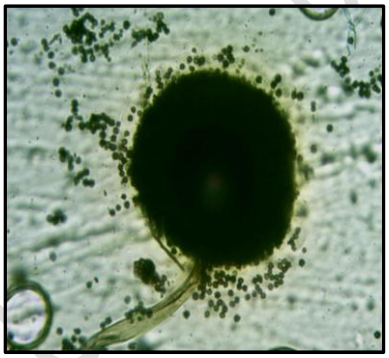
(B)*Colletotrichum* spp.



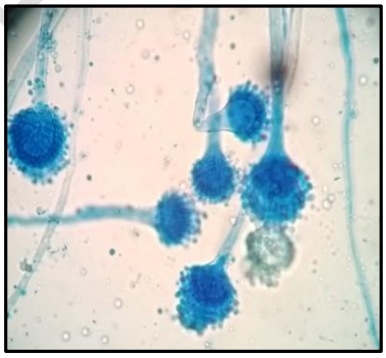
(C) *Fusarium* spp.



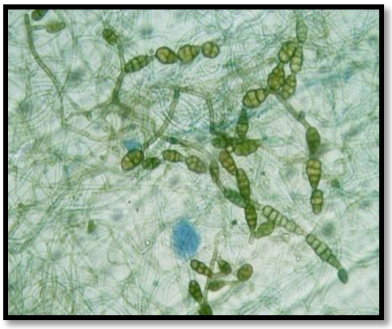
(D) *Rhizopus* spp.



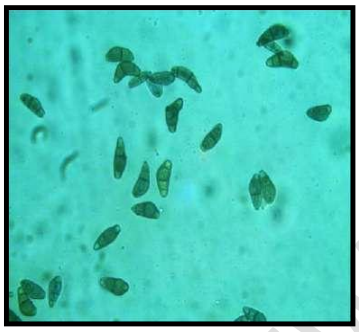
(E) *Aspergillus niger*



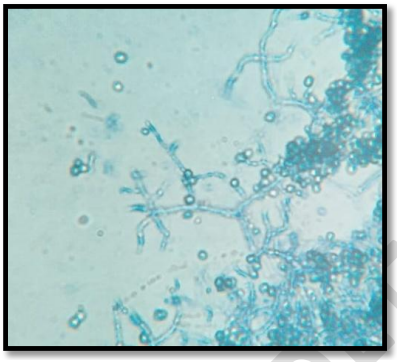
(F) *Aspergillus flavus*



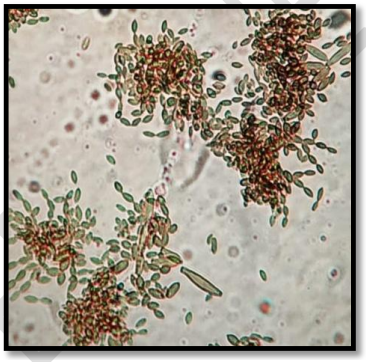
(G) *Alternaria* spp.



(H)*Curvularia*spp.



(I) *Trichoderma*spp.



(J)*Cladosporium*spp.

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

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

The c~~C~~omparative 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.2,4 D blotter method ~~were~~ waspresented in ~~table~~ Table 5 ~~(a)~~. Among them, agar plate method was found to be 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.

In terms of percentage efficiency~~Per cent efficiency wise~~, the agar plate method recorded maximum efficiency (116.36%) ~~over~~ compared to2,4 D blotter method, followed by (90.67%) ~~over~~ compared to thedeep freeze method (34.73%) ~~over~~ in comparedto theroll paper towel method, (7.39%) ~~over~~ compared to thestandard blotter paper method.

SUMMARY

~~The s~~Studies onthe 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~~ improvedof black gram production practices, seed health, and overall crop productivity.

CONCLUSION

When t~~T~~hese seed samples ~~when~~ weresubjected to incubation methods, ~~varying~~ differentfrequency frequenciesand types of mycoflora associated with black gram seeds were recorded. The mycofloraseed recordedassociated ~~mycoflora~~ with the seed~~recorded were~~ was*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 the local variety, which may be due to the presence of seed-associated mycoflora with maximum frequency, ~~whereas~~ while theKU-96 variety has the most extreme germination percentage dueto the minimum frequency of seed-associated mycoflora~~as~~ compared to other varieties of black gram taken in the ~~examination~~study.

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