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Detection of mycoflora of sesamum seed (*Sesamum indicum* L.)

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Abstract
In the present investigation five seed samples *viz.*, AKT-101, GT-10, JLT-408, N-8, PKV NT-11 were tested to detect the association of seed borne mycoflora by standard blotter paper method, pre-treatment blotter method and agar plate method at department of Plant Pathology College of Agriculture, Nagpur. From the present study it was revealed that six fungal species associated with sesamum varieties *viz.*, *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Alternaria alternata, Curvularia lunata and Cladosporium* spp. *Fusarium oxysporum* and *Aspergillus flavus* is most associative fungus with all varites. Standard blotter paper method was found superior method of detection of seed borne mycoflora of sesamum.

Keywords: Sesamum seeds, seed mycoflora, *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Alternaria alternata, Curvularia lunata and Cladosporium Spp*, standard blotter paper method, pre-treatment blotter method, agar plate method

Introduction
Sesamum is called as "Queen of edible oils" in view of the rich oil content (40-50%), seed protein (20%), carbohydrates and minerals such as calcium (1%), and phosphorous (0.7%). It is rich source of vitamin E. Sesamum is the 6th most important oilseed crop in the world. World sesame seed production is estimated at around 4.8 million tonns. India rank first in both area and production of sesamum. In India 17.30 lakh ha area and 7.46 tonnes production of sesamum crop. The average yield of sesamum is 431 kg per ha. In India Uttar Pradesh leads in area and production followed by Rajasthan, Gujrat, Orissa and Karnataka. Oilseeds infected with mycoflora experience a variety of undesirable modifications, rendering them unfit for human consumption and sowing. Further mycoflora association has a negative impact on seed quality and health. *Alternaria* spp., *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp., *Penicillium* spp., *Mommoniella* spp., *Aspergillus* spp., *Mucor* spp., and *Rhizophus* spp. were found in seed samples, with *Alternaria* spp. and *Aspergillus* spp. being the most destructive pathogens of oilseeds *viz.*, Sesamum, Groundnut and Mustard (Ghosh *et al.*, 2018) [2].
Among the cultivated crops of India, sesamum is a unique plant and it have a wide range of uses not only in daily life of people but also in industries. So it became essential to determine seed health of a crop through detect a seed mycoflora.

Methodology
Collection of Seeds
The seeds of sesamum variety PKV-NT-11, AKT-101, N-8, JLT-408, GT-10 were collected from oilseed research unit of Dr. P.D.K.V. Akola.

Detection of Seed Mycoflora
a) Standard blotter paper method
For this 100 seeds of each sample were placed on two layer of moist blotter in surface disinfected transparent plastic petri plate of 90 mm diameter. Each plate containing 25 seeds at equal distance infour replication such a manner that 16 place on outer ring, eight in inner ring and one in centre. The petri plates were incubated at 26$\pm$2 ^{0}C for 7 days. Distilled water was added regularly on blotter paper to keep it moist. After seven days of incubation the seed examined under stereoscopic-binocular microscope for associated fungi.

b) Pre-treatment blotter method

For pretreatment seed treated with 2% available chlorine of NaOCl (Sodium hypochlorite) for 2 minutes and washed sterilized distilled water before plating. Treated seed placed on three layer of moist blotter paper as like blotter paper method.

c) Agar plate method

Arrange the seed on PDA media as like blotter paper method.

Isolation of seed borne mycoflora from sesamum seeds

The fungal colonies on seeds of sesamum varities were picked up with the help of a sterilized inoculating needle and transferred on Potato Dextr ose Agar (PDA) petri plates and slants and incubated at 26±2°C for seven days.

Result

Standard blotter paper method

From the table 1 it was revealed that the maximum association of *Fusarium oxysporum* was recorded in all the varieties (39.25%) followed by *Aspergillus niger* (35.00%) and *Aspergillus flavus* (16.50%) where as least association of *Alternaria alternata*, *Curvularia lunata* and *Cladosporium* spp. recorded 3.25, 2.50 and 1.50 per cent respectively.

The earlier work revealed the difference in type of fungal species associated with seeds of different varieties. The result were correlated with Gooya *et al.,* (2000) [3]. Mashoda – Begum *et al.,* (2003) [5].

Table 1: Detection of seed borne mycoflora by standard blotter paper method

Variety	Per cent association of seed borne Mycoflora						Total
	F.O	A.N	A.F	A.A	C.L	CL	
N-8	10.25	9.75	4.25	1.75	0.75	0.75	27.5
AKT-101	9.50	9.50	4.50	0.75	1.00	0.25	25.25
PKV NT-11	9.25	10.25	3.75	0.75	0.75	0.50	25.25
GT-10	5.50	3.25	1.75	0.00	0.00	0.00	10.50
JLT-408	4.75	2.25	2.25	0.00	0.00	0.00	9.25
Total	39.25	35.00	16.50	3.25	2.50	1.50	98.00
Mean	7.85	7.00	3.30	0.65	0.50	0.30	19.60

F.O - Fusarium oxysporum A.A – Alternaria alternata
A.N - Aspergillus Niger C.L – Curvularia lunata
A.F – Aspergillus flavus CL – Cladosporium spp.

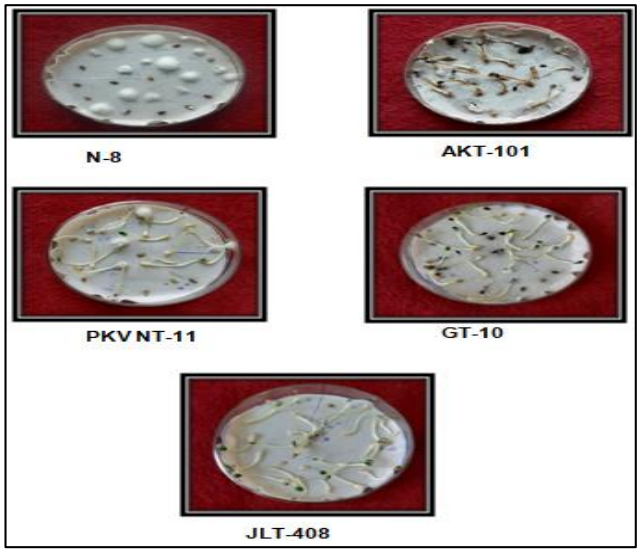

Plate 1: Seed borne mycoflora by standard blotter paper method

Pre-treatment blotter method

Among all the varieties highest association of *Fusarium oxysporum* was recorded, followed by *Aspergillus niger* recorded 4.85 and 3.55 per cent respectively. Other fungal association was found in the range of 0.15 to 0.90 per cent. The result showed that association of seed borne fungi is less than blotter paper and agar plate method. Haider *et al.,* (2020) [4] During the detection experiment, *Alternaria alternata, Fusarium moniliforme, F oxysporum, A. tenuis, S.rolfsi, Cercospora sesami, Curvularia lunata, Macrophomina phaseolina, Aspergillus flavus, A. ochraceus, A. versicolor, A. terreus, A. candidus, Haplosporangium* spp, *Penicillium citratum, Rhizopus nigricans* and *R. stolonifer* were isolated from local variety of sesamum seed. In all seed health test methods, standard blotter methods were more superior for detection of seed borne fungi over the other methods.

Table 2: Detection of seed borne mycoflora by pre-treatment blotter method

Variety	Per cent association of seed borne mycoflora						Total
	F.O	A.N	A. F	A.A	C.L	C.L	
N-8	7.25	5.75	1.25	0.50	0.50	0.25	15.50
AKT-101	6.75	5.25	0.75	0.50	0.25	0.25	13.75
PKV NT-11	7.75	4.25	1.25	0.25	0.25	0.25	14.00
GT-10	1.75	1.25	0.75	0.00	0.00	0.00	3.75
JLT-408	0.75	1.25	0.50	0.00	0.00	0.00	2.50
Total	24.25	17.75	4.50	1.25	1.00	0.75	49.50
Mean	4.85	3.55	0.90	0.25	0.20	0.15	9.90

F.O - Fusarium oxysporum A.A – Alternaria alternata
A.N - Aspergillus Niger C.L – Curvularia lunata
A.F – Aspergillus flavus CL – Cladosporium sp.

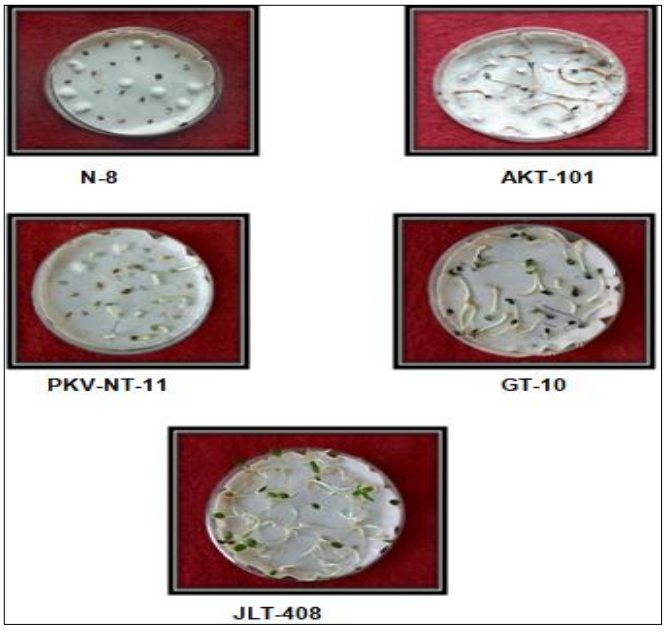

Plate 2: Seed borne mycoflora by pre-treatment blotter method

Agar Plate Method

The data presented in the Table 3 indicated that the per cent association of seed borne mycoflora through agar plate method were higher in N-8 variety (21.50%) ranging from (0.25-8.75%) followed by AKT-101 (20.50%) ranging from (0.25-8.25%), PKV NT-11 (18.75%) ranging from (0.50-7.25%), GT-10 (6.50%) ranging from (0.25-3.75 %) and JLT-408 (5.25%) ranging from (0.00-3.25%). *Fusarium oxysporum* showed higher association in all varieties

(31.25%) followed by *Aspergillus niger* (25.25%), *Aspergillus flavus* (10.75%), *Alternaria alternata* (2.75%), *Curvularia lunata* (1.25%) and *Cladosporium* spp (1.25%).The result were correlated with earlier workers with Pillai *et al.,* (2003) [6].

Table 3: Detection of seed borne mycoflora by agar plate method

Variety	Per cent association of seed borne mycoflora						Total
	F.O	A.N	A.F	A.A	C.L	C.L	
N-8	8.75	8.25	2.75	1.00	0.50	0.25	21.50
AKT-101	8.25	8.25	2.25	1.25	0.25	0.25	20.50
PKV NT-11	7.25	6.75	3.25	0.50	0.50	0.50	18.75
GT-10	3.75	1.25	1.25	0.00	0.00	0.25	6.50
JLT-408	3.25	0.75	1.25	0.00	0.00	0.00	5.25
Total	31.25	25.25	10.75	2.75	1.25	1.25	72.25
Mean	6.25	5.05	2.15	0.55	0.25	0.25	14.45

F.O - Fusarium oxysporum, A.A - Alternaria alternata
A.N - Aspergillus Niger, C.L - Curvularia lunata
A.F - Aspergillus flavus, CL - Cladosporium spp.

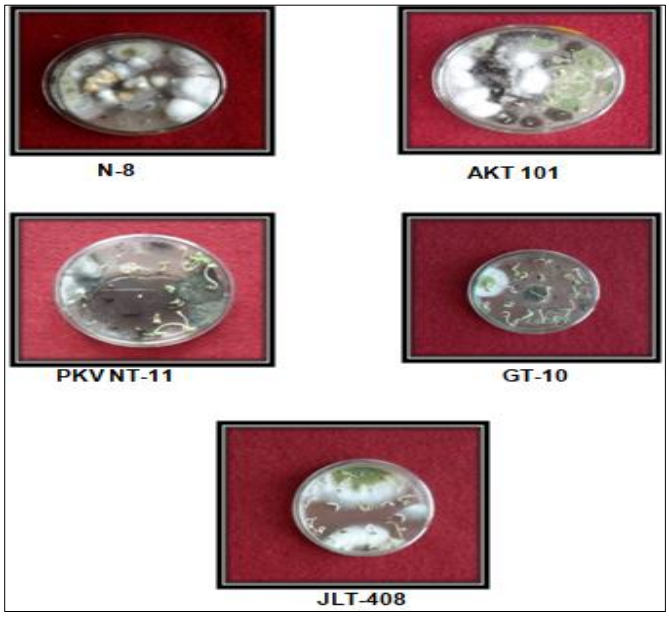

Plate 3: Seed borne mycoflora by agar plate method

Detection of seed borne mycoflora with different method

The observation recorded in all the methods were presented in Table 4 revealed that the association of *Fusarium oxysporum* had the highest mean incidence (31.58%), followed by *Aspergillus Niger* (26.00%) and *Aspergillus flavus* (10.58%). The other remaining fungi *viz.*, *Alternaria alternata*, *Curvularia lunata* and *Cladosporium* spp had shown least mean association recorded 2.41, 1.58 and 1.16 per cent respectively. *Fusarium oxysporum* was found to be the most common fungus, followed by *Aspergillus niger* and *Aspergillus flavus*.

It was observed that the standard blotter paper method had a highest per cent association of seed borne mycoflora in the range (1.50 to 39.25%) followed by agar method (1.25 to 31.25%) and in pre-treatment blotter method had association of seed borne mycoflora represented in the range of (0.75 to 24.25%) Similar result observed by Tobin-West *et al.,* (2018) [8] and Ranasingh *et al.,* (2019) [7].

Table 4: Detection of seed borne mycoflora with different methods

Fungi	Per cent association of seed borne mycoflora in different methods			Total	Mean
	Blotter	Pre-treatment blotter	Agar plate method		
Fusarium oxysporum	39.25	24.25	31.25	94.75	31.58
Aspergillus niger	35.00	17.75	25.25	78.00	26.00
Aspergillus flavus	16.50	4.50	10.75	31.75	10.58
Alternaria alternata	3.25	1.25	2.75	7.25	2.41
Curvularia lunata	2.50	1.00	1.25	4.75	1.58
Cladosporium spp.	1.50	0.75	1.25	3.50	1.16

Conclusion

The five varieties of sesamum were collected from Dr. P.D.K.V Akola. These five varieties were tested for seed mycoflora by standard blotter paper method, pre treatment blotter paper method and agar plate method. The result indicated that total six fungal species belonging to five genera *viz.*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Altrernaria alternata*, *Curvularia lunata*, *Cladosporium sp* associated with three varieties *viz.*, N-8, PKV NT-11 and AKT 101. Three fungal species *viz.*, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus* associated with variety JLT-408 and GT-10. Per cent association of seed mycoflora varied in the different detection methods adopted and the variety tested. Among the fungi, *Fusarium oxysporum* association was highest (31.91%) followed by *Aspergillus Niger* (25.66%), while *Cladosporium* spp. was found to be lowest (1.16%). Standard blotter paper method was found superior in recording more number of fungal colonies than pre-treatment blotter method and agar plate method.

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