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Standardization of detection methods for seed borne fungi in sesame

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Abstract
An investigation was conducted to detect the seed borne mycoflora associated with 28 seed samples of sesame collected from farmers and different research stations in Andhra Pradesh and Telangana. A total of seven fungal species belonging to six genera were found to be associated with the seed of sesame indicating their seed borne nature. Detection of seed mycoflora revealed that the mean incidence of *Alternaria sesami* was highest (31.44%) followed by *Aspergillus flavus* (16.85%) and *Aspergillus niger* (16.03%), while the mean incidence of *Helminthosporium* sp. was found to be the lowest (4.82%). Among the six methods employed for detection of seed mycoflora, standard blotter method was found to be superior and recorded maximum fungal colonies (31.48%) in all the 28 samples, while the minimum number was observed in water agar method (9.50%).

Keywords: Sesame, seed borne fungi, detection methods, *Alternaria sesami*, standard blotter method

Introduction
Sesame (*Sesamum indicum* L.) is an important oil seed crop being cultivated in the tropics as well as temperate zone of the world. It is popularly known as 'Queen of Oilseeds' due to its stabilized keeping quality contributed by high degree of resistance to oxidation and rancidity (Bedigian and Harlan, 1986) [1]. Sesame is renowned for its high oil content (up to 60% oil) with potent antioxidants (Sesamolin and Sesamol). Sesame seeds are chemically composed of 44–57% oil, 18–25% protein and 13–14% carbohydrates (Borchani *et al*., 2010) [2]. Sesame is used in different food items all over the world. It is also consumed as a traditional health food for its specific antihypertensive effect, anticarcinogenic, anti-inflammatory and antioxidative activity (Pathak *et al*., 2017) [3]. Besides food, sesame also finds its uses in application areas such as pharmaceutics, industries and as biofuel. Sesame cake is also used as soil amendment for the control of various diseases (Maiti *et al*., 1988) [4].
Sesame crop is attacked by many phytopathogens, most of them are seed borne, causing accountable quantitative and qualitative losses. Different fungi *viz*., *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Mommoniella* and *Rhizopus* sp. were associated with sesame seed (ISTA, 1999) [5]. Seed is the carrier of diseases and can spread them to unknown areas. The plants emerging from infected seed may not only be diseased but can serve as infection foci for secondary infection. Moreover, farmer-saved sesame seed planted by most farmers are infected with seed borne fungal pathogens. Therefore, testing of seed is very essential to know various fungi associated with it and their influence on seed quality. In order to know the different mycoflora associated with sesame seed collected from different locations from different farmers' and research stations and analysed for the seed borne fungi.

Material and Methods
The present investigation was carried out in the laboratory of Plant Pathology, Regional Agricultural Research Station, Lam and the Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Guntur, Andhra Pradesh during 2017-2018. A total of 28 samples were collected from different sesame growing areas *viz.*, five samples from Agricultural Research Station (ARS), Yellamanchili; four samples from Regional Agricultural Research Station (RARS), Lam, Guntur and 16 farmers' saved samples from villages of Darsi and Marturu mandals of Prakasam district of Andhra Pradesh and three samples from RARS, Jagtial, Telangana. Different detection methods *viz*., standard blotter method, deep freezing blotter method, 2, 4-D blotter method,

Water agar method, agar plate method with potato dextrose agar (PDA) and paper towel method were employed to standardize the detection of seed borne fungi in sesame seed samples as detailed below:

Standard blotter method (SBM): Hundred seed of each sample was tested using standard blotter method in three replications. Twenty five seed were placed in each Petri plate on the blotters at equal distance. The Petri plates were incubated at 25±2°C under alternate cycles of 12 h light and 12 h darkness in BOD incubator. After eight days of incubation the seed was examined under stereoscopic-binocular microscope for associated fungi.

Deep freezing blotter method: Hundred seed of each sample was placed at the rate of 25 seed per plate on moistened blotters in the same way as described under standard blotter method in three replications. The Petri plates were incubated at 25±2°C for 24 h under alternate cycles of 12 h near UV light and darkness in BOD incubator, for next 24 hours the plates were incubated at -20°C in dark and then kept again under alternate light and dark conditions in BOD incubator as explained above for next six days. After eight days of incubation, the seed were examined under stereo-binocular microscope.

2, 4-D Blotter method: Hundred seed of each sample was placed at the rate of 25 seed per Petri plate with moistened blotters dipped in 0.2% solution of sodium salt of 2,4-dichlorophenoxy acetic acid in three replications. The Petri plates were incubated as described under standard blotter method. After eight days of incubation, the fungal growth on seeds was examined by using stereo-binocular microscope.

Water agar method: Hundred seed of each sample was placed at the rate of 25 seed per Petri plate containing 20 ml of 1% water agar in three replications. The Petri plates were incubated as described under standard blotter method. After eight days of incubation, the fungal growth on seeds was examined by using stereo-binocular microscope.

Agar plate method with potato dextrose agar (PDA): Hundred seed of each sample were placed at the rate of 25 seed per Petri plate containing 20 ml of potato dextrose agar in three replications. The Petri plates were incubated as described under standard blotter method. After eight days of incubation the fungal growth on seed was examined under stereoscopic binocular microscope.

Paper towel method (Rolled towel method): Hundred seed of each sample in each replication was placed on two layers of moist germination paper at ten seed per row in ten rows, which were then placed on a polythene paper and rolled carefully to avoid any excess pressure on seed. These towels in four replications were incubated under ambient conditions for six days. After six days of incubation the fungal growth on seed was examined under stereoscopic binocular microscope.

Observations Recorded

Observations on the per cent infection by different fungi in each method were recorded and frequency of the fungus was calculated. The various fungal cultures obtained were identified using key given by Barnett (2003) [6], Booth (1971) [7] and Ellis (1976) [8]. The number of infected seed and fungal colonies developed were recorded in terms of percentage frequency.

Per cent infection of fungal species: The incidence of fungus was recorded as suggested by Aslam *et al.* (2015) [9] by counting the number of seed colonized by fungus in each replication and the per cent colonization of seed was calculated as follows:

$$\text{Per cent Infection} = \frac{\text{Number of infected seed}}{\text{Total number of seed}} \times 100$$

Frequency of the fungus (%): The frequency of the fungus was calculated by the following formula (Neha and Razia, 2013) [10]:

$$\text{Frequency of fungus (\%)} = \frac{\text{Number of seed containing a particular fungus}}{\text{Total number of seed}} \times 100$$

Results and Discussion

The results indicated (Table 1) that a total of seven fungal species belonging to six genera *viz*., *Alternaria sesami*, *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Rhizopus* sp., *Aspergillus flavus* and *Aspergillus niger* were found to be associated with the seed of sesame indicating their seed borne nature. Per cent incidence of seed mycoflora varied across different detection methods adopted and the samples tested. Among these fungi, the mean incidence of *Alternaria sesami* was highest (31.44%) followed by *Aspergillus flavus* (16.85%) and *Aspergillus niger* (16.03%), while the mean incidence of *Helminthosporium* sp. was found to be the lowest (4.82%) over all the detection methods.

Among the different detection methods, agar plate with PDA method (38.40%) was found superior in the recovery of *Alternaria sesami* in sesame seed (Fig. 1) followed by standard blotter method (30.78%). Deep freezing blotter method (11.89%) was found superior in the isolation of *Curvularia* sp. Higher recovery of *Fusarium* sp. was obtained in water agar method (11.27%) followed by deep freezing blotter method (11.15%). *Helminthosporium* sp. was found higher in 2, 4-D blotter method (8.31%), whereas *Rhizopus* sp. was observed to be high in paper towel method (22.90%). Standard blotter method was found superior in the isolation of *Aspergillus flavus* (21.03%) and *A. niger* (21.50%).

The major seed borne pathogenic fungi *viz*., *Alternaria alternata*, *A. sesami*, *A. helianthi*, *Rhizoctonia bataticola*, *Macrophomina phaseolina* and *Fusarium oxysporum* as well as saprophytic fungi *viz*., *Aspergillus niger*, *A. flavus*, *R. stolonifer etc*. were reported in sunflower (Abdullah and Al-Mosavi, 2010[11] and Afzal *et al*., 2010) [12] and in sesame (Radha *et al*., 2015) [13] by applying various detection methods.

Among the six methods employed for the detection of seed mycoflora, standard blotter method was found superior and recorded maximum incidence of mycoflora (31.48%) (Table 2) from all the test samples followed by agar plate method with PDA (17.53%), deep freeze blotter (15.53%), 2,4-D blotter (13.36%), paper towel method (12.60%) and water agar method (9.50%) (Fig. 2). Among the 28 samples, total fungal colonies were found to be highest in farmers' samples (33.56% to 49.68%) followed by samples collected from RARS, Lam, Guntur (28.16 to 38.59%), while the lowest incidence of fungal colonies (13.15 to 20.40%) were observed

in foundation seed samples collected from ARS, Yellamanchili in all the six detection methods.

Out of the seven fungal species detected in the present investigation, *Alternaria sesami* was found to be associated with all the test samples, while *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Aspergillus flavus* and *Aspergillus niger* were found only in some of the test samples. These results clearly indicated that *Alternaria sesami* was more predominant in the sesame growing areas, whereas *Curvularia* sp., *Fusarium* sp. and *Helminthosporium* sp. were the common pathogenic fungi observed and species of *Aspergillus i.e*., *A. niger* and *A. flavus*, and *Rhizopus* sp. were the common saprophytic fungi found associated as seed mycoflora.

Detection of seed mycoflora was higher in blotter method (31.48%) as compared to agar plate method (17.53%). This variation might be due to the reason that some of the weak and slow growing fungi could not able to grow on agar medium in comparison to fast growing saprophytic fungi. Similarly, visual sporulation of the fungus on the seed was generally heavier in the SBM method than in water agar method, PDA and 2,4-D methods (Nagaraja and Krishnappa, 2011) [14]. Pre-surface sterilization of seed and use of substratum in the method employed may be another reason (De Tempe, 1961) [15]. Agar plate method was found to be superior in isolating more number of fungal colonies over blotter method in sesame by Mahatma *et al*. (2001) [16]. Hence verification by both the methods will give complete spectrum of the mycoflora.

The present results are in conformity with the findings of Radha and Chattannavar (2017) [17] who found that standard blotter method was statistically superior for the detection of *Alternaria* sp. The efficacy of this method was proven to be superior by various workers in sesame (Nagaraja *et al*., 2009) [18] and safflower (Nagaraja and Krishnappa, 2011[14], Pushpavathi *et al*., 2012) [19].

Table 1: Detection of seed borne fungi in sesame seed samples by different methods

S. No	Fungal flora	Incidence of mycoflora (%)							
		Standard blotter method	Deep freezing blotter method	2,4-D blotter method	Water agar method	Agar plate with PDA method	Rolled paper towel method	Total fungal colonies (%)	Mean fungal colonies (%)
1.	*Alternaria sesami*	30.78	27.15	34.61	30.39	38.40	27.31	188.64	31.44
2.	*Curvularia* sp.	4.46	11.89	8.31	8.85	7.16	4.18	44.85	7.48
3.	*Fusarium* sp.	3.64	11.15	9.98	11.27	7.80	6.60	50.44	8.41
4.	*Helminthosporium* sp.	2.93	5.51	8.31	7.01	3.16	1.98	28.90	4.82
5.	*Rhizopus* sp.	15.63	12.38	8.02	14.78	16.4	22.90	90.11	15.02
6.	*Aspergillus flavus*	21.03	15.52	15.53	14.40	14.13	20.48	101.09	16.85
7.	*Aspergillus niger*	21.50	16.47	15.23	13.63	12.86	16.51	96.20	16.03

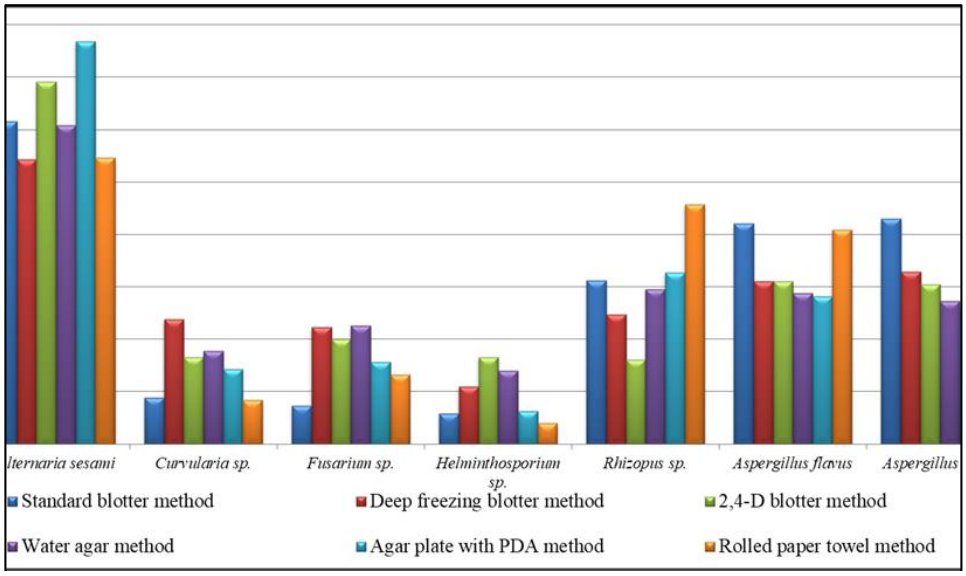

Fig 1: Detection of seed borne fungi in sesame seed samples by different methods

Table 2: Efficacy of different methods for detection of seed-borne fungal infections of sesame

Place	Sample	Sample no.	Incidence of mycoflora (%)							Remarks
			Standard blotter method	Deep freezing blotter method	2,4-D blotter method	Water agar method	Agar plate with PDA method	Rolled paper towel method	Total fungal colonies (%)	
ARS, Yellamanchili	YLM-11	1	2.67	3.33	1.66	0.99	3.99	3.50	16.14	Foundation seed
	YLM-17	2	6.66	3.66	1.33	2.66	3.34	2.75	20.40	
	YLM-66	3	4.00	2.33	0.66	1.33	2.33	2.50	13.15	
	Gouri	4	6.00	3.32	1.33	1.67	3.00	1.00	16.32	
	Madhavi	5	4.34	0.66	3.33	2.00	3.66	2.00	15.99	
RARS, Lam, Guntur	YLM-11	6	8.01	5.67	7.00	2.33	3.65	4.75	31.41	Seed harvested from experimental
	YLM-17	7	8.68	4.99	5.67	2.32	6.99	5.50	34.15	

	YLM-66	8	9.32	6.67	8.01	1.67	9.67	3.25	38.59	plot
	Gouri	9	7.00	4.67	5.00	3.33	4.66	3.50	28.16	
Maruturu, Prakasam district	YLM-17	10	14.00	6.99	4.66	3.33	5.00	4.25	38.23	Farmers' saved seed
		11	12.64	5.67	6.00	2.33	5.33	3.50	35.47	
		12	14.67	5.33	3.33	3.33	5.00	5.75	37.41	
		13	12.33	6.34	6.33	4.66	14.02	6.00	49.68	
		14	10.33	7.66	4.67	5.99	7.34	3.75	39.74	
		15	10.67	4.66	6.67	3.67	8.65	4.50	38.82	
Darsi, Prakasam district	Local variety	16	16.01	5.33	6.70	2.33	5.33	3.50	39.26	
		17	11.32	4.34	8.01	3.99	6.01	3.25	36.92	
		18	10.33	7.33	4.99	4.99	11.01	6.25	44.90	
		19	11.00	5.00	5.00	3.33	4.66	7.75	36.74	
		20	13.67	5.00	5.33	5.00	7.00	3.25	39.25	
		21	19.01	3.99	5.33	2.66	8.33	4.75	44.07	
		22	13.67	5.68	3.33	2.66	3.66	6.25	35.25	
		23	13.00	6.34	5.33	4.00	4.66	4.75	38.08	
		24	10.32	7.33	2.67	4.99	5.00	3.25	33.56	
		25	12.34	6.67	2.66	4.34	8.67	4.25	38.93	
RARS, Jagitial	Hima	26	8.66	2.66	2.34	0.66	2.99	3.25	20.56	Foundation seed
	Swetha	27	6.66	3.99	2.33	2.67	1.00	3.50	20.15	
	Rajeswari	28	6.33	4.33	0.66	2.32	3.01	3.00	19.65	Breeder seed
Total			283.64	139.94	120.33	85.55	157.96	113.50	900.92	
Percentage			31.48	15.53	13.36	9.50	17.53	12.60		

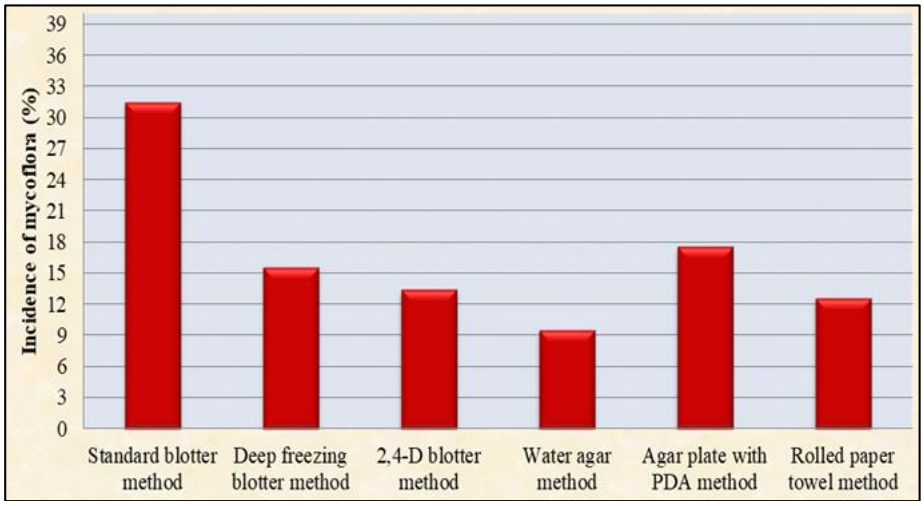

Fig 2: Efficacy of different methods for detection of seed-borne fungal infections of sesame

Conclusion

Hence, it can be concluded that standard blotter method can be recommended for routine diagnosis of seed borne fungal infection in sesame as the method is simple and sensitive.

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