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Detection and identification of the seed mycoflora associated with safflower seed samples

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Abstract

The present investigation was undertaken with the main objective to determine studies on seed borne fungi of safflower were conducted at Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad. A total of 41 safflower seed samples were collected from ARS, Tandur. Blotter method and agar plate methods were used for detection of seed mycoflora of safflower seeds. Across the two methods adopted, a total of seven fungal genera including *Alternaria* sps., *Aspergillus nigar*, *Aspergillus flavus*, *Chaetomium* sps., *Rhizopus* sps, *Curvularia* sps, *Macrophomina phaseolina* and *Fusarium* sps were detected. The fungi detected were identified based on their cultural and morphological characteristics. The fungal species namely *A. flavus* and *A. niger* was found associated with all the tested varieties/germplasms while species of *Alternaria* sps., *Chaetomium*, *Rhizopus* sps., *Curvularia* sps., *Macrophomina phaseolina* and *Fusarium* sps were not detected in some of the varieties/germplasms. Among the eight fungal species detected the occurrence of was high 42.52 per cent followed by *Aspergillus flavus*. The cultivar, PBNS-12 showed higher per cent incidence of seed mycoflora. Out of 41 genotypes tested the total percentage incidence of mycoflora was highest in variety PBNS-12 (90-92.5 per cent) while it was lowest in variety TSF-86 and SSF -1305 (25 -27.5 Per cent) respectively. Incidence of seed mycoflora varied across the methods adopted and cultivars tested. The highest per cent incidence of 42.52% was observed with the fungus on *A. niger* in agar plate method. Out of the two methods tested blotter method was found superior over agar plate method in which higher number of fungi were recorded.

Keywords: Safflower, seed borne, germplasm, *A. niger*

Introduction

Safflower, *Carthamus tinctorius L.*, is an annual, broad leaf crop which belongs to the family of Compositae. Safflower occupies prominent place in the agricultural wealth and economy of India. It is a rich source of proteins and edible oil and so many farmers plant it. Safflower is known to be infected by 57 pathogens including 40 fungi, two bacteria, 14 viruses and one mycoplasma (Patil *et al.* 1993) [5]. Seed borne fungi are carried over by infested seeds. They cause deterioration in soil, before seed germination causing seedling mortality and infection of foliage at adult stages. Fungi including *Alternaria* sps, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium*, *Macrophomina*, *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp., were found associated with safflower, *Aspergillus niger* was the most destructive pathogen of safflower. Disease free quality seed production in safflower was utmost important to sustain the productivity and maintain the quality of the crop. The infected seeds failed to germinate or seedlings and plants developed in the field from infected seeds may escape the early infection but often may be infected at the later stages of the crop growth. Besides, pathogens can spread over a longer distance and uninfected field may be infected by the seeds in which different pathogens are present. Seed health testing methods like blotter paper method and agar plate method have been employed for detection of internal and external seed borne mycoflora of safflower. The frequency in occurrence of such potentially pathogenic fungi on safflower cultivars poses a potential threat in crop production programme. However, information on seed borne fungi associated with safflower seeds and its detection by different methods is meager. Keeping this in view, the present investigation was taken up

Material and Methods

The seed samples of 41 safflower cultivars were collected from major growing areas of Andhra Pradesh. The seeds were collected in polythene bags and stored at room temperature of

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25±2 °C. The collected seed samples were analyzed for the presence of seed mycoflora by employing standard blotter method and agar plate method (ISTA, 1976). In all the methods 400 seeds taken randomly from each sample were subjected to analysis without sterilization. For conducting incubation tests sterile glass Petri plates of 9 cm diameter were used. In blotter method, the seeds were placed on three layers of moistened blotter papers in Petri plates. In agar plate method, the seeds were placed over the surface of solidified potato dextrose agar medium in Petri plates. In both the cases, the seeds were plated in Petri plates at the rate of 10 seeds/plate at equidistance and incubated in an incubator set to 25±2 °C temperature for seven days. The incubated seeds were observed on eighth day by using steriobinocular and compound microscope. The mycoflora associated with seed were identified using key given by Barnett (2003)^[2], Booth (1972) and Subramanian (1971)^[9].

Results and Discussion

The seed samples of safflower cultivars viz., Nira, PBNS-12, TSF-1, GMU-7578, GMU-1095, GMU-4610, GMU-7583, GMU-1777, GMU-977, GMU-1070, GMU-2424, GMU-7585, GMU-777, GMU-961, GMU-4546, GMU-1693, GMU-7633, GMU-7608, GMU-7634, GMU-1799, GMU-1193, GMU-253, GMU-1802, GMU-7618, GMU-184, GMU1830, GMU-6098, GMU-1217, GMU-6886, GMU-7574, TSF-84, TSF-64, TSF-28, TSF-87, TSF-85, TSF-86, TSF-71, SSF-1350, SSF-1305, Manjira and A1 collected from ARS, Tandur were analyzed following standard blotter paper and agar plate methods to detect the mycoflora associated with the seeds of safflower.

Standard Blotter Paper Method

Total 41 genotypes of safflower seeds were collected from ARS, Tandur and the mycoflora associated with seeds were detected following standard blotter paper method (Table 1 and plate 1-2.A). The results indicated that eight fungal species viz., *Alternaria* sps, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium* sp., *Rhizopus* sp., *Curvularia* sp., *Macrophomina phaseolina* and *Fusarium* sp., were found associated with seeds of safflower cultivars and the total per cent incidence ranged from 25-92.5. Out of these eight fungal species the occurrence of *A. niger* was found predominant (40.28 per cent). While *M. phaseolina* was least (2.39 per cent). Out of 41 genotypes tested the total percentage incidence of mycoflora was highest in variety PBNS-12 (92.5 per cent) while it was lowest in variety TSF-86 (25 per cent). The per cent incidence of other fungi ranged from 2.39 – 27.62 per cent with the low and high incidence of *M. phaseolina* and *A. flavus*, respectively. The fungal species namely *A. flavus* and *A. niger* were found associated with all the tested genotypes while species of *Fusarium* sp., *Chaetomium* sp., *Curvularia* sp., *Rhizopus* sp and *Macrophomina phaseolina* were not detected in some of the genotypes. Among the eight fungal species detected the occurrence of *A. niger* was high 40.28 per cent followed by *A. flavus* (27.62 per cent).

In safflower seeds, maximum (80-90) per cent incidence of seed mycoflora was observed with safflower cultivars Nira, PBNS-12, GMU-7583, GMU-7634, GMU-6098 and A1. Among seed samples of different genotypes tested such as Nira showed maximum infection of *Fusarium* sp., (15 per cent) respectively. Maximum per cent incidence of *A. flavus* was observed in seeds of safflower cultivars GMU-2424 and

GMU-7634 (25 per cent). GMU-1777 showed maximum per cent incidence of *A. niger* (35 per cent). Maximum per cent incidence of *Chaetomium* sp., was observed in seeds of safflower cultivars GMU-7583, GMU-7608, GMU-7574 and GMU-6098. Maximum per cent incidence of *Curvularia* sp., (10 per cent) was observed in seeds of safflower cultivars GMU-4546, GMU-6098 and TSF-64. Maximum per cent incidence of *Alternaria* sps., (22.5 per cent) was observed in seeds of safflower cultivars A1. Maximum per cent incidence of *Rhizopus* sp., (10 per cent) was observed in seeds of safflower cultivars GMU-7583, GMU-2424 and GMU-6098. Maximum per cent incidence of *M. phaseolina* was observed in seeds of safflower cultivars PBNS-12, GMU-4610, GMU-977 and A1 (7.5 per cent). The results also indicated that, the percent incidence of *Fusarium* sp., (0-15 per cent), *Rhizopus* sp., (0-10 per cent), *Chaetomium* sp., (0-7.5 per cent), *Curvularia* sp., (10 per cent) and *M. phaseolina* (0-7.5) and were found less. Occurrence of the fungal colonies of *Fusarium* sp., *A. flavus*, *A. niger*, *Chaetomium* sp., *Alternaria* sps., *Rhizopus* sp., *Curvularia* sp and *M. phaseolina* and on the safflower seeds indicates their seed borne nature. Similar findings were reported earlier by Singh *et al.* (1987), Awadhya (1991), Rajeswari *et al.* (2012)^[8] and Pushpavathi *et al.* (2012)^[6] in safflower seeds. The highest per cent incidence of 46 was observed with the fungus *A. carthami* on Nari NH1 seeds in blotter method. It was also found to be the most frequent and the predominant fungus followed by *A. niger* (3 - 29 per cent) and *Curvularia* sp (0-35 per cent) which were detected by standard blotter method.

Agar Plate Method

Total 41 genotypes of safflower seeds were collected from ARS, Tandur and the mycoflora associated with seeds were detected following agar plate method (Table.2 and plate 1-2.B). The results indicated that eight fungal species viz., *Fusarium* sp., *A. flavus*, *A. niger*, *Chaetomium* sp., *Alternaria* sps., *Rhizopus* sp., *Curvularia* sp and *M. phaseolina* were found associated with seeds of safflower cultivars and the total per cent incidence ranged from 27.5-90 per cent. Out of these eight fungal species the occurrence of *A. niger* was found predominant (42.52 per cent). While *M. phaseolina* was least (2.02 per cent). Out of 41 genotypes tested the total percentage incidence of mycoflora was highest in variety PBNS-12 (90 per cent) while it was lowest in variety SSF - 1305 (27.5 per cent). The per cent incidence of other fungi ranged from 2.48 – 29.86 per cent with the high and low incidence of *M. phaseolina* and *A. flavus*, respectively. The fungal species namely *A. flavus* and *A. niger* were found associated with all the tested genotypes while species of *Fusarium* sp., *Chaetomium* sp., *Curvularia* sp., *Rhizopus* and *M. phaseolina* were not detected in some of the genotypes. Among the eight fungal species detected the occurrence of *A. niger* was high 42.52 per cent followed by *A. flavus* (29.86 per cent).

The number of colonies of seed mycoflora recorded was less in agar plate method than in standard blotter paper method. In safflower seeds, maximum (80-90 per cent) incidence of seed mycoflora was observed with safflower cultivars Nira, PBNS-12 and A1. Among seed samples of different genotypes tested such as PBNS-12 showed maximum infection of *Fusarium* sp., (10 per cent, respectively). Maximum per cent incidence of *A. flavus* was observed in seeds of safflower cultivars GMU-7585 (27.5 per cent). GMU-1193 showed maximum per cent incidence of *A. niger* (32.5 per cent). Maximum per

cent incidence of *Chaetomium* sp., was observed in seeds of safflower cultivars TSF-28 and A1 (7.5 per cent). Maximum per cent incidence of *Curvularia* sp., (20 per cent) was observed in seeds of safflower cultivars GMU-7618. Maximum per cent incidence of *Alternaria* sps., (22.5 per cent) was observed in seeds of safflower cultivars Manjira. Maximum per cent incidence of *Rhizopus* sp., (7.5 per cent) was observed in seeds of safflower cultivars GMU-4610 and GMU-1777. Maximum per cent incidence of *Macrophomina phaseolina* was observed in seeds of safflower cultivars PBNS-12, GMU-1830 and GMU-1217 (7.5 per cent). The results also indicated that, the percent incidence of *Fusarium* sp., (0-10 per cent), *Rhizopus* sp., (0-7.5 per cent), *Chaetomium* sp., (0-7.5 per cent), *Curvularia* sp (20 per cent) and *Macrophomina phaseolina* (0-7.5) and were found less (Table 1) Occurrence of the fungal colonies of *Fusarium* sp., *A. flavus*, *A. niger*, *Chaetomium* sp., *Alternaria* sps., *Rhizopus* sp., *Curvularia* sp., *M. phaseolina* and on the safflower seeds indicated their seed borne nature.

Several criteria are involved in selecting a suitable method for the detection of different fungi on seeds. The primary criterion being its capacity to reveal the fungi in maximum percentage, another is versatility and capacity to reveal a wide range of pathogens. Keeping these two points in view, a study to compare the efficacy of two routine seed health testing

methods, in detecting the seed-borne fungal pathogens of safflower was undertaken. Out of two methods employed to ascertain their efficacy in detecting the seed borne fungal infections of safflower, standard blotter paper method was found to be superior for detecting the seed-borne infection. The similar efficacy of standard blotter paper method in detecting seed mycoflora of safflower was reported earlier (Rajeswari *et al.*, (2012) [8] and Pushpavathi *et al.* (2012) [6].

Similar findings were reported by Gayathri *et al.*, 2014 studied seed mycoflora associated with safflower. A total of 19 safflower seed samples were collected from major growing areas of safflower. Blotter method and agar plate methods were used for detection of seed mycoflora. the two methods adopted, a total of seven fungal genera including *Alternaria*, *Aspergillus*, *Chaetomium*, *Rhizopus*, *Curvularia* and *Fusarium* were detected. The highest per cent incidence of 46% was observed with the fungus *Alternaria carthami* on Nari, NH1 in blotter method.

The results were reported by other workers (Raghuwanshi and Deokar, 2002) [7] who reported that mycoflora associated with seeds of safflower seeds reduced germination and seedling vigour. Most of the fungal species detected in this work have been reported earlier as pathogens of safflower and affected seed germinability and vigour of the safflower seedlings. (Awadhiya, 1992) [1].

Table 1: Incidence of seed mycoflora in different safflower cultivars (Standard blotter paper method)

S. No	Genotypes	<i>Fusarium</i> sp (%)	<i>Aspergillus Flavus</i> (%)	<i>Aspergillus Niger</i> (%)	<i>Chaetomium</i> sp (%)	<i>Curvularia</i> Sp (%)	<i>Alternaria</i> Sp (%)	<i>Rhizopus</i> Sp (%)	<i>Macrophomina phaseolina</i> (%)	Total Mycoflora (%)
1	NIRA	10	17.5	27.5	2.5	5	15	2.5	5.0	85.0
2	PBNS-12	15	15.0	22.5	5.0	2.5	17.5	7.5	7.5	92.5
3	TSF-1	0.0	15.0	30.0	5.0	0.0	5.0	0.0	0.0	55.0
4	GMU-7578	2.5	22.5	25	0.0	0.0	0.0	0.0	0.0	50.0
5	GMU-1095	7.5	25	20	0.0	0.0	7.5	7.5	0.0	67.5
6	GMU-4610	2.5	12.5	22.5	5.0	0.0	0.0	0.0	7.5	50.0
7	GMU-7583	0.0	17.5	30.0	7.5	7.5	7.5	10.0	0.0	80.0
8	GMU-1777	5.0	15.0	35.0	0.0	0.0	0.0	0.0	0.0	55.0
9	GMU-977	0.0	17.5	30	0.0	0.0	15.0	0.0	7.5	70.0
10	GMU-1070	0.0	10.0	20.0	5.0	7.5	10.0	5.0	0.0	57.5
11	GMU-2424	0.0	20.0	17.5	0.0	0.0	5.0	10.0	2.5	55.0
12	GMU-7585	10.0	22.5	17.5	0.0	7.5	0.0	0.0	2.5	60.0
13	GMU-777	0.0	20.0	25.0	0.0	0.0	20.0	0.0	0.0	65.0
14	GMU-961	0.0	17.5	32.5	5.0	0.0	0.0	7.5	0.0	62.5
15	GMU-4546	7.5	7.5	25.0	2.5	10.0	5.0	0.0	5.0	62.5
16	GMU-1693	0.0	22.5	25.0	5.0	5.0	5.0	7.5	0.0	70.0
17	GMU-7633	10	17.5	27.5	0.0	0.0	15.0	0.0	2.5	72.5
18	GMU-7608	0.0	12.5	20.0	7.5	0.0	5.0	0.0	0.0	45.0
19	GMU-7634	5.0	25.0	27.5	0.0	5.0	15.0	5.0	0.0	82.5
20	GMU-1799	0.0	15.0	32.5	0.0	0.0	0.0	0.0	0.0	47.5
21	GMU-1193	0.0	17.5	25.0	0.0	0.0	15.0	0.0	0.0	57.5
22	GMU-253	5.0	17.5	22.5	0.0	5.0	5.0	0.0	5.0	60.0
23	GMU-1802	2.5	20.0	27.5	0.0	5.0	0.0	0.0	0.0	55.0
24	GMU-7618	0.0	15.0	25.0	5.0	0.0	10.0	2.5	0.0	57.5
25	GMU-1840	0.0	12.5	20.0	5.0	5.0	17.5	0.0	0.0	60.0
26	GMU1830	2.5	15.0	25	0.0	0.0	10.0	0.0	0.0	52.5
27	GMU-6098	0.0	15.0	22.5	7.5	10.0	15.0	10.0	0.0	85.0
28	GMU-1217	0.0	22.5	25.0	0.0	0.0	5.0	0.0	0.0	52.5
29	GMU-6886	0.0	15.0	20.0	0.0	0.0	5.0	0.0	0.0	40.0
30	GMU-7574	0.0	22.5	27.5	7.5	5.0	10.0	0.0	0.0	72.5
31	TSF-84	0.0	20.0	22.5	0.0	5.0	10.0	5.0	0.0	62.5
32	TSF-64	0.0	15.0	25.0	0.0	10.0	5.0	0.0	5.0	60.0
33	TSF-28	0.0	17.5	20.0	5.0	0.0	0.0	5.0	0.0	47.5
34	TSF-87	0.0	20.0	27.5	0.0	0.0	12.5	5.0	0.0	65.0
35	TSF-85	0.0	20.0	25.0	0.0	5.0	5.0	0.0	0.0	55.0
36	TSF-86	0.0	2.5	22.5	0.0	0.0	0.0	0.0	0.0	25.0

37	TSF-71	5.0	12.5	17.5	5.0	5.0	12.5	7.5	2.5	67.5
38	SSF-1350	0.0	15.0	22.5	0.0	0.0	5.0	0.0	0.0	42.5
39	SSF-1305	2.5	20.0	27.5	0.0	0.0	0.0	2.5	0.0	52.5
40	Manjira	7.5	15.0	27.5	0.0	0.0	12.5	5.0	0.0	67.5
41	A1	7.5	15.0	20.0	5.0	5.0	22.5	2.5	7.5	85.0
	Total	112.50	692.50	1010.00	90.00	110.00	325.00	107.50	60.00	2507.5
	Per cent mean	4.49	27.62	40.28	3.59	4.39	12.96	4.29	2.39	

Table 2: Incidence of seed mycoflora in different safflower cultivars (Agar plate method)

S. No	Genotypes	<i>Fusarium</i> Sp (%)	<i>Aspergillus</i> <i>Flavus</i> (%)	<i>Aspergillus</i> <i>Niger</i> (%)	<i>Chaetomium</i> sp (%)	<i>Curvularia</i> Sp (%)	<i>Alternaria</i> Sp (%)	<i>Rhizopus</i> Sp (%)	<i>Macrophomina phaseolina</i> (%)	Total Mycoflora (%)
1	NIRA (SC)	7.5	15.0	22.5	5.0	5.0	15.0	5.0	5.5	80.5
2	PBNS-12	10.0	17.5	27.5	2.5	7.5	15	2.5	7.5	90.0
3	TSF-1(LC)	0.0	17.5	22.5	0.0	0.0	5.0	0.0	0.0	45.0
4	GMU-7578	5.0	25.0	20.0	0.0	2.5	5.0	0.0	2.5	60.0
5	GMU-1095	0.0	22.5	17.5	0.0	12.5	0.0	0.0	0.0	52.5
6	GMU-4610	5.0	12.5	20.0	5.0	0.0	0.0	7.5	0.0	50.0
7	GMU-7583	0.0	15	25	2.5	7.5	7.5	0.0	0.0	57.5
8	GMU-1777	5.0	10.0	30.0	0.0	7.5	5.0	7.5	0.0	65.0
9	GMU-977	0.0	20.0	25.0	0.0	0.0	0.0	0.0	0.0	45.0
10	GMU-1070	10.0	7.5	20.0	5.0	0.0	10.0	0.0	0.0	52.5
11	GMU-2424	0.0	12.5	20.0	0.0	5.0	5.0	0.0	0.0	42.5
12	GMU-7585	0.0	27.5	15.0	0.0	0.0	7.5	0.0	0.0	50.0
13	GMU-777	0.0	12.5	25	0.0	0.0	5.0	0.0	0.0	42.5
14	GMU-961	0.0	20.0	22.5	5.0	0.0	0.0	5.0	0.0	52.5
15	GMU-4546	5.0	7.5	20.	2.5	0.0	5.0	0.0	0.0	40.0
16	GMU-1693	0.0	22.5	25.5	5.0	5.0	5.0	5.0	0.0	68.0
17	GMU-7633	0.0	15.0	20.0	0.0	5.0	10.0	0.0	0.0	50.0
18	GMU-7608	2.5	10.0	20.0	0.0	0.0	5.0	2.5	0.0	40.0
19	GMU-7634	5.0	17.5	27.5	0.0	0.0	2.5	0.0	0.0	52.5
20	GMU-1799	0.0	17.5	27.5	0.0	0.0	5.0	0.0	0.0	50.0
21	GMU-1193	0.0	15.0	32.5	0.0	0.0	0.0	0.0	0.0	47.5
	GMU-253	0.0	20.0	25.0	2.5	0.0	0.0	0.0	2.5	50.0
23	GMU-1802	2.5	20.0	25.0	0.0	0.0	12.5	0.0	0.0	60.0
24	GMU-7618	0.0	12.5	22.5	0.0	20.0	10.0	5.0	0.0	70.0
25	GMU-1840	0.0	10.0	27.5	0.0	10.0	0.0	0.0	0.0	47.5
26	GMU1830	5.0	15	20	0.0	0.0	5.0	0.0	7.5	52.5
27	GMU-6098	0.0	15.0	15.0	0.0	0.0	0.0	0.0	0.0	30.0
28	GMU-1217	0.0	17.5	27.5	0.0	0.0	5.0	2.5	7.5	60.0
29	GMU-6886	0.0	12.5	12.5	5.0	0.0	0.0	0.0	0.0	30.0
30	GMU-7574	0.0	15.0	20.0	0.0	0.0	10.0	0.0	0.0	45.0
31	TSF-84	0.0	20.0	22.5	0.0	5.0	5.0	5.0	5.0	62.5
32	TSF-64	0.0	15.0	27.5	0.0	15.0	0.0	0.0	0.0	57.5
33	TSF-28	0.0	15.0	15.0	7.5	0.0	0.0	0.0	0.0	37.5
34	TSF-87	0.0	20.0	17.5	0.0	5.0	2.5	2.5	0.0	47.5
35	TSF-85	0.0	17.5	22.5	0.0	5.0	7.5	0.0	0.0	52.5
36	TSF-86	0.0	2.5	22.5	0.0	0.0	0.0	5.0	0.0	30.0
37	TSF-71	0.0	17.5	20.0	0.0	2.5	0.0	2.5	0.0	42.5
38	SSF-1350	0.0	15.0	22.5	0.0	12.5	0.0	2.5	0.0	52.5
39	SSF-1305	0.0	10.0	15.0	0.0	0.0	0.0	0.0	0.0	27.5
40	Manjira	7.5	7.5	15.0	0.0	0.0	22.5	0.0	0.0	52.5
41	A1	5.0	15.0	22.5	7.5	5.0	20.0	2.5	5.0	82.5
	Total	80	632.5	903	55	132.5	215	62.5	43.0	2123.5
	Per cent	3.77	29.79	42.52	2.59	6.24	10.12	2.94	2.02	

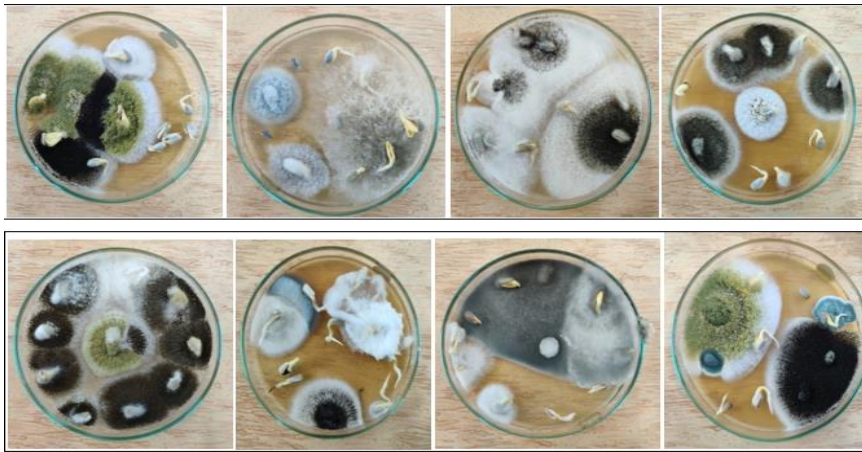


Plate 1a: Detection of Seed mycoflora by agar plate method



Plate 1b: Detection of Seed mycoflora by standard blotter paper method

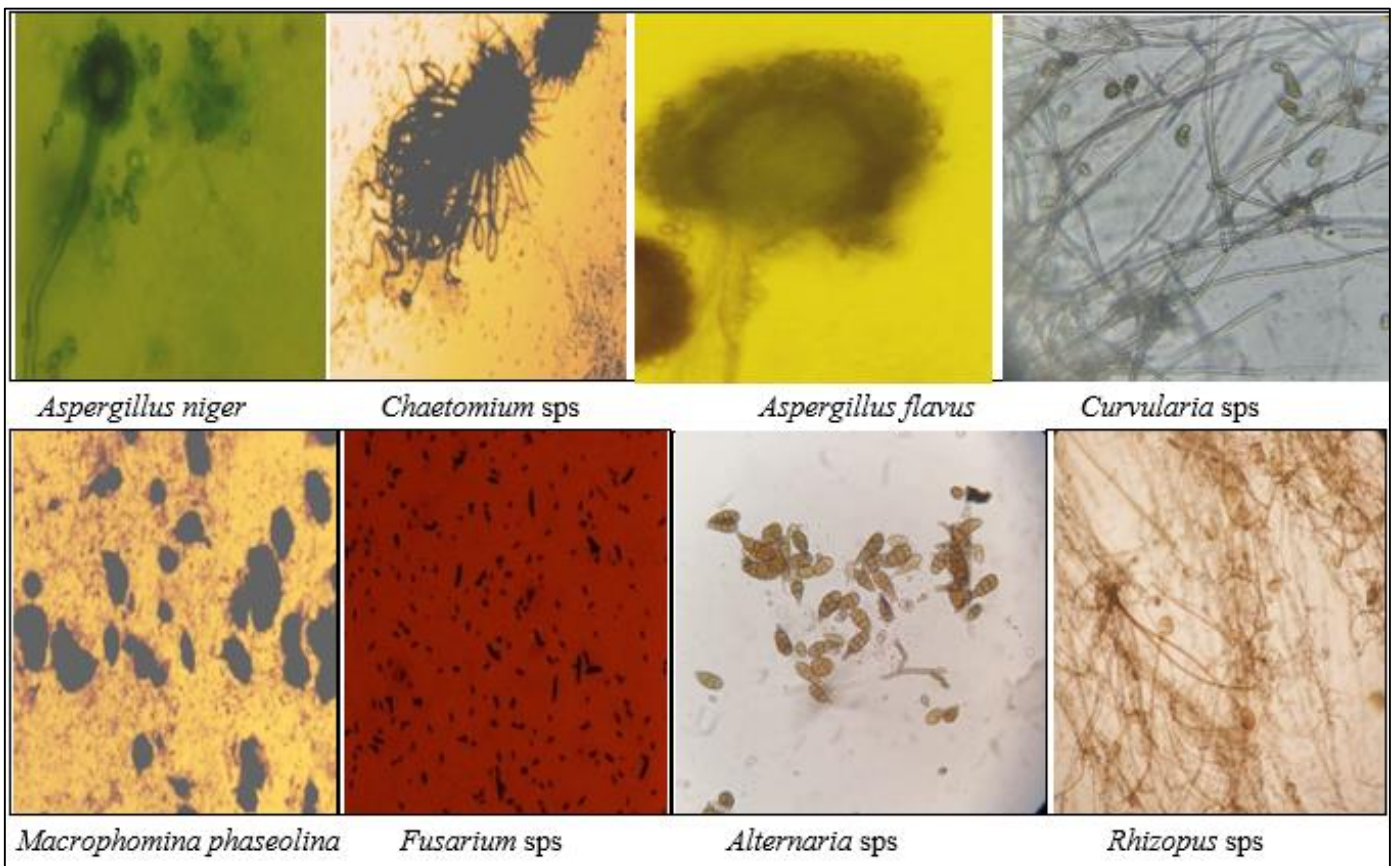


Plate 2A: Identification of the seed mycoflora associated with safflower varieties (standard blotter paper method)

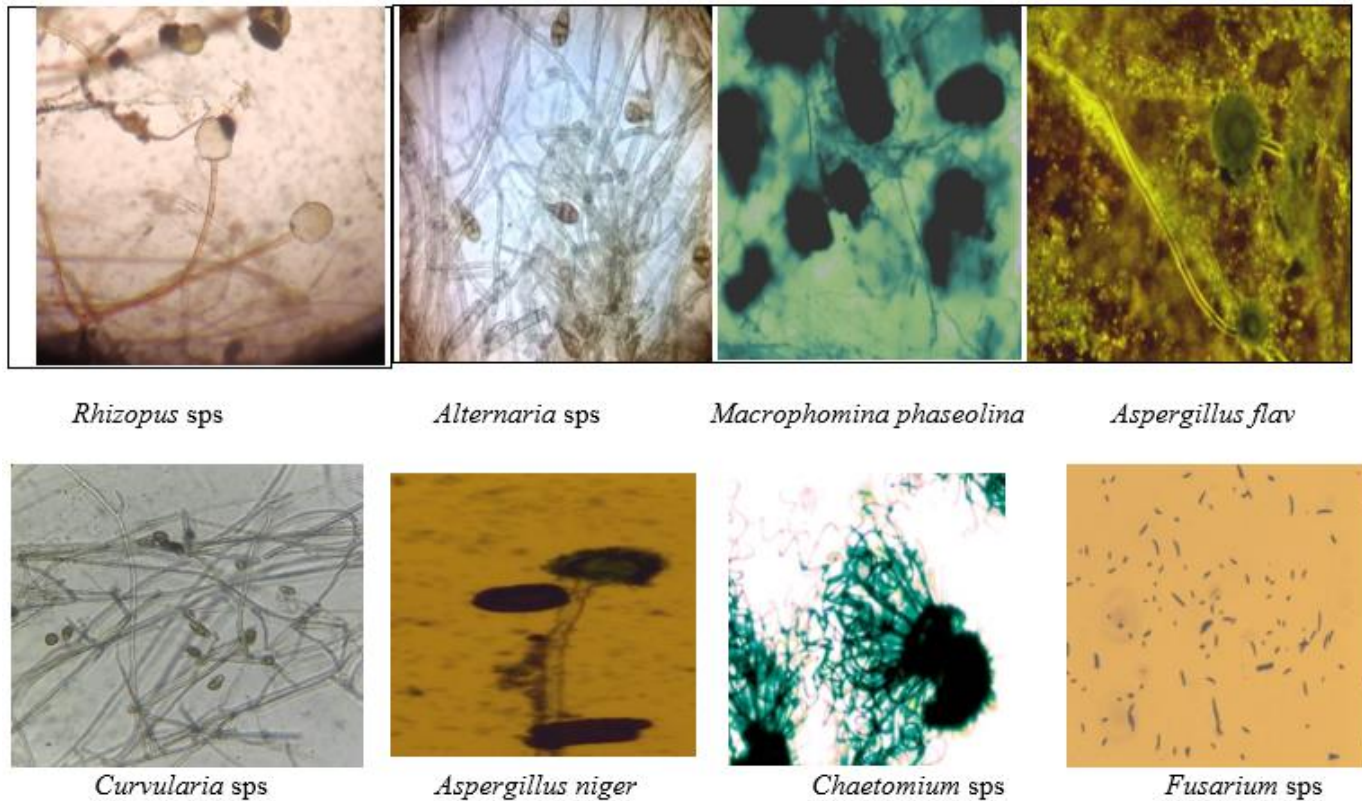


Plate 2B: Identification of the seed mycoflora associated with safflower varieties (agar plate method)

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