



International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 2 Number 1 (January, 2014) pp. 41-45

www.ijcrar.com



Detection and Identification of Seed Mycoflora of Safflower

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KEYWORDS

Safflower;
Mycoflora;
Alternaria carthami;
seed borne.

A B S T R A C T

An investigation was conducted to detect the associated seed mycoflora in safflower and its control. 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 of safflower seeds. Across the two methods adopted, a total of seven fungal genera including *Alternaria*, *Aspergillus*, *Chaetomium*, *Rhizopus*, *Curvularia* and *Fusarium* were detected. The fungi detected were identified based on their cultural and morphological characteristics. The fungal species namely *Alternaria carthami*, *Aspergillus niger* was found associated with all the tested hybrids/varieties/germplasms while species of *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp were not detected in some of the hybrids/varieties/germplasms. Among the seven fungal species detected the occurrence of *Alternaria carthami* was high 47.94 per cent followed by *Aspergillus niger*. The cultivar, Nira showed higher per cent incidence of seed mycoflora. Per cent incidence of seed mycoflora varied across the methods adopted and cultivars tested. The highest per cent incidence of 46% was observed with the fungus *Alternaria carthami* on Nari NH1 in blotter method. Out of the two methods tested blotter method was found superior over agar plate method in which higher number of fungi were recorded.

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the major *rabi* oilseed crops in India, it belonging to the family *Asteraceae* is an important source of oil and proteins. The

crop is cultivated over an area of 287 thousand hectares with the production of 658 kg ha⁻¹ (CMIE, 2009) and production of 179 thousand t. (CMIE, 2010) in India. One

of the major constrain in safflower production is the lack of quality seed at the time of planting. The health of safflower plant is affected by number of fungal diseases. The diseases are Fusarial wilt (*Fusarium oxysporum* f.sp. *carthami*), Alternaria leaf spot (*Alternaria carthami*), Rust (*Puccinia carthami*), Bud blight (*Phytophthora drechslera*), Ramularia leaf spot (*Ramularia carthami*), Cercospora leaf spot (*Cercospora carthami*), Bacterial spot (*Pseudomonas syringe*) and Cucumber mosaic virus. 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 carthami*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium*, *Macrophomina*, *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp. Were found associated with safflower, *Alternaria carthami* was the most destructive pathogen of safflower, as it cause leaf spot and blight.

Materials and Methods

The seed samples of 19 safflower cultivars were collected from major growing areas of Andhra Pradesh. The seeds were collected in polythene bags and stored at room temperature of $25\pm 2^{\circ}\text{C}$.

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 $25\pm 2^{\circ}\text{C}$ temperature for seven days. The incubated seeds was observed on eighth day by using steriobinocular and compound microscope. The mycoflora associated with seed were identified using key given by Barnett (2003), Booth (1972) and Subramanian (1971).

Result and Discussion

The analysis of seed mycoflora of 19 safflower cultivars using standard blotter and agar plate methods showed the association of seven fungal species. The fungi detected were identified based on their cultural and morphological characteristics. The fungal species detected through standard blotter method include *Alternaria carthami*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp (Table 1) while, the agar plate method yielded six fungal species except *Curvularia* sp.(Table 2).

Per cent incidence of seed mycoflora varied across the methods adopted and cultivars tested. The highest per cent incidence of 46% was observed with the fungus *Alternaria carthami* on Nari NH1 in blotter method. Among the mycoflora detected *Alternaria carthami* (2-46 per cent) was recovered from all the cultivars in both the methods. Among the seven fungal species detected the occurrence of *Alternaria carthami* was high 46 per cent followed by *Aspergillus niger* (29 per cent). The cultivar, Nira showed higher per cent incidence of seed mycoflora.

Table.1 Incidence of safflower seed mycoflora in different cultivars and in different locations of Andhra Pradesh*
* standard blotter method

S. No	Name of the district	S. No	Genotypes	<i>Alternaria carthami</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Chaetomium</i> sp	<i>Curvularia</i> sp	<i>Fusarium</i> sp	<i>Rhizopus</i> sp	Total mycoflora
Per cent incidence of seed mycoflora											
1.	RangaReddy (DOR)	1.	A-1	32	11	24	-	-	-	3	70
		2.	JSF414	37	10	22	-	23	-	-	92
		3.	Nari 38	13	-	29	-	35	-	-	77
		4.	Bhima	38	9	21	12	-	-	-	80
		5.	Nari 6	36	-	20	4	-	-	4	64
		6.	SSF 658	8	10	11	-	-	-	5	34
		7.	Nari NH 1	46	12	18	2	-	-	-	78
		8.	Nari NH 15	15	10	21	-	-	-	-	46
2.	Khammam	9.	Manjeera	39	4	4	-	3	-	4	54
3.	Adilabad	10.	Bhima	25	7	10	10	1	-	6	59
4.	Nalgonda	11.	Manjeera	32	3	5	2	5	0	4	51
5.	Mahaboobnagar	12.	Sagara mutyalu	28	4	3	6	3	0	5	49
		Ranga Reddy (Tandur)	13.	Sagara mutyalu	45	-	20	4	22	--	-
	14.		Manjeera	16	5	3	6	19	1	-	50
	15.		Nira	32	15	10	4	20	-	6	95
	16.		TSF-1	30	-	26	-	-	2	-	58
	17.		GMU-5536	38	-	13	-	-	-	-	51
	18.		GMU-5653	36	11	5	-	5	4	-	61
	19.		IVT-1002	22	13	10	4	10	-	3	62
		Total		560	92	255	54	146	7	40	1168
	Per cent		47.94	7.87	21.8	4.62	12.5	0.59	3.42		

Table 2. Incidence of safflower seed mycoflora in different cultivars and in different locations of Andhra Pradesh*

*Agar Plate method

S.No.	Name of the district	S.No.	Hybrid/variety/ germplasm	<i>Alternaria carthami</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Chaetomium sp</i>	<i>Fusarium sp</i>	<i>Rhizopus sp</i>	Total mycoflora
Per cent incidence of seed mycoflora										
1.	RangaReddy (DOR)	1.	A-1	20	-	13	-	-	1	34
		2.	JSF414	26	6	11	-	4	2	49
		3.	Nari 38	2	3	18	-	-	-	23
		4.	Bhima	27	-	19	-	-	3	49
		5.	Nari 6	27	-	10	-	-	1	38
		6.	SSF 658	2	-	3	-	-	2	7
		7.	Nari NH 1	32	5	12	-	-	-	49
		8.	Nari NH 15	5	-	15	-	-	1	21
2.	Khammam	9.	Manjeera	34	1	12	7	7	1	62
3.	Adilabad	10.	Bhima	22	2	15	6	4	3	52
4.	Nalgonda	11.	Manjeera	30	4	16	5	-	3	58
5.	Mahaboobnagar	12.	Sagara mutyalu	32	2	14	-	4	-	52
6.	Ranga Reddy (Tandur)	13.	Sagara mutyalu	21	2	15	4	-	-	42
		RangaReddy (DOR)	14.	Manjeera	21	1	20	7	1	1
	15.		Nira	18	-	6	9	-	-	33
	16.		TSF-1	15	-	4	-	-	2	21
	17.		GMU-5536	14	-	8	-	5	-	27
	18.		GMU-5653	3	6	-	-	-	-	9
	19.		IVT-1002	17	-	5	-	4	2	28
		Total		368	32	216	38	29	23	705
	Per cent		52.2	4.5	30.6	5.4	4.1	3.3		

The fungal species viz., *Alternaria carthami*, *Aspergillus niger* was found associated with all the tested hybrids/varieties/germplasms while species of *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp were not detected in some of the hybrids/varieties/germplasms. The results also indicated that, the percent incidence of *Rhizopus* sp (0-6%), *Chaetomium* sp (0-12%) and *Fusarium* sp (0-7%) was found less (Table 1 and 2).

Appearance of the colonies of *Alternaria carthami*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium* sp, *Rhizopus* sp, *Curvularia* sp and *Fusarium* sp on the safflower seeds indicates their seed borne nature. Similar findings were reported by Padaganur and Anil kumar (1976), Singh *et al.*, (1987), Awadhiya (1991), Rajeswari *et al.*, (2012), Pushpavathi *et al.*, (2012).

The predominance nature of *Alternaria carthami* is in agreement with the findings of Raghuvanshi and Deokar (2002) and Singh *et al.* (1987) who reported the highest percentage of association of *Alternaria carthami* with safflower seed. It was also proved as an externally seed borne fungus causing discoloration of seed in several safflower cultivars (Borkar and Shinde 1989).

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