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Seed-borne mycoflora of safflower (*Carthamus tinctorius* L.) and their impact on seed germination

M. Ismail, M. Irfan Ul-Haque and A.Riaz

Department of Plant Pathology, University of Arid Agriculture, Rawalpindi

Abstract

The incidence and frequency of fungi on safflower seed with their role in seed germination was investigated. Three samples each of five safflower cultivars/lines were collected from National Oil Development Programme (NODP), NARC Islamabad. Eleven different fungal species belonging to seven genera i.e., *Alternaria, Aspergillus, Chaetomium, Curvularia, Fusarium, Helminthosporium* and *Rhizopus* were observed. The incidence and frequency of these fungi varied with cultivar and lines with maximum fungal prevalence in Thori-78 (7.7) and minimum in Dholka Sindh (5.4). Maximum germination was noticed on Dholka Sindh (85%) with least fungal incidence (23%) and minimum seed germination was observed on Thori-78 (60%) with higher fungal incidence (55%).

Key words:- *Carthamus tinctorius,*. Safflower, Seed germination, Seed mycoflora.

Introduction

Safflower (*Carthamus tinctorius* L.) is gaining importance as a source of good quality edible and industrial oil (Chaudhry1985). It has potential oil contents of 30-47% with 90% useful fats. Being a non-traditional oil seed crop, it has a limited acreage of 266 hectares in Pakistan mainly in Balochistan and Sind yielding 119 tones in total. The leading safflower producing countries round the globe include India (62%) and USA (16%) where it is mostly used for the industrial purposes (Beg1993). Knowl (1955) reported that safflower was cultivated in Pakistan as a fodder crop with wheat. Total oil consumption in Pakistan during 2001-2002 was 20 million tones with local oil production estimated at 0.582 million tones to meet the 29% of domestic consumption while 71% through imports. Inspite of its importance as a source of good quality oil, safflower has a nominal share of 0.02 % in oil production in Pakistan (Anonymous 2002). Safflower can play a vital role in reducing this gigantic burden of oil import through renewed interest. Among many factors contributing towards this low yield of Safflower in Pakistan, seed-borne mycoflora is a major factor. Khannum (1993) reported the occurrence of seed mycoflora on safflower and observed that these fungal pathogens can reduce the yield of safflower. Borkar and Shinde (1988) examined seed samples of 32 safflower cultivars for the occurrence of *Alternaria carthami* on seed using blotter paper method and found out 70-100% incidence of *A. carthami* while the seedlings from these samples showed 5% pre-emergence mortality and 10% seedling blight. Both of which were related to the number of seed-borne conidia. Seed transmission of safflower rust has been reported by Singh and Pandaya (1998). The present study reports the relative occurrence of seed- borne mycoflora of five different Safflower cultivars/lines.

Materials and Methods

Collection of seed samples

Three samples of each five Safflower cultivars/lines were obtained from National Oil Development Programme, (NODP), NARC Islamabad Pakistan. All safflower cultivars samples are listed in Table 1. A composite sample of one kilogram (Kg) drawn from two Kg random samples was transported in polyethylene bags for investigation in Plant Pathology lab., University of Arid Agriculture Rawalpindi.

Testing procedure

Seeds of all cultivars/lines were analyzed for their association of seed mycoflora by Standard Blotter Paper Method (ISTA, 1985). A working sample of 400 seeds was taken at random from each cultivars/lines and plated on 40, 9cm diameter, sterilized petri dishes. Ten seeds were placed on three layered blotter paper soaked with sterilized water. The seeds were disinfested with Chlorox (1:4) for about 2-3 minutes and rinsed with sterilized water before plating. The plated seeds were incubated at 25$\pm$2^{o}C for a day. On second day, seeds were subjected to freezing for 24 hrs to avoid germination. After freezing seeds were allowed to

incubate at 25±2ºC for 7 days. The seeds were examined under stereomicroscope for associated mycoflora. These fungi with different growth characteristics were cultured on Potato Dextrose Agar (PDA) medium and examined under compound microscope for identification. The plated seeds were classified as infected and non-infected to determine the fungal incidence. The frequency was assessed from the mycoflora associated with infected seeds.

Effect on germination

Germination test was carried out by using standard Rolled Paper Towel Method (ISTA, 1985). Hundred seeds of each cultivar were randomly selected and allowed to germinate between two blotter paper layers at 25±2ºC for 7 days.

At the end of incubation the number of ungerminated (including rotten) and germinated seeds were counted. The emerged seedlings were graded as normal and abnormal as defined by Anwar *et al*. (1994) as under:

Normal seedlings: Seedlings with well developed root and shoot and free of disease symptoms.

Abnormal seedlings: Seedlings with under developed either root or shoot or both and exhibiting disease symptoms.

The fungi were examined under stereomicroscope on abnormal seedlings and rotten seeds. Diseased portions of the seedlings including roots and shoots were cut and plated on PDA medium to confirm the association of pathogens.

Results and Discussion

The analysis of fifteen seed samples of five safflower cultivars/lines Thori-78, Pawari-95, Dholka Sindh, BI 251978 and BI 209296 yielded eleven different fungi belonging to seven genera i.e., *Alternaria, Aspergillus, Chaetomium, Curvularia, Fusarium, Helminthosporium* and *Rhizopus* with variable frequency and incidence (Table-1). Among these fungal pathogens *Aspergillus* sp. and *Rhizopus* spp. are the storage fungi while species belonging to *Alternaria, Chaetomium, Curvularia, Fusarium* and *Helminthosporium* are field fungi. Among storage fungi, *Aspergillus flavus* was noticed on all cultivars with an incidence range of 4-26 with an average of 14. *Aspergillus niger* was recorded on all cultivars/lines except Dholka Sindh with an incidence of 0-14 while *Aspergillus terreus* was found on lines BI 251978 and BI 209296 with a range of 13 and 5 respectively. *Rhizopus* sp. was observed on all safflower samples except those of Dholka Sindh with an incidence of 0-14. Hassan (1999) has observed the reduction in the seed germination with increasing moisture contents and the length of storage. It has also been reported that the ability of the seed fungi to produce aflotoxin reaches the maximum level after one month of seed infection and then decreases which could play an important role in the poor seed germination.

Table I: Frequency of seed mycoflora on safflower

Fungi	Safflower Cultivars/Lines						
	Thori-78	**Pawari-85**	**Dholka Sindh**	**BI 251978**	**BI 209296**	**Range**	**Mean**
Alternaria alternata	24	18	22	2	16	2-24	16.4
Alternaria sp	4	2	12	22	4	2-22	8.8
Aspergillus flavus	4	16	16	8	26	4-26	14
Aspergillus niger	6	3	-	14	2	0-14	5
Aspergillus terreus	-	-	-	13	5	0-13	3.6
Chaetomium sp	9	7	2	-	-	0-9	3.6
Curvularia sp	12	4	6	3	-	0-12	5
Fusarium moniliforme	7	4	2	0	3	2-9	5
Fusarium sp	7	-	-	3	1	0-7	2.2
Helminthosporium sp	2	5	-	4	6	0-6	3.4
Rhizopus sp	10	14	-	5	2	0-14	6.2
Mean	7.7	6.6	5.4	6.7	5.9		

Table-II: Effect of seed mycoflora on seed germination

Cultivars	Normal seedling	Infected seedling	Rotted seeds	Germi-nation %	Fungal inci-dence %	Fungi recovered
Thori-78	55	25	20	60	55	*Alternaria* sp, *A. alternata, Aspergillus flavus, A. niger, Chaetomium* sp, *Curvularia* sp, *Fusarium* sp, *F. moniliforme, Helminthosporium* sp, *Rhizopus* sp.
Pawari-95	45	30	25	70	43	*Alternaria* sp, *A. alternata, Aspergillus flavus, A. niger, Chaetomium* sp, *Curvularia* sp, *Fursarium moniliforme.*
Dholka Sindh	60	23	17	85	23	*Alternaria* sp, *A. alternata, Aspergillus niger, A. flavus, A. terreus, Curvularia* sp, *Fusarium* sp. *F. moniliforme, Helminthosporium* sp, *Rhizopus* sp.
BI 251978	62	26	12	65	40	*Alernaria* sp, *A. alternata, Aspergilus niger, A. flavus, A. terreus, Curvularia* sp, *Fusarium* sp, *F. moniliforme, Helminthosporium* sp, *Rhizopus* sp.
BI 209296	57	35	8	80	27	*Alternaria* sp, *A.alternata, Aspergillus flavus, A. niger, A.terreus, Fusarium moniliforme, Helminthosporium* sp, *Rhizopus* sp.

The field fungi also showed a variable frequency and incidence on all safflower cultivars/lines. All the samples yielded *Alternaria alternata* with a range of 2-24. *Alternaria* sp was observed on all safflower cultivars/lines with an incidence range of 2-22. Borkar (1997) in a study showed that the spines present on the leaf margins in the safflower serves as a new site of infection by *Alternaria carthami*. The infection through the spines occurred when the pathogen was seed borne serving as a primary source of inoculum. Some available breeding material was screened against *Alternaria carthami* by Desai (1998) under the conditions of natural infection for resistance against the pathogens. *Chaetomium* sp. was observed on Thori-78, Pawari-95 and Dholka Sindh with a range of 9, 7 and 2 respectively. The line BI 209296 showed no incidence of *Curvularia* sp among all, its incidence on other fungi ranged from 3-12. *Fusarium moniliform* was observed on all cultivars/lines and *Fusarium* sp. was noticed on Thori-78, BI 251978 and BI 209296 only. *Helminthosporium* sp was found on four cultivar/lines Dholka Sindh showed no *Helminthosporium* prevalence On plating experiment the mean incidence of *Alternaria alternata* was maximum and among safflower cultivars Thori-78 showed maximum occurrence of seed mycoflora, and minimum fungal incidence was noticed on cultivar Dholka Sindh. *Fusarium* sp was the least to occur on plating. During germination test (Table-II), maximum seed germination was observed in the cultivar Dholka Sindh (85%) with minimun fungal incidence (23%). Minimum seed germination was found in the cultivar Thori-78 (60%) with maximum fungal incidence 55%. Other cultivars/lines i.e., Pawari-95, BI 251978 and BI 209296 showed fungal incidence as 43%, 40%, 27% with germination as 70%, 65% and 80% respectively.

It is evident that the cultivar Dholka Sindh can perform well against seed borne mycoflora and Thori-78 showed more susceptibility to these fungal pathogens. Healthy seeds are the bases of better quality and yields of crops. The use of poor quality seeds even in the absence of epiphytotic diseases can not meet the goals of higher yields. Seeds with good health should be used to get good results.

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