The Pharma Innovation Journal 2023; 12(3): 5757-5760

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 5757-5760

www.thepharmajournal.com
Received: 28-01-2023
Accepted: 15-03-2023

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Evaluation of different seed health testing methods for detection of seed borne leaf spot causing organism in safflower

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Abstract
Safflower is as an important rabi oilseed crop and the diseases are taking heavy toll of the productivity. The seed borne organisms not only reduce the quality but also have an effect on germination. In view of these, understanding of seed borne mycoflora responsible for leaf spot disease, their detection, and management are important. The present investigation on different seed health testing was undertaken during 2020-21 at Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. Among the six different methods employed for detection of seed borne fungal infections, Standard blotter test with untreated seeds was found effective for detection of overall pathogens which shows the highest incidence of seed mycoflora i.e. (12.00%) followed by modified PDA method (9.83%). While for detection of leaf spot causing seed borne, *A. carthami* and *A.alternata* the modified PDA method was found effective which detect 46.66% and 19.33% respectively followed by Standard blotter method (34.32% and 20.33%).

Keywords: Safflower, seed borne fungi, leaf spot disease, seed health testing methods

Introduction
Carthamus tinctorius L. is commonly known as Safflower is one of the important rabi oilseed crops of the world. It is popularly called as '*Kardai*' in Marathi grown for its much-valued edible oil having world-wide acceptability for its health benefits especially to heart patients which belong to family Asteraceae or Compositae with the chromosome number of 2n=24. Safflower seed contains about 25-32 percent oil. India is the largest producer of safflower in the world accounting for 47 per cent area and 27 per cent of total production mainly cultivated for its orange red colour dye and valuable oil. Safflower, apart from superior adaptability to scanty moisture conditions, produces oil rich polyunsaturated fatty acids which play an important role in reducing the blood cholesterol level.

A large proportion of Safflower is used for producing edible oil. Safflower oil is used for manufacturing perfumed oils and for medicinal purposes and the cake is a rich source of protein, carbohydrates and mineral nutrients such as calcium and phosphorous. It is also a valuable and nutritious feed for milk cattle.

The safflower production in India is limited because of the hard spiny nature of the crop. Today the area and production of safflower is increasing due to use of the combined harvesters for harvesting and also due to the availability of non-spiny varieties. Production and productivity of safflower in India is less, when compared to other countries because of biotic and abiotic factors. Biotic factors which include diseases caused by fungi, bacteria, virus and parasitic nematodes are the main reasons for low yield. Among the biotic agents, major diseases are caused by fungal pathogens *viz.*, *Alternaria* leaf spot (*Alternaria carthami*), *Cercospora* leaf spot (*Cercospora carthami*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *carthami*), *Ramularia* leaf spot (*Ramularia carthami*), Root rot (*Phytophthora drechsleri)* and Rust (*Puccinia carthami*). Minor diseases include Bacterial leaf blight (*Pseudomonas syringae*), viral diseases; Cucumber mosaic virus, Lettuce mosaic virus, Tobacco mosaic virus and Root knot nematode (*Meloidogyne incognita*). Among the diseases, leaf spots were the most important and destructive disease of safflower. Leaf spots caused by *Alternaria carthami* and *Alternaria alternata* are a serious threat to successful cultivation of safflower. The disease was reported for the first time from India by Choudhury (1944) at Pune. Up to 50 per cent seed yield loss was recorded due to this disease (Indi *et al.*, 1987). This disease plays an important role in safflower cultivation and causes 25-60 per cent yield loss every year (Singh

and Prasad, 2005) [19]. Some times as high as 80-90%, when the disease appears at early stage of crop growth (Krishna Prasad, 1988) [11].

Safflower is resistant to drought and tolerant to insect pests but it is susceptible to diseases like *Alternaria* and *Cercospora* leaf spots. Seed borne mycoflora are carried over by infected seeds and they cause deterioration in seed, in soil affecting germination, causing seedling mortality and further infection of foliage is observed at adult stage.

Materials and Methods

The present investigations were carried out in the plant pathology and Seed Pathology laboratory of Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. The details of materials used and the methodology followed in conducting the experiments are presented here under.

Borosil and corning glasswares were used for all the laboratory experimental studies. They were kept for a day in the cleaning solution containing 60 g potassium dichromate, 60ml of concentrated sulphuric acid, in one liter of water. Then they were cleaned by washing with detergent solution followed by rinsing several times in tap water and finally with distilled water. All glassware used in the studies were sterilized in autoclave at 1.1 kg/cm pressure for 20 min and kept in hot air oven at 60º C for one hour. Both solid and liquid media were sterilized at 1.1 kg /cm pressure for 15 min.

Evaluation of different seed health testing methods for detection of leaf spot causing *Alternaria carthami, A. alternata* and other seed borne mycoflora associated with safflower seed

To know the efficacy of different seed health testing methods in detecting seed borne leaf spot causing *Alternaria carthami, A. alternata* and other seed borne mycoflora associated with safflower seed, following methods were employed as described below.

1. Standard blotter method

The standard blotter method was developed by Doyer in 1938 which was later included in the International Seed Testing Association Rules of 1966. (ISTA, 1999) [9]. Four hundred seed of each variety were tested by employing standard blotter method in three replications. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri plates after draining excess water. Untreated seeds were placed at the rate of 10 seeds per Petri plate at equal distance. The plates were incubated at room temperature (20± 2 ºC) under alternate cycles of 12 hrs NUV light and darkness. After eight day of incubation the seeds were examined under stereoscopic –binocular microscope for the associated fungi and they were identified based on "morphological and colony characters (Neergaard, 1979) [14].

2. Agar plate method

Four hundred infected seeds of safflower were placed at the rate of 10 seeds per petriplate containing 20 ml of two per cent water agar. The petriplates were incubated for seven days as described under Standard agar plate method. After seven days of incubation, the fungal colony growth was examined under stereo-binocular microscope (Khare, 1996) [10].

3. Standard Deep freezing blotter method

This method was developed by Limonard (1968) to detect slow growing pathogens. Four hundred infected seeds of safflower were placed at the rate of 10 seeds per plate on moistened blotters in the way as described under Standard blotter method. The petriplates were incubated at 20± 2 °C for 24 hrs under alternate cycles of 12 hrs NUV light and darkness, for next 24 hours the plates are incubated at 20 °C in dark and then kept back under original conditions for next five days. After eight days of incubation, the seeds were examined under stereo-binocular microscope.

4.2 4-D blotter method

Four hundred infected seeds of safflower were placed at the rate of 10 seeds per petriplate with moistened blotter dipped in 0.2 per cent solution of sodium salt of 2,4-dichlorophenoxy acetic acid. The petriplates were incubated in the way as described under Standard blotter method. After eight days of incubation, the fungal growth on seeds was examined using stereo-binocular micro scope (Khare, 1996) [10].

5. Test tube water agar seedling symptom test

Safflower seed samples were examined for seedling symptom test. Culture tubes (100 x 16 mm) were filled with 10 ml of 2 per cent water agar and solidified to have slight slant. Hundred seeds of each sample was placed individually in each tube and incubated at 20± 2°C with alternate cycles of 12 hrs light and dark periods for 15 days. The cotton plug was removed after seedling reached to rim portion of the tube and observation was taken on symptom expressed in the seedling (Khare, 1996) [10].

6. Modified P.D.A. method

Four hundred seeds of safflower variety Manjira were placed at the rate of 10 seeds per petriplate containing 20 ml of acidified Potato Dextrose Agar (pH 4.5). Seeds were placed after pretreatment with one per cent sodium hypochlorite solution for two minutes and the plates were kept for incubation as described under Standard blotter method for seven days. After seven days of incubation, the fungal colony growth was examined under stereo-binocular microscope (Jeffrey *et al*., 1985).

Results and Discussion

The objective of this study was to find out the effective, economical and quick method for detection of seed borne mycoflora as well as leaf spot causing seed borne pathogens, *Alternaria carthami and Alternaria alternata*. For this purpose six different methods *viz.*, Standard blotter method, Agar plate method, Standard Deep-freeze blotter method, 2, 4-D blotter method, Test tube water agar seedling symptom test and Modified P.D.A. method were studied.

Among the six different methods employed for detection of seed borne fungal infections, Standard blotter test with untreated seeds was found effective for detection of overall pathogens which shows the highest incidence of seed mycoflora i.e. (12.00%) followed by modified PDA method (9.83%).

While for detection of leaf spot causing seed borne, *A. carthami* and *A.alternata* the modified PDA method was found effective which detect 46.66% and 19.33% respectively followed by Standard blotter method (34.32% and 20.33%). (Table 1 and Plate 1.) The findings of the present study were

in concurrence with earlier findings of Irwin (1976) [8], Krishna Prasad (1988) [11], Dawar and Ghaffar (1990) [5], Khare (1996) [10], Prasad *et al*., (2009), Nagaraja and Krishnappa (2011) [13], Pushpavathi *et al*., (2012) [17], Patharkar *et al.* (2013) [15], Amrutha Gayathri *et al*. (2014) [1], Monica and Rajeshwari (2014) [12] and Radha and Chattannavar (2017) [18]. Irwin (1976) [8] detected *Alternaria carthami* from safflower seed by standard blotter test and component plating method. He found the infection of *Alternaria carthami* on cotylendons and seed coats of safflower by component plating method. Khare (1996) [10] gave brief account on various isolation techniques *viz.* visual inspection, incubation test (standard blotter method, 2,4-D method, deep freeze blotter method, agar plate method and ulster method) seedling symptoms test (Hiltners method, multi plots and test tube agar method), embryo count method, histopathological technique and Immuno diagnostic assay for isolation of seed association fungi. Pushpavathi *et al*., (2012) [17] tested different seed health methods *viz*., standard blotter, agar plate and seed washing methods by using 12 safflower cultivars and found 10 different fungal species associated with the seeds reported that standard blotter method was best for the detection of seed mycoflora associated with safflower seed followed by agar plate method. Amrutha Gayathri *et al.* (2014) [2] detected *Alternaria carthami* by using Blotter method and agar plate methods. Out of the two methods tested blotter method was found superior over agar plate method in which the highest per cent incidence of 46% was observed with the fungus *Alternaria carthami* on Nari. Radha and Chattannavar (2017) [18] Evaluate different six seed health testing methods *viz*., Standard blotter method, Water Agar method, Potato Dextrose Agar method, Deep freezing blotter method, 2,4- D blotter method and Paper towel method for detection of *Alternaria sesame* in sesame. Among six different seed health testing methods used to assess their efficacy and reliability, standard blotter method was found to be good for the detection of seed borne infection of *A. sesami* in sesame.

Table 1: Efficacy of different seed health testing methods for detection of seed-borne *Alternaria carthami, A. alternata* and other fungal infections of safflower (Variety: Manjira)

Sr. No	Detection methods		Pathogens associated (%) Seed borne pathogens of significance								Mean Seed borne pathogens (%)
			Alternaria carthami	*Alternaria alternata*	*Macrophomina phaseolina*	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus fumigatus*	*Curvularia spp*	*Fusarium oxysporum*	
1	Standard blotter test	Un-treated Seeds	34.32 (35.86)	20.33 (26.78)	11.67 (19.97)	8.00 (16.43)	6.00 (14.15)	3.00 (9.88)	6.00 (14.17)	9.67 (18.11)	12.00
		Pre-treated seeds	30.00 (33.20)	8.33 (16.77)	7.33 (15.70)	00 (4.05)	00 (4.05)	00 (4.05)	1.33 (6.53)	2.67 (9.36)	6.21
2	Standard Agar Plate		25.66 (30.42)	10.67 (19.06)	12.00 (20.27)	00 (4.05))	00 (4.05)	00 (4.05	3.00 (9.97)	7.33 (15.70)	7.33
3	Standard deep freeze blotter		12.66 (20.83)	11.67 (19.92)	6.33 (14.57)	3.00 (9.88)	2.00 (8.13)	2.00 (8.13)	4.00 (11.54)	15.33 (23.05)	7.12
4	2,4-D blotter soak		18.66 (25.60)	17.33 (24.60)	2.33 (8.74)	2.00 (8.13)	2.00 (8.13)	00 (4.05	3.00 (9.97)	0.33 (4.62)	5.71
5	Test tube water agar seedling symptom test		3.00 (9.88)	0.67 (5.18)	0.33 (4.62)	00 (4.05)	00 (4.05)	00 (4.05	00 (4.05)	00 (4.05)	0.50
6	Modified PDA method		46.66 (43.09)	19.33 (26.08)	9.67 (18.11)	00 (4.05))	00 (4.05)	00 (4.05	1.00 (5.73)	2.00 (8.13)	9.83
S. E. ±			0.74	0.63	0.41	0.37	0.26	0.37	0.30	0.38	-
CD at 5%			2.24	1.93	1.07	1.12	0.80	1.12	0.91	1.15	-
C.V. (%)			4.51	5.57	4.86	8.90	6.87	11.7	5.00	5.52	-

Plate 1: Different Seed health testing methods for Detection of seed borne mycoflora of Safflower

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