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RESEARCH ARTICLE :

Efficacy of fungicides on seed mycoflora of sunflower at different storage periods

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SUMMARY : The efficacy of seven fungicides *viz*., captan, mancozeb, carboxin + thiram, carbendazim, tebuconazole, carbendazim + iprodione and metalaxyl against seed mycoflora of sunflower at recommended dosages and at different storage periods (1 day to 3 months) were studied. A total of 16 seed borne fungi belonging to 13 genera *viz*., *Alternaria* sp., *Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Fusarium* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Rhizopus* sp., *Trichoderma* sp. and *Penicillium* sp. were recovered from untreated and treated seeds at different storage periods. Among the fungicides tested, seed treatment with carboxin + thiram (4.19%) was found significantly superior in reducing the per cent seed infection followed by carbendazim + iprodione (11.84%) and the least of that was carbendazim (62.47%).the per cent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora *viz.*, *Alternaria* sp., *Macrophomina phaseolina, Fusarium* sp. and *Drechslera* sp. and gradual increase in storage mycoflora *viz*., *Aspergillus flavus, Aspergillus niger, Cladosporium* sp., *Curvularia* sp. etc. was found with the increase in storage period.

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BACKGROUND AND OBJECTIVES

Sunflower (*Helianthus annuus* L.) is one of the most popular oilseed crops grown in India. Sunflower seeds contain 40-50% oil, 23% of protein and constitute excellent source of unsaturated fats, fibre and important nutrients, selenium, copper, zinc, vitamin E and B complex as well (Afzal *et al*., 2010). It is a rich source of linoleic acid (64%) which helps in reducing the cholesterol deposition. The total area of sunflower in India is 0.69Mha with a production of 0.50Mt. It occupies 6th place among the oilseed crops grown in India in terms of production. United Andhra Pradesh constitutes 0.16Mha area under sunflower with a production of 0.14Mt (Indiastat, 2013-14). Karnataka and Andhra Pradesh are the major sunflower growing states in India.

Seed health plays an important role in successful cultivation and yield exploration of a crop. Fungi are the main component of microflora associated with seeds and are the main cause of deterioration and loss observed

during storage (Tanaka *et al.*, 2001). The associated micro-rganisms may be pathogenic or non-pathogenic in nature. Major seedborne diseases of sunflower include, leaf blight (*Alternaria helianthi*), head rot (*Rhizopus arrhizus*), collar rot (*Sclerotium rolfsii*) and downy mildew (*Plasmopara halstedii*). In addition to these seedborne pathogens, seeds are also known to harbour several other fungi which may cause seed rot, seedling mortality, reduced seedling vigour and seed viability which leads to poor plant stand in the field. The seed quality also affects the rate and uniformity of emergence and the dynamics of initial plant growth. The seedborne fungi may also cause systemic or local infections, resulting in development of diseases at later stages of the crop growth. It was reported that, 20-30 per cent loss in germinability of sunflower was due to seedborne diseases (Jamaria *et al.*, 1975). Therefore, management of seedborne fungi is extremely important for realization of full yield potential of cultivars.

Seed treatment is one of the best methods to manage seedborne diseases. It has become a common practice to use fungicides as seed dressers for reducing the seedborne infections under field conditions. Fungicides form a zone of protection over the seed surface that reduces seed decay and seedling blight, resulting in healthy and vigorous seedlings. Treating the seeds with fungicides may eradicate pathogens in or on seeds and can also protect seeds and seedlings from soil-borne pathogens (Maude, 1996). In the present study, efficacy of different fungicides against sunflower seed mycoflora was evaluated over a period of three months of storage after seed treatment.

RESOURCES AND METHODS

Seeds of sunflower hybrid DRSH-1 were collected from IIOR, Rajendranagar, Hyderabad and stored at ambient storage temperature of 28 ± 2^0C. This experiment was conducted at SRTC, Rajendranagar, Hyderabad.The seeds were treated with fungicides *viz*., captan (0.25%), mancozeb (0.25%), carboxin+thiram (0.3%), carbendazim (0.2%), tebuconazole (0.1%), carbendazim + iprodione (0.2%) and metalaxyl (0.6%). The treated seeds were stored in butter paper bags along with untreated control for further use.

The effect of fungicides on seed mycoflora was assessed by employing standard blotter method(ISTA, 1996). The randomly selected 400 treated seeds were subjected to seed health testing at different intervals *viz*., immediately after treatment, one day after treatment, one week after treatment, two weeks after treatment, three weeks after treatment, one month after treatment, two months after treatment and three months after treatment consecutively along with controls to estimate seed borne mycoflora. The data on number of seeds infected by different fungi and a specific fungus was recorded separately to calculate per cent seed infection and frequency of a specific fungus.

Detection of seed mycoflora by standard blotter method :

Sterilized blotting paper discs of 90mm diameter were placed in sterile Petri plates and moistened with sterile distilled water. The excess water was drained off from the plates. Seeds were transferred to the plates containing moist blotting paper discs. Ten seeds per plate were placed at equidistance, 10 such plates were maintained under each replication. The experiment was conducted with four replications and under each replication hundred seeds were tested. The plates were incubated at 24 ± 2^0C for seven days in an incubator. The mycoflora observed on seeds were isolated and identified.

Data recording :

On 8th day, the incubated seeds were examined under stereo binocular microscope. The mycelium and the fungal structures obtained from the seeds were further observed critically under 10 x and then under 40 x objective lens of a compound microscope by preparing water mount slides.

Data on number of seeds infected by different fungi and a specific fungus were recorded separately to calculate per cent seed infection and frequency, respectively. To calculate per cent seed infection (Aslam *et al.*, 2015) and frequency of the species (Neha and Razia, 2013) the following formulae were used.

$$\text{Per cent seed infection} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \text{x100}$$

$$\text{Frequency} = \frac{\text{No. of seeds containing a specific fungus}}{\text{Total number of seeds}} \text{x 100}$$

Isolation of fungi :

Fungal colonies or sporulating structures obtained from seeds after incubation through both the methods

were isolated separately onto fresh PDA medium in Petri plates. Pure cultures of the fungi isolated were obtained by adopting hyphal tip method or single spore isolation technique (Tuite, 1969). Pure cultures thus, obtained were maintained on PDA slants.

Identification of fungi :

Identification of various seed mycoflora was done using relevant keys given by Subramanian (1971); Booth (1971); Barnett (1965) and descriptions of CMI (1970).

OBSERVATIONS AND ANALYSIS

A total of 16 seed borne fungi belonging to 13 genera *viz.*, *Alternaria* sp., *Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus ustus, Emericella nidulans, Fusarium* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Rhizopus* sp., *Trichoderma* sp. and *Penicillium* sp. (Table 2) were recovered from untreated and treated seeds at different storage periods. It was observed that, the per cent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora *viz.*, Alternaria sp., *Macrophomina phaseolina*, *Fusarium* sp. and *Drechslera* sp. and gradual increase in storage mycoflora *viz., Aspergillus flavus, Aspergillus niger, Cladosporium* sp., *Curvularia* sp. etc. was found with the increase in storage period.

All the fungicides were highly significant in suppressing seed mycoflora when compared to control. The fungi recovered from seeds treated with different fungicides at different storage periods include, *Alternaria* sp., *Macrophomina phaseolina*, *Rhizopus* sp., *Fusarium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Emericella nidulans*, *Epicoccum* sp., *Cladosporium* sp. and *Curvularia* sp. (Table 2). The fungi *viz.*, *Aspergillus ochraceus*, *Aspergillus ustus*, *Trichoderma* sp., *Penicillium* sp., *Chaetomium* sp. and *Drechslera* sp. were observed only in control but not in treated seeds. Among the fungicides, carboxin + thiram

Table 1 : Efficacy of fungicides against seed mycoflora of sunflower at different storage periods

Fungicide	Per cent seed infection								
	IAT	1 DAT	1 WAT	2 WAT	3 WAT	1 MAT	2 MAT	3 MAT	Mean
Captan	10.00*	11.50	11.50	13.00	13.25	15.00	15.00	21.75	13.88
	(18.41)**	(19.80)	(19.80)	(21.09)	(21.32)	(22.77)	(22.77)	(27.79)	
Mancozeb	15.00	16.75	16.75	21.75	23.50	25.00	26.75	28.50	21.75
	(22.78)	(24.14)	(24.14)	(27.79)	(28.99)	(29.99)	(31.14)	(32.26)	
Carboxin + thiram	3.25	3.25	3.50	3.50	5.00	5.00	5.00	5.00	4.19
	(10.29)	(10.29)	(10.75)	(10.75)	(12.88)	(12.88)	(12.88)	(12.88)	
Carbendazim	53.25	55.00	56.75	60.00	63.25	70.00	70.00	71.50	62.47
	(46.86)	(47.87)	(48.88)	(50.77)	(52.68)	(56.79)	(56.79)	(57.75)	
Metalaxyl	32.75	33.25	36.75	38.25	40.00	41.75	50.00	53.25	40.75
	(34.89)	(35.20)	(37.31)	(38.20)	(39.22)	(40.24)	(45.00)	(46.86)	
Tebuconazole	25.00	26.75	30.00	30.00	33.25	41.75	46.67	56.75	36.27
	(29.98)	(31.13)	(33.19)	(33.19)	(35.20)	(40.24)	(43.13)	(48.88)	
Carbendazim	10.00	10.00	11.50	11.50	11.75	13.25	13.25	13.50	11.84
+ iprodione	(18.40)	(18.40)	(19.77)	(19.77)	(20.01)	(21.32)	(21.32)	(21.54)	
Control	65.00	68.25	70.00	71.75	72.00	74.00	75.00	77.50	71.69
	(53.73)	(55.71)	(56.79)	(57.90)	(58.06)	(59.35)	(60.01)	(61.70)	
Mean	26.78	28.09	29.78	31.22	32.56	35.72	37.71	40.97	
	Storage period			Fungicide			Storage period x Fungicide		
S.E.±	0.21			0.21			0.61		
C.D. (P=0.05)	0.60			0.60			1.71		

IAT - Immediately after treatment, DAT - Day (s) after treatment, WAT - Week (s) after treatment, MAT- Month (s) after treatment

* Mean of four replications

** Figures in parenthesis are angular transformed values

Table 2 : Seed mycoflora recovered from sunflower seeds treated with fungicides

Fungicide	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
Captan																
IAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 MAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Mancozeb																
IAT	++	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
2 MAT	+	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
3 MAT	+	-	-	-	+	+	-	-	-	-	-	+	+	-	+	-
Carboxin + thiram																
IAT	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3 MAT	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbendazim																
IAT	++++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-
3 WAT	++	+	++	-	+	-	-	-	-	-	-	-	+	-	-	-
1 MAT	++	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-
2 MAT	++	-	+++	+	-	-	-	-	-	-	-	-	+	-	+	-
3 MAT	++	-	+++	+	+	+	-	-	-	-	+	-	+	-	+	-
Fungicide metalaxyl																
IAT	++	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
1 DAT	++	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
1 WAT	++	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
3 WAT	++	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

Table 2 : Contd.……..

Table 2 : Contd……..

1 MAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
2 MAT	+	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-
3 MAT	+	-	+	+	+	-	-	-	-	-	+	+	+	-	+	-
Tebuconazole																
IAT	++	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	++	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
2 MAT	++	-	++	-	-	+	-	-	-	-	-	-	-	-	+	-
3 MAT	+	-	++	-	+	+	-	-	-	-	+	-	+	-	+	-
Carbendazim + iprodione																
IAT	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3 MAT	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Control																
IAT	+++	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-
1 DAT	+++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 WAT	+++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+++	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	++	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-
1 MAT	++	+	++	+	++	++	-	-	-	-	+	+	+	-	-	-
2 MAT	++	-	++	+	++	++	+	+	+	+	-	+	+	+	+	+
3 MAT	++	-	++	+	++	++	-	+	-	+	-	-	+	-	+	-

Alt - *Alternaria* sp., Mp – *Macrophomina phaseolina*, Rhi - *Rhizopus* sp.,Fus - *Fusarium* sp., An – *Aspergillus niger*, Af – *Aspergillus flavus*, Ao –*Aspergillus ochraceus*, Au – *Aspergillus ustus*, Pen - *Penicillium* sp., Tri - *Trichoderma* sp., En – *Emericella nidulans*, Epi - *Epicoccum* sp., Cla - *Cladosporium* sp., Cha - *Chaetomium* sp., Cur - *Curvularia* sp., Dre - *Drechslera* sp. IAT - Immediately after treatment, DAT – Day(s) after treatment, WAT - Week(s) after treatment, MAT - Month(s) after treatment

(4.19%) was found significantly superior followed by carbendazim + iprodione (11.84%), captan (13.88%) and the least effective (62.47%) was carbendazim (Table 1). The least significant per cent seed infection (3.25%) was observed with Carboxin + thiram treated seeds plated immediately after treatment and maintained on par significance upto 2 weeks after seed treatment.

It was also observed that, *Alternaria* sp. followed by *Macrophomina phaseolina* and *Fusarium* sp. were commonly recovered from all the fungicides tested at different storage periods, while the fungus *Rhizopus* sp. was not observed from the seeds treated with carboxin + thiram, captan and mancozeb. Across the storage periods, *Aspergillus niger* and *A. flavus* were recovered with less abundancy from metalaxyl, tebuconazole, mancozeb and carbendazim treated seeds and were not recovered from carbendazim + iprodione, carboxin + thiram and captan treated seeds. Other seed mycoflora *viz., Emericella nidulans, Epicoccum* sp., *Cladosporium* sp. and *Curvularia* sp.were rarely recovered from

metalaxyl, tebuconazole, mancozeb and carbendazim treated seeds and not at all recovered from carbendazim + iprodione, carboxin + thiram and captan treated seeds at different storage periods tested (Table 2). The present findings are in conformity with the findings of Anbhule and Kareppa (2009) and Nghiep and Gaur (2005) who reported the superiority of carboxin as seed dresser in eradicating seedborne fungi of groundnut and rice, respectively.

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