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Efficacy of Biocontrol Agents against Seed Mycoflora of Sunflower at Different Storage Periods

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ABSTRACT

The efficacy of three bioagents viz., Trichoderma viride, Bacillus subtilis, Pseudomonas fluorescens tested both alone and in combinations @ 10 g kg^{-1} seed against seed mycoflora of sunflower and at different storage periods (upto 3 months) were studied. A total of 16 seedborne 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 biocontrol agents, Trichoderma viride (20.66%) was found significantly superior to other biocontrol agents in inhibiting the seed mycoflora followed by Pseudomonas fluorescens + Trichoderma viride (23.59%) and the least effective (60.66%) was Pseudomonas fluorescens. 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.

Key words: *Sunflower seed mycoflora, Biocontrol seed treatments, storage mycoflora, standard blotter method*

INTRODUCTION

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, fiber, linoleic acid and important nutrients, selenium, copper, zinc, vitamin E and B complex as well[1]. 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[7]. Karnataka and Andhra Pradesh are the major sunflower growing states in India.

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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[13]. The associated microorganisms 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. It was reported that, 20-30 per cent loss in germinability of sunflower was due to seedborne diseases[9]. 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. Though fungicides have played an important role in increasing production and management of diseases, their indiscriminate use has led to several problems such as development of resistance in fungi to fungicides, destruction of beneficial organisms and direct and indirect influence on human health. Thus, exploration of other alternative disease management options need to be considered. Use of biological control agents for seedborne diseases is likely to be less spectacular than chemical control but is usually also more stable and long lasting. The biocontrol agents have the ability to colonize the root surfaces and the cortex[10]. They release certain antibiotics and plant growth promoting substances in rhizosphere by which they offer protection from seed and soilborne pathogens and promote plant growth. In spite of biological control having been used in agriculture for centuries, as an industry biological control is still in its infancy.

In the present study, efficacy of different biocontrol agents against sunflower seed mycoflora was evaluated over a period of three months of storage after seed treatment.

MATERIAL 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 commercial formulations of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and their combinations @ 10 g kg^{-1} seed and were stored in butter paper bags along with chemical (Carbendazim - 0.2%) and untreated control for further use.

The effect of biocontrol agents on seed mycoflora was assessed by employing standard blotter method[8]. 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. Seeds treated with a standard seed dressing fungicide carbendazim and untreated seeds were served as controls. 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^{0}C 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 10x and then under 40x 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[2] and frequency of the species[11] the following formulae were used.

$$\text{Per cent seed infection} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

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

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[14]. 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[12], Booth[5], Barnett[4] and descriptions of CMI[6].

RESULTS AND DISCUSSION

A total of 16 seedborne 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 biocontrol agents were found significantly effective in suppressing seed mycoflora when compared to both the controls. The fungi *viz.*, *Alternaria* sp., *Macrophomina phaseolina*, *Rhizopus* sp., *Fusarium* sp., *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Epicoccum* sp., *Cladosporium* sp., *Chaetomium* sp. and *Curvularia* sp. (Table 2) were observed at different storage periods from the seeds treated with different biocontrol agents. The fungi *viz*., *Aspergillus ochraceus, Aspergillus ustus, Trichoderma* sp., *Emericella nidulans* and *Drechslera* sp. were recorded only in control but not in treated seeds. Among the biocontrol agents, *Trichoderma viride* (20.66%) was found significantly superior to other biocontrol agents in inhibiting the seed mycoflora followed by *Pseudomonas fluorescens* + *Trichoderma viride* (23.59%), *Bacillus subtilis* + *Trichoderma viride* (27.47%) and the least effective (60.66%) was *Pseudomonas fluorescens* (Table 1). The least significant per cent seed infection (18.25%) was also observed with *Trichoderma viride* treated seeds plated immediately after treatment and maintained on par significance upto 2 weeks after seed treatment.

Across the storage periods tested, *Alternaria* sp. followed by *Aspergillus niger*, *A. flavus* and *Fusarium* sp. were the predominant fungi occurring in all the treatments tested, while the fungus *Macrophomina phaseolina* was not recovered from *Trichoderma viride* and *Bacillus subtilis* + *Trichoderma viride* treated seeds. *Rhizopus* sp. was also not recovered from the seeds treated with *Trichoderma viride* at all the storage periods studied. Other seed mycoflora *viz.*, *Penicillium* sp., *Epicoccum* sp., *Cladosporium* sp., *Curvularia* sp. and *Chaetomium* sp. were absent in *Trichoderma viride* treated seeds and were found with less frequency in all other treatments including untreated control at different storage periods under evaluation (Table 2). The present findings are in conformity with the findings of Baig *et al.*[3] who reported the efficacy of *Trichoderma viride* in inhibiting seed mycoflora of oilseeds.

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

Biocontrol agent	Per cent seed infection								
	IAT	**1 DAT**	**1 WAT**	**2 WAT**	**3 WAT**	**1 MAT**	**2 MAT**	**3 MAT**	**Mean**
Bacillus subtilis	40.00***** (39.22)**	40.00 (39.22)	43.25 (41.11)	43.25 (41.11)	43.50 (41.26)	46.75 (43.13)	46.75 (43.13)	50.00 (45.00)	44.19
Pseudomonas fluorescens	56.75 (48.88)	58.25 (49.75)	58.50 (49.89)	60.00 (50.77)	60.25 (50.91)	60.25 (50.91)	65.50 (54.03)	65.75 (54.18)	60.66
Trichoderma viride	18.25 (25.27)	18.50 (25.46)	20.00 (26.53)	20.25 (26.72)	21.50 (27.61)	21.75 (27.79)	21.75 (27.79)	23.25 (28.82)	20.66
Bacillus subtilis* + *Trichoderma viride	23.25 (28.82)	23.25 (28.81)	26.50 (30.97)	26.50 (30.97)	26.75 (31.14	30.00 (33.20)	30.00 (33.20)	33.50 (35.35)	27.47
Pseudomonas fluorescens* + *Trichoderma viride	21.50 (27.60)	21.50 (27.60)	21.75 (27.78)	23.25 (28.81)	23.50 (28.99)	25.00 (29.99)	25.50 (30.32)	26.75 (31.13)	23.59
Control (Carbendazim)	53.25 (46.86)	55.00 (47.87)	56.75 (48.88)	60.00 (50.77)	63.25 (52.69)	70.00 (56.80)	70.00 (56.80)	71.50 (57.73)	62.47
Control (Untreated)	71.75 (57.90)	73.50 (59.03)	73.50 (59.03)	75.25 (60.18)	75.50 (60.34)	75.50 (60.34)	76.75 (61.19)	78.50 (62.40)	75.03
Mean	40.68	41.43	42.89	44.07	44.89	47.04	48.04	49.89	
	Storage period			**Biocontrol agent**			**Storage period x Biocontrol agent**		
SE(m)±	0.22			0.20			0.58		
CD at 5%	0.62			0.58			1.64		

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 biocontrol agents

Biocontrol agent	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
Bs																
IAT	++	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
1 DAT	++	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
1 WAT	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-
2 MAT	+	-	++	+	++	++	-	-	+	-	-	-	+	+	-	-
3 MAT	+	-	++	+	++	++	-	-	+	-	-	-	+	-	+	-
Pf																
IAT	+	-	++	-	+	+	-	-	-	-	-	-	-	-	-	-
1 DAT	+	+	++	-	+	+	-	-	-	-	-	-	-	-	-	-
1 WAT	+	+	++	-	+	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	+	++	-	+	+	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	++	+	+	+	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	++	-	++	+	-	-	-	-	-	-	-	+	-	-
2 MAT	+	-	+++	+	++	++	-	-	-	-	-	-	+	-	+	-
3 MAT	+	-	+++	+	++	++	-	-	-	-	-	+	+	-	-	-
Tv																
IAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
3 MAT	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Bs+Tv																
IAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	-	+	+	-		-	-	-	-	-	-	-	-	-	-
3 WAT	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	-
3 MAT	+	-	+	+	+	+	-	-	+	-	-	-	+	-	+	-

Biocontrol agent	Alt	Mp	Rhi	Fus	An	Af	Ao	Au	Pen	Tri	En	Epi	Cla	Cha	Cur	Dre
Pf+Tv																
IAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
2 WAT	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 WAT	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
1 MAT	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 MAT	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	-
3 MAT	+	-	+	+	+	+	-	-	+	-	-	-	+	-	-	-
Control (Carbendazim)																
IAT	++++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 DAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1 WAT	+++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 WAT	++	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-
3 WAT	++	+	++	-	+	-	-	-	-	-	-	-	+	-	-	-
1 MAT	++	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-
2 MAT	++	-	+++	+	-	-	-	-	-	-	-	-	+	-	+	-
3 MAT	++	-	+++	+	+	+	-	-	-	-	+	-	+	-	+	-
Control (Untreated)																
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., Bs - *Bacillus subtilis*, Pf - *Pseudomonas fluorescens*, Tv - *Trichoderma viride*. IAT - Immediately after treatment, DAT – Day(s) after treatment, WAT - Week(s) after treatment, MAT - Month(s) after treatment.

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