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Evaluation of Microbial Biocontrol Agents and Fungicides against *Alternaria helianthi* Causing Leaf Blight of Sunflower

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A B S T R A C T

The efficacy of twelve microbial biocontrol agents *viz.*, *Trichoderma viride T. viride* strain 16 *T. viride* strain 60 *T. harzianum T. harzianum* strain 2 *T. harzianum* strain 55 *Pseudomonas fluorescens and Bacillus subtilis* were evaluated by dual culture technique against *Alternaria helianthi*. *In vitro* efficacy of six fungicides viz., Mancozeb, Iprodione, Propiconozole, Chlorothalonil, Hexaconozole and Difenconozole were evaluated by food poison technique. Among the biocontrol agents, maximum inhibition of radial growth of *A. helianthi* was observed in observed in *T. viride* (85.33%) which was followed by *T. viride* strain 16 (79.33%), and *T. harzianum* (76.44%). Among the fungicides, Propiconozole recorded maximum inhibition of mycelial growth of *A. helianthi* (90.47%) followed by Mancozeb (90.42%) and Iprodione+Caebendazim (89.75%). In *in vivo* evaluation spraying of *T. viride* @ 1:0 dilution was effective and Propiconozole @ 0.1% was found effective.

Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop, ranks second to soybean in worldwide vegetable oil production. Commercially available sunflower contains 39 to 49 per cent oil in the seed. It is extensively grown in Argentina, France, Spain, USA, China, Ukraine and India. In India, sunflower occupies the fourth place among oilseed crops in terms of acreage and production. The area under sunflower cultivation in India was 0.72 m ha, with a total production of 0.50 m t and productivity of 692 kg/ha (Anonymous, 2013). Over 70 per cent of the sunflower crop is being grown across Karnataka, Maharastra and Andhra Pradesh. In Karnataka it occupies an area of about 3.84 lakh ha with a production of 1.93 lakh tonnes and productivity of 503 kg/ha.

Among the major diseases of sunflower, Alternaria blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is most devastating disease in Karnataka. The disease is known to cause more than 80 per cent of yield loss under severe epiphytotic conditions. Several effective pesticides have been recommended for the control of Alternaria leaf blight but they are not

considered to be long term solutions, due to concerns of expense, exposure to heal the risk, fungicide residue and other environmental hazards. In the recent past most of the work has been directed towards biological disease management, use of antagonistic fungi appears logical and safe (Latha *et al.,* 2009). Bio control agents compete with fungal phytopathogens either competing for nutrients and space, or indirectly modifying environmental conditions, promoting plant growth and plant defense mechanism and antibiosis, or directly through mechanisms such as mycoparasitism (Shakeri and Foster, 2007; Reino *et al.,* 2008). *Trichoderma* spp. suppresses disease by antagonizing the pathogen (Shah and Nasreen, 2011). In the present study some of the new chemicals and microbial biocontrol agents were tested under laboratory and field conditions to find out their efficacy against *A.helianthi*.

Materials and Methods

In vitro evaluation of biocontrol agents and fungicides against *A. helianthi*

Six fungal and two bacterial antagonists were evaluated in vitro against *A. helianthi* applying dual culture technique. In dual culture technique, 20ml of sterilized and cooled potato dextrose agar medium was poured into sterilized petriplates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the petriplate and the antagonist was inoculated exactly on opposite side of the same plate by leaving 3-4cm gap. For this, actively growing cultures were used. In case of bacterial antagonist evaluation, two mycelia discs of pathogen were inoculated and bacterial antagonist was streaked at the centre of the plate. Each treatment was replicated three times. After required period of incubation *i.e*., after mycelia growth in control plate reached 90mm diameter, the radial growth of pathogen was measured. Per cent inhibition of the test fungus over untreated control was calculated by applying the formula (Arora and Upadhyay, 1978).

$$I = \frac{C-T}{C} \times 100$$

I = Per cent growth inhibition
C = Colony growth in control plate
T = Colony growth in intersecting plate

In vitro evaluation of fungicides

Efficacy of six fungicides against *A. helianthi* was assessed by food poison technique. Five mm diameter of culture disc of *A. helianthi* was kept at the center of each Petri plate containing the fungicides of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27±1°C for ten days and colony diameter was recorded.

Chemical fungicides used with their concentration are presented in table 1.

Per cent inhibition of mycelial growth was calculated by using the formula

$$I = \frac{C-T}{C} \times 100$$

I = Per cent inhibition of mycelium.
C = growth of mycelium in control.
T= Growth of mycelium in treatment.

Field evaluation of biocontrol agents and fungicides against Alternaria leaf blight

Field experiment was conducted at Zonal Agricultural Research Station during kharif 2013-14 to know the efficacy of fungicides and different dilutions of *T. viride* which was found effective in vitro and under glass house against Alternaria blight of sunflower.

Plot size: 4.8 m x 3.6 m
Hybrid: KBSH-44

Space: (60 x 30) cm
Design: RCBD
Treatment: 7
Replication: 3

Treatments

T1 = Seed treatment and spraying with *T. viridae* @ 1:0 dilution
T2 = Seed treatment and spraying with *T. viridae* @ 1:1 dilution
T3 = Seed treatment and spraying with *T. viridae* @ 1:2 dilution
T4 =Seed treatment and spraying with Propiconozole @ 0.1%
T5= Seed treatment and spraying with Iprodione+Carbendazim @ 0.2%
T6= Seed treatment and spray with Mancozeb @ 0.3%
T7= Control

Mancozeb, Iprodione, *T. viride* were treated to seeds and two sprays were given at 45 and 60 DAS. Propiconozole was sprayed thrice at 30, 45 and 60 DAS. Observations on growth parameters like plant height, number of leaves and stem girth were recorded at 60 DAS. Per cent disease severity was recorded at 50% of flowering stage. Yield of each treatment was calculated separately. The data were analyzed statistically.

Results and Discussion

In vitro evaluation of bioagents by dual culture technique

Six fungal and two bacterial bioagents were evaluated *in vitro* against *A. helianthi* applying dual culture technique and using Potato dextrose agar (PDA) as basal medium. Results (Table 2) revealed that all the bioagents evaluated exhibited fungistatic activity and significantly inhibited mycelial growth of *A. helianthi*. The least growth of the pathogen was observed in *T. viride* (0.66 cm) followed by *T. viride* strain 16 (0.93 cm) and *T. harzianum* (1.06 cm).Maximum inhibition of mycelial growth of *A. helianthi* was recorded in *T. viride* (85.33%), followed by *T. viride* strain 16 (79.33%), *T. harzianum* (76.44%), and the least inhibition (49.77%) of mycelial growth was noticed in *P. fluorescens*. Thus, all the biocontrol agents evaluated in vitro were found fungistatic against *A. helianthi*; the fungal bioagent was found effective than bacterial bioagent, for inhibition of test pathogen are in conformity to those reported earlier by several workers (Imtiaj and Lee, 2008; Vihol *et al.*, 2009; Ambuse *et al.*, 2009).

Table.1 *In vitro* evaluation of bioagents against *A. helianthi* by dual culture technique

Treatments	Radial growth of pathogen (mm)	Per cent inhibition over control (%)
Alternaria helianthi	45	
T. viride	6.6	85.33
***T. viride* 16**	9.3	79.33
***T. viride* 60**	16	64.44
T. harzianum	10.6	76.44
***T. harzianum* 2**	15.6	65.33
***T. harzianum* 55**	18.6	58.66
B. subtilis	21.2	52.88
P. fluorescens	22.6	49.77
S. Em±	0.16	2.03
C.D @ 1%	0.671	8.55

Table.2 *In vitro* evaluation of fungicides against *A. helianthi* by food poison technique

Sl.No.	Fungicides	Per cent inhibition (%)			
		Concentration (ppm)			
		50	100	250	500
1	Mancozeb	79.68	84.12	89.84	90.42
2	Iprodione+Carbendazim	88.94	75.23	88.18	89.75
3	Propiconazole	72.82	90.47	90.47	90.47
4	Chlorothalonil	65.20	69.52	78.15	88.51
5	Difenoconazole	58.86	61.39	62.03	68.19
6	Hexaconazole	49.84	51.75	56.95	62.15
S.Em±		1.2	0.79	1.01	1.8
C.D @ 1%		5.36	3.54	4.54	8.2

Table.3 Effect of seed treatment with *T. viride* and fungicides on growth and yield parameters of sunflower

Treatments		Plant height (cm)	No. of leaves	Stem girth (cm)	Seed yield (Kg ha^{-1})	Disease severity (%)
T1:	*T. viride* @ 1:0 dilution	173.15	24.65	2.15	1851.27	23.23
T2:	*T. viride* @ 1:1 dilution	170.45	22.75	1.95	1776.04	27.98
T3:	*T. viride* @ 1:2 dilution	164.05	21.95	1.88	1759.25	33.74
T4:	Propiconozole @ 0.1%	189.12	27.37	2.42	2181.71	9.85
T5:	Iprodione+Carbendazim @ 0.2%	180.40	24.65	2.19	1942.71	18.11
T6:	Mancozeb @ 0.3%	185.65	25.45	2.29	2160.30	13.91
T7:	Control	151.93	20.05	1.68	1472.80	46.03
S.Em		2.57	0.73	0.94	1.27	0.38
C.D @ 5%		7.99	2.27	2.92	3.78	0.53
CV (%)		20.9	11.6	12.9	14.54	11.7

In vitro evaluation of fungicides

Application of fungicides for the management of plant diseases in sunflower has become an inevitable method as no resistant cultivars for Alternaria blight are available. Among the six fungicides evaluated through food poison technique, the results revealed that Propiconozole and Mancozeb (50, 100, 250 and 500ppm) were effective. Iprodione+Carbendazim and Chlorothalonil were also found effective to some extent.

The results obtained in present studies in respect of in vitro effect of fungicides on mycelial growth inhibition of the test pathogen for the combination of SAAF, Azoxystrobin, Mancozeb, Propiconazole, Chlorothalonil and Hexaconazole fungicides effect is similar with earlier workers (Akbari and Parakhia, 2007; Mathivanan and Prabavathy, 2007; Dighule *et al.*, 2011).

Effect of seed treatment and spraying with different dilutions *T. viride* and fungicides on growth, yield parameters and disease severity of sunflower

The results presented in Table 3 revealed that all treatments were found significantly superior over the control. Among the different concentrations of *T. viride*, T_1 (seed treatment and spraying with *T. viride* at 1:0 dilution) recorded maximum stem girth of 2.15 cm, an yield of 1851.27kg/ha and recorded disease severity of 23.23 per cent. These results are in accordance with the results obtained by earlier research workers in sunflower (Arunakumara *et al.*, 2010; Reshu and Mahmud Khan, 2012).

Among the three fungicides evaluated, spraying with Propiconozole (0.1%) recorded highest yield of 2181.71kg/ha and a least disease severity of 9.85 per cent followed by T6 (seed treatment and spraying with Mancozeb)

13.91per cent and T5 (seed treatment and spraying with Iprodione+Carbendazim) 18.11 per cent. The highest disease severity (46.03 per cent) was recorded in control (T7). These results are in accordance with the results obtained by earlier research workers in sunflower (Murumkar *et al.*, 2007; Singh and Singh, 2007; Mesta *et al.*, 2011).

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