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Exploiting a combination of host plant resistance and *Trichoderma* species for the management of safflower wilt caused by *Fusarium oxysporum* f. sp. *carthami* Klisiewicz and Houston

R. D. PRASAD and K. ANJANI
Directorate of Oilseeds Research, Rajendranagar, Hyderabad -500 030, India.
E-mail: ravulapalliprasad@yahoo.com

ABSTRACT: In an integrated management approach against safflower wilt, *Trichoderma* species application to susceptible and moderately susceptible varieties of safflower was tried over two seasons in fusarial wilt affected plots. Though the disease incidence levels compared to a pathogen only check (96.7%) are significantly low in var. Nira with *T. viride* and *T. harzianum* seed applications (44.5 and 50.9 % respectively), the disease incidence in general is very high in bioagent treatments since the variety Nira is highly susceptible. *T. harzianum* soil application in var. A1 resulted in significantly low disease compared to pathogen check and fungicide treatments but comparable to soil application of bioagents over two seasons. In general, there was an increase in bioagents populations and reduction in *Fusarium* population in the rhizosphere. By adopting a moderately susceptible variety like A1 in place of susceptible varieties of safflower and application of *Trichoderma* species either to the soil or on the seed the wilt disease in safflower can be managed more efficiently.

KEY WORDS: *Fusarium oxysporum* f. sp. *carthami*, safflower wilt, *Trichoderma* species

INTRODUCTION

The wilt of safflower caused by *Fusarium oxysporum* f. sp. *carthami* Klisiewicz and Houston is a major soilborne disease in safflower particularly where safflower is cultivated without crop rotation. In India this disease was first reported during 1975 by Singh *et al*. The disease is widely distributed in safflower growing areas of Maharashtra state with an incidence of 25 to 40% (Nirmal *et al.*, 1989; Deokar *et al.*, 1997). In India it has appeared as a serious threat to safflower cultivation, destroying up to 25% of plants, amounting to considerable yield loss in the Gangetic valley (Chakrabarti, 1980). The disease caused yield losses ranging from 7.2 to 100% (Sastry *et al.*, 1994). Successful use of *Trichoderma* against fusarial diseases in many crop plants in India was reported (De *et al.*, 1996; Prasad *et al.*, 2002; Singh *et al.*, 2006). Many workers reported the effect of antagonists like *T. viride*, *T. harzianum*, *T. virens* and *Aspergillus fumigatus* against wilt disease in safflower mostly through laboratory and greenhouse tests (Chattopadhyay and Sastry, 1997; Gaikwad and Behere, 2001; Patibanda and Prasad, 2004). Many resistant lines have been identified from cultivated safflower germplasm lines and wild germplasm maintained at DOR, Hyderabad, and some exotic accessions from US and other countries. Considerable number of parents and advanced breeding lines of safflower with various levels of resistance to wilt have been identified through screening in wilt affected plots under All India Coordinated Research Project on Safflower (Anon, 2003 and 2004). Hybrids DSH-129, NARI-NH-1 and varieties A1, HUS-305, PBNS-12 and NARI-6 are moderately susceptible to this wilt disease. In the present study efforts were made to develop an integrated diseases management strategy against safflower wilt by exploiting host plant resistance and biological control.

MATERIALS AND METHODS

Field trials were conducted during *rabi* seasons of 2003-04 and 2004-05 in fusarial wilt affected plots of DOR, Rajendranagar, Hyderabad. The soil is light sandy loam with a pH of 6.2. Two fungal bioagents *T. harzianum* and *T. viride* found effective in greenhouse studies during previous year were further tested in wilt affected plots (pathogen concentration 2-3 x 10^4cfu/g of soil) during 2003-04. The highly susceptible variety Nira that had no seedling resistance and A1 which is moderately susceptible to wilt disease are selected to study the performance of two bioagents.

The biological control potential shown by *Trichoderma* spp. was further confirmed in a field trial during 2004-05 at DOR, Hyderabad. During the year, proliferation of *Trichoderma* and *F. oxysporum* f. sp. *carthami* was monitored at 30 days interval from the beginning.

Bioagents production and formulation

Trichoderma spp. were grown on molasses-soy medium (Prasad and Rangeshwaran, 2000) by inoculation of 2 ml of spore suspension from sporulating cultures on potato dextrose agar slants to 100 ml of medium in 250-ml Erlenmeyer flasks. Inoculated flasks were kept in shaking incubator for 3 days at 200rpm and allowed to sporulate in incubation room for 2 days. The five-day old inoculum was homogenized in a blender and mixed with talc powder (1:2 v/w). The powder formulation was air-dried by spreading as thin layer in a clean room for 2 days to reduce moisture to 8-10% and carboxymethyl cellulose (CMC) as sticker was added @ 5 g kg^{-1} formulation at the end. The colony forming units (cfu) were determined by plating serial dilutions of homogenized suspensions on *Trichoderma* specific medium.

Application of Bioagents

Bioagents were applied as seed treatment @ 10 g kg^{-1} seed and as soil application by mixing powder formulation of bioagents (3 x10^8 cfu/g) @ 2.0 kg in 2.5t farm yard manure (FYM)/ha. All plots other than soil treatment also uniformly received FYM. Each treatment was replicated three times in a randomized block design (RBD) with a plot size of 5.4 x 4 m and 45 x 20cm spacing. In one treatment fungicide carbendazim 50WP was applied at 2 g kg^{-1} as it was one of the local recommended measures for the control of the disease. Plots applied with FYM alone served as pathogen check.

Recording of data and statistical analysis

The disease incidence was recorded periodically at 30 days interval and final disease incidence was recorded at 100 days after sowing. During the 2004-05 field trial, pathogen and bioagent populations were recorded in experimental plots by collecting soil samples from rhizosphere zone at 30 day intervals by plating on selective media. The data on disease incidence, pathogen and bioagent populations and yield were subjected to ANOVA after necessary transformations, and means were compared by critical difference at $p=0.05$.

RESULTS AND DISCUSSION

During the 2003-04 field trial in variety Nira, seed treatment with *T. harzianum* and *T. viride* resulted in significantly less disease i.e. 21 and 14% respectively compared to bioagent soil application treatments, fungicide treatment and pathogen check at 35 days after sowing (Table 1). Since in seed treatment, *Trichoderma* is immediately available for the germinating seeds and roots to get protection against the wilt pathogen, a highly susceptible variety also showed good plant stand. Seed treatment with *T. viride* has given significantly less disease incidence compared to *T. harzianum* seed treatment. At 35 days in variety Nira, soil application of *T. harzianum* and *T. viride* resulted in a wilt incidence of 58.5% and 65.6%. Even carbendazim

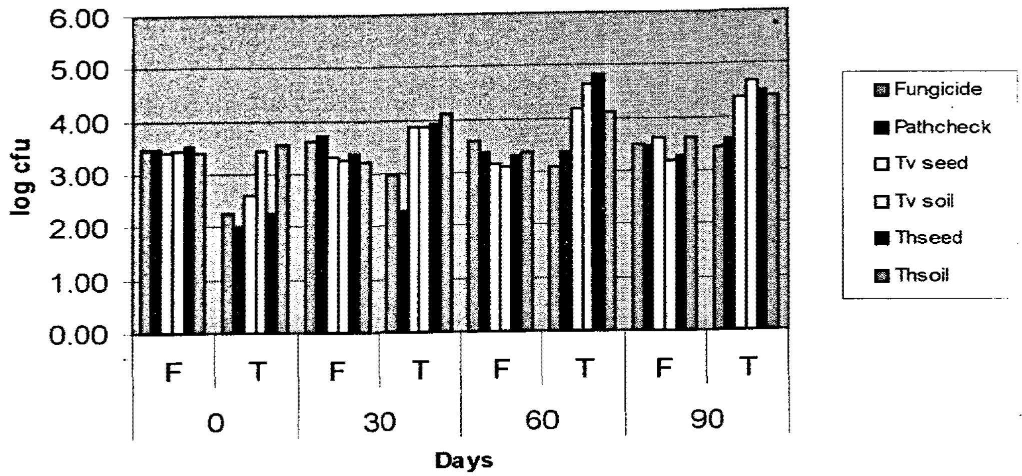
Table 1. Effect of biological control agents on wilt incidence and seed yield of safflower (2003-04)

Treatments	Disease incidence (%)				Seed Yield (kg/ha)	
	35 days		100 days		Nira	A1
	Nira	A1	Nira	A1		
T. harzianum seed treatment (@10g/kg)	21.0 (27.1)	4.6 (10.3)	50.9 (48.7)	22.6 (28.3)	121	667
T. harzianum soil application (@2 kg/2.5t FYM /ha)	58.5 (53.1)	5.2 (10.4)	83.3 (65.8)	16.6 (23.9)	18	849
T. viride seed treatment (@10g/kg)	14.0 (24.5)	2.5 (5.6)	44.5 (45.6)	12.9 (20.9)	318	461
T. viride soil application (@ 2 kg/2.5t FYM /ha)	65.6 (55.8)	2.9 (8.1)	84.2 (66.7)	9.6 (17.9)	8	521
Carbendazim seed treatment (@2g/kg)	60.5 (54.1)	0.6 (3.0)	87.9 (69.9)	40.4 (39.5)	33	276
Pathogen check	88.2 (64.5)	9.6 (18.0)	96.7 (81.5)	39.4 (38.8)	10	320
CD (P=0.05)						
Variety	(3.5)		(2.2)		26.9	
Treatments	(6.0)		(3.8)		46.7	
Variety x Treatments	(8.6)		(5.4)		66.8	

*Values in the parentheses are angular transformations

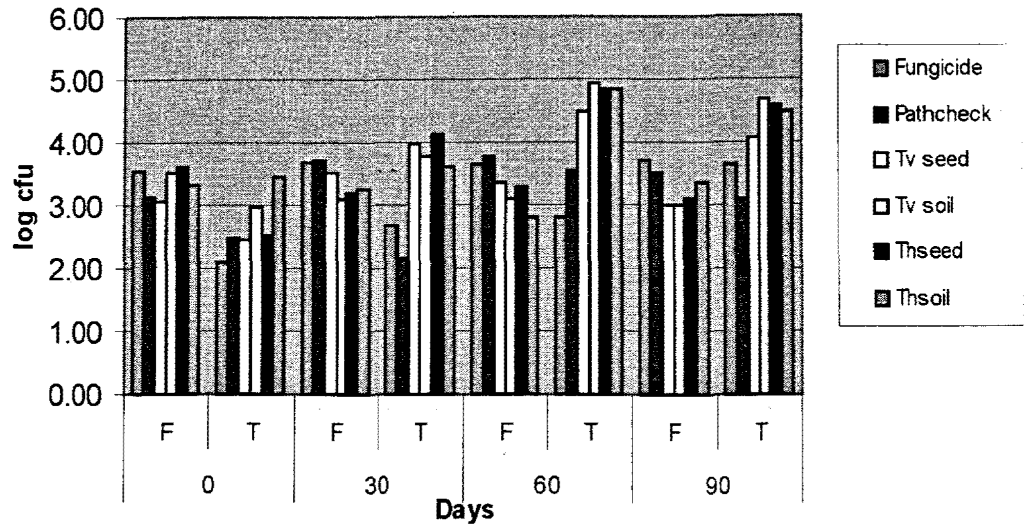
Table 2. Effect of *Trichoderma* species on safflower wilt incidence, pathogen population and seed yield (2004-05)

Treatments	Disease incidence (%)*		*Fusarium* Population at 60DAS (log cfu)		*Trichoderm* population at 60DAS(log cfu)		Seed Yield (kg/ha)	
	Nira	A1	Nira	A1	Nira	A1	Nira	A1
Carbendazim seed treatment (@2g/kg)	88.2 (69.9)	37.5 (37.7)	3.51	3.71	3.43	3.69	69	319
Pathogen Check	94.1 (75.8)	38.1 (38.0)	3.46	3.51	3.59	3.10	40	357
T. viride seed treatment @ 10g/kg	58.1 (49.6)	25.2 (30.1)	3.44	3.00	4.36	4.07	339	461
T. viride soil application @ 2kg in 2.5t FYM/ha	80.1 (63.4)	22.6 (28.4)	3.20	3.00	4.66	4.67	83	576
*T. harzianum*seed treatment @ 10g/kg	64.7 (53.6)	26.9 (31.2)	3.28	3.10	4.50	4.59	267	515
*T. harzianum*soil application @ 2kg in 2.5t FYM/ha	85.3 (67.3)	22.1 (27.9)	3.44	3.36	4.40	4.47	175	588
CD (P = 0.05)								
Variety	5.9	0.66	0.62	63.6				
Treatments	4.8	0.06	0.51	25.0				
Interaction	8.4	0.94	0.88	43.3				



F-*Fusarium*, T-*Trichoderma*, Tv-*T. viride*, Th-*T. harzianum*

Fig. 1. ***Fusarium*** **and** ***Trichoderma*** **populations in variety Nira treated with seed and soil application of** ***Trichoderma*** **sp.**



F-*Fusarium* T-*Trichoderma*, Tv-*T. viride*, Th-*T. harzianum*

Fig. 2. ***Fusarium*** **and** ***Trichoderma*** **populations in variety A1 treated with seed and soil application of** ***Trichoderma*** **sp.**

seed treatment was unable to protect Nira from wilt, which had a disease incidence of 60.5%. At 35 days in variety A1, all treatments recorded significantly less disease incidence compared to same treatments with Nira since A1 was not susceptible at seedling stage. In variety A1, pathogen check resulted in 9.6% wilt incidence which was significantly higher as compared to bioagent and fungicide treatments. At 100 days *T. viride* seed treatment in Nira showed the least disease incidence (44.5%) and *T. harzianum* seed treatment recorded a wilt incidence of 50.9%. Bioagent soil application treatments, fungicide and pathogen check have recorded more than 80% wilt incidence. In variety A1, *T. viride* soil application resulted in the least wilt incidence (9.6%), followed by *T. viride* seed treatment (12.9%). *T. harzianum* seed and soil application treatments with this variety resulted in 22.6 and 16.6% wilt incidence. Fungicide seed treatment and pathogen check recorded a disease incidence of 40.4% and 39.4%, respectively.

In Variety Nira, *T. viride* seed treatment recorded the highest yield (318 kg/ha) compared to other bioagent treatments, fungicide treatment and pathogen check with same variety (Table 1). In variety A1, *T. harzianum* soil application and seed treatment recorded a yield of 849 and 669 kg ha^{-1} respectively, which were significantly higher as compared to all other treatments. There were no significant differences in yields of *T. viride* seed and soil application treatments. Fungicide treatment and pathogen check recorded lowest yields of 276 and 320 kg ha^{-1} respectively. From this study it can be concluded that with a highly seedling susceptible variety like Nira bioagent seed treatments were found effective whereas in the case of A1, which has at seedling storage resistance to wilt, bioagent soil applications resulted in significant but smaller improvements in disease reduction and higher yield.

During 2004-05 in variety Nira, seed treatment with *T. viride* and *T. harzianum* resulted in significantly less disease i.e 58.7 and 67.4% respectively, compared to bioagent soil application treatments, fungicide treatment and pathogen check at 100 days after sowing (Table 2). Seed treatment with *T. viride* resulted in significantly less disease incidence compared to *T. harzianum* seed treatment. *T. harzianum* and *T. viride* soil application treatments recorded a wilt incidence above 80% disease incidence. Even carbendazim seed treatment was not able to protect Nira from wilt, which resulted in a disease incidence of 88.2%. In variety A1, *T. harziaum* soil application resulted in low wilt incidence (22.1%), followed by *T. viride* soil treatment (22.6%). *T. harzianum* seed application treatments resulted in 26.9% wilt incidence. The bioagent treatments did not differ significantly in their performance with this variety but these treatments were significantly superior to fungicide treatment and check. Similarly *T. harzianum* soil application resulted in low safflower wilt incidence and higher seed yield in earlier studies (Anon, 2001). In another field investigation, carbendazim tolerant mutant of *T. viride* and carbendazim seed treatment resulted in higher seed yield and low safflower wilt incidence (Chattopadhyay and Sastry, 1997). There was a significant increase in bioagents population up to 60 days with corresponding decrease in pathogen population (Table 2, Figs. 1 & 2) which was reflected in reduced disease incidence in the present investigation.

In variety Nira, *T. viride* seed treatment resulted in the highest yield (339kg ha^{-1}) compared to other treatments (Table 2). In variety A1, *T. harzianum* soil application and seed treatments resulted in a yield of 588 and 576 kg ha^{-1}, respectively, which were significantly high compared to all other treatments. Though disease incidence levels are very high, susceptible variety like Nira responded better to bioagent seed treatments whereas in the case of A1, which has resistance to wilt, soil and seed applications of bioagents were equally effective. By adopting a moderately susceptible variety like A1 in place of susceptible varieties of safflower and application of *Trichoderma* species either to the soil or on the seed, the wilt disease can be managed more efficiently.

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