RESEARCH ARTICLE

Field evaluation of talc formulation of *Trichoderma* for the management of Fusarium wilt in watermelon

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ABSTRACT: Wilt of watermelon caused by *Fusarium oxysporum* f.sp. *niveum* is one of the major constraints in watermelon production in coastal regions. In this study, twenty seven isolates of *Trichoderma* were evaluated for their ability to inhibit the pathogen growth. Talc formulations of four selected *Trichoderma* isolates were evaluated in the field. Incidence of wilt in the biocontrol treatments was less than 10 per cent in trial 1 and less than seven percent in trial 2 and less than four per cent in trial 3 during 2012-13 while control plot recorded 21 to 34 per cent incidence. During 2013-14, incidence of wilt was less than seven per cent in trial 1 and less than 15 percent in trial 2 in the biocontrol treatments while control plot recorded 19 and 28 per cent incidence. From the pooled data of all the five trials it was observed that 70 to 80 per cent reduction of wilt and 27 to 30 per cent increase in number of fruits in biocontrol treated plots over untreated control. From the study, it is concluded that seedling drench with talc formulation of *Trichoderma* reduced Fusarium wilt and increased yield in watermelon.

Keywords: *Fusarium oxysporum*, Fusarium wilt, *Trichoderma*, watermelon

Fusarium wilt is one of the most important diseases of watermelon and is caused by *Fusarium oxysporum* f.sp. *niveum*. Fusarium wilt of watermelon has been reported in all countries where watermelon is cultivated (Egel and Martyn, 2013). Yield losses due to Fusarium wilt has increased (Everts and Himmelstein, 2015) and incidence was higher in the fields where cultivation was taken in successive years (Wu *et al.*, 2013). In India, watermelon wilt was first reported in 1955 in Thane district of Mumbai and the causal agent was identified as *F. oxysporum* f.sp. *niveum* (Hegde and Bhide, 1958). In Coastal regions of Goa, watermelon is cultivated in rice fallows during *rabi* season. Farmers use local non-descript varieties and improved hybrids. In both the cases incidence of Fusarium wilt was reported; yield and economic loss is high. Early reports on survival of *F. oxysporum* f.sp. *niveum* in soil indicated that the fungus persisted for 16 years (Martyn, 2014) primarily as thick-walled chlamydospores.

Crop rotation has been traditionally practiced to manage wilt in watermelon. Rotation with non-host crops whose root exudates do not stimulate the spore germination might suppress *F. oxysporum* f.sp. *niveum* (Everts and Himmelstein, 2015). Crop rotation with non-cucurbitaceous crops reduced the population of *F. oxysporum* f.sp. *niveum* (Naik and Sen, 2002). Fallowing also reduced the population of this fungus (Wu *et al.*, 2013). Grafting on non-host cucurbits has been successful in controlling wilt of watermelon in many countries (Davis *et al.*, 2008; King *et al.*, 2008) and

grafting reported to be effective in eliminating Fusarium wilt (Keinath and Hassell, 2014).

Use of chemicals for management of Fusarium wilt had been mainly restricted to the fumigant, Methyl bromide (MeBr) (King *et al.*, 2008) and to a lesser extent 1, 3-dichloropropene and metam sodium. However, MeBr use has been discontinued due to environment and other regulatory concerns. Efforts have sought non-fumigant alternatives to MeBr use (Antoniou *et al.*, 2014). Alternative soil fumigants are not as effective as MeBr at controlling wilt. They are expensive and also negatively impact environment (Sande *et al.*, 2011). Application of fungicides *viz.* prothioconazole, acibenzolar-S-methyl, and thiophanate-methyl had resulted into less incidence of Fusarium wilt of watermelon (Everts *et al.*, 2014).

Biological control is one of the promising, effective and eco-friendly methods of control of Fusarium wilt. Antagonists (Fravel *et al.*, 2005) as well as compost amendments (Boulter-Bitzer *et al.*, 2006) have been used but with inconsistent results. Several organisms such as *Penicillium oxalicum* (De Cal *et al.*, 2009), *Paenibacillus polymyxa* (Wu *et al.*, 2009), *Pseudomonas fluorescens*, *P. chlororaphis* (Tziros *et al.*, 2007) and species of *Trichoderma* (Pandey and Gupta, 2012) have been evaluated for Fusarium wilt suppression. *Trichoderma* species are soil inhabitants, competitive saprophytes, and facultative mycoparasites that can colonize the soil and rhizosphere (Harman *et al.*, 2004). *Trichoderma* spp. are the most widely studied biological control agents for soil borne diseases and are also applied in post-harvest disease management (Woo *et al.*, 2014).

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Fig. 1. Wilting of watermelon plant (A: complete wilt; B: partial wilt)

In some countries, watermelon seeds were treated with biocontrol agents and the seedlings were also applied with biocontrol agents during transplanting (Tziros *et al.*, 2007). However in India, the seeds were directly sown in the field, hence we tried the seedling drench instead of seedling dip. The aim of the present study was to assess the antagonistic features of isolates of *Trichoderma* and their efficacy in controlling Fusarium wilt in watermelon, in order to design an effective biological control strategy.

MATERIALS AND METHODS

Isolation of *F. o. f.sp. niveum*

F. oxysporum f. sp. *niveum* was isolated from infected watermelon plants (Augusta-Hybrid from Syngenta and local non-descript type), grown in an open field. The first visible symptom of Fusarium wilt in watermelon is partial wilting of the plant i.e. one side of a plant wilts (Fig. 1). Roots of the infected plant are white, but the vascular tissue is brown and discolored (Fig. 2). *F. oxysporum* f. sp. *niveum* was consistently isolated from the base of the plant/ roots on potato dextrose agar (PDA) medium and cultures were routinely grown on PDA. *Fusarium* was identified morphologically as *F. oxysporum* by the thick walled chlamydospore production and colonies forming microconidia on short monophialides. The susceptible local variety was inoculated with single spore cultures and all plants developed typical Fusarium wilt, often one sided and the *Fusarium* was re-isolated. Pathogenicity tests demonstrated that the *F. oxysporum* fungus caused wilt on inoculated watermelon plants, and so by definition is *F. oxysporum* f.sp. *niveum*. Inoculated plants wilted in eight to 30 days after inoculation.

Fig. 2. Browning of vascular tissues in the wilt affected watermelon root

Screening of *Trichoderma* isolates for the inhibition of *F. o. f.sp. niveum*

Antagonistic properties of 27 selected isolates of *Trichoderma* (Table 1) were tested on PDA plates using a dual culture technique. Fifteen isolates were obtained from ICAR-IISR, Kozhikode under the PhytoFuRa project and 10 isolates were obtained from ICAR-PDBC (Now NBAIR), Bengaluru. Five days old mycelial discs (7mm) of the pathogen was placed on one side of the Petri dish and a disc of *Trichoderma* growth (7mm dia) of four days old was placed on the opposite side of the plate and incubated at 28 ± 2 °C for 4 days for initial observation. Final observation on antagonism was recorded after 10 days of incubation. Diameter of *Trichoderma* growth and pathogen growth were recorded. Further, inhibition zone created by *Trichoderma* was also recorded.

Field evaluation of talc formulation of promising *Trichoderma* isolates

Based on the *in vitro* evaluation, four strains of *Trichoderma* (Pf2, Pf3, TA23 and Tv1) were selected for field evaluation for the management of wilt in watermelon. Talc based formulation of the *Trichoderma* was prepared according to the standard method (Ramesh and Korikanthimath, 2006). Population of *Trichoderma* at the time of application was about 10^7 CFU g⁻¹.

One week-old watermelon plants were treated by drenching the water suspension of biocontrol formulation @ 1.25g per plant. Carbendazim was used @ 0.1 g per plant. During 2012-13, three field trials were conducted; two trials with Augusta hybrid and one trial with local variety. During 2013-14, two trials were conducted using Augusta hybrid. All experiments were conducted in RBD with 4 replications. Mean plot size was 32 m² and twenty plants per replication were maintained with 1x 1m spacing. Standard crop management practices were followed in all the trials. Incidence of wilt was recorded periodically and number of fruits per plot was recorded.

RESULTS

Screening of *Trichoderma* isolates for the inhibition of *F. o. f.sp. niveum*

Twenty seven *Trichoderma* isolates were used in the study. *In vitro* screening indicated that, most of the

Table 1. Details of *Trichoderma* isolates used and their *in vitro* inhibitory potential against *F. oxysporum* f.sp. *niveum*

Isolates	Source	Growth of FON (cm)	% inhibition of FON over control
PhytoFuRa 1 (Pf1)	ICAR-IISR, Kozhikode	3.0	66.7
PhytoFuRa 2 (Pf2)	ICAR-IISR, Kozhikode	1.2	86.7
PhytoFuRa 3 (Pf3)	ICAR-IISR, Kozhikode	0.6	93.3
PhytoFuRa 4 (Pf4)	ICAR-IISR, Kozhikode	2.1	76.3
PhytoFuRa 5 (Pf5)	ICAR-IISR, Kozhikode	1.0	88.5
PhytoFuRa 6 (Pf6)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 7 (Pf7)	ICAR-IISR, Kozhikode	3.4	62.6
PhytoFuRa 8 (Pf8)	ICAR-IISR, Kozhikode	2.1	77.0
PhytoFuRa 9 (Pf9)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 10 (Pf10)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 11 (Pf11)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 12 (Pf12)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 13 (Pf13)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 14 (Pf14)	ICAR-IISR, Kozhikode	0.0	100.0
PhytoFuRa 15 (Pf15)	ICAR-IISR, Kozhikode	2.5	72.6
NABII-TA10	ICAR-NBAIR, Bengaluru	0.0	100.0
NABII-TA23	ICAR-NBAIR, Bengaluru	0.0	100.0
NABII-TH10	ICAR-NBAIR, Bengaluru	0.9	90.4
NABII-TH07	ICAR-NBAIR, Bengaluru	1.5	83.0
NABII-TH19	ICAR-NBAIR, Bengaluru	0.8	91.5
NABII-TH09	ICAR-NBAIR, Bengaluru	1.3	85.2
NABII-TVS05	ICAR-NBAIR, Bengaluru	1.1	87.4
NABII-TVS08	ICAR-NBAIR, Bengaluru	0.0	100.0
NABII-TVS12	ICAR-NBAIR, Bengaluru	1.2	87.0
NABII-TH16	ICAR-NBAIR, Bengaluru	1.2	86.7
TV1	ICAR-CCARI, Goa	0.0	100.0
TV2	ICAR-CCARI, Goa	1.8	79.6
Control		9.0	0.0
CD (0.05)		0.182	-
CD (0.01)		0.243	-

*Mean of three replications. FON: *F. oxysporum* f.sp. *niveum*

isolates inhibited the pathogen growth (Table 1). Results revealed that out of 27 *Trichoderma* cultures seven isolates viz. Pf 2, Pf3, Pf 10, Pf 13, TA 23, TH-10, TH-7, TVS 08 and Tv1 effectively inhibited pathogen growth in repeated assays. Based on the fast growth, sporulation and parasitism of the pathogen four isolates viz. Pf2, Pf3, TA-23 and Tv1 were selected for making formulation and testing in the field.

Field evaluation of talc formulation of promising *Trichoderma* isolates

Results of field trials during 2012-13 indicated that biocontrol treatments reduced the incidence of wilt and increased the number of fruits. Incidence of wilt in the biocontrol treatments is less than 10 per cent in trial 1 and less than 7 percent in trial 2 and less than 4 per cent in trial 3. Plants treated with Pf2 recorded the least wilt incidence (5.35%, 3.88%, and 1.39%) followed by Tv1 treatment (6.26%, 4.88% and 2.08%) in all the trials. Wilt

incidence in biocontrol treatments was significantly less compared to control plot which recorded 21-34% incidence. Number of fruits per plot in biocontrol treatments ranged between 42-46 in trial 1; 53-59 in trial 2 and 72-76 in trial 3. Higher number of fruits per plot was recorded in Pf2 treatment followed by Tv1 treatment (Table 2). Per cent reduction of wilt was 76-84% and per cent increase in number of fruits was 26-30% based on consolidated data of all the three trials during 2012-13.

Results of field trials during 2013-14 also indicated a similar trend. Incidence of wilt in the biocontrol treatments is less than seven per cent in trial 1 and less than 15 percent in trial 2. Plants treated with Pf2 and Pf3 recorded the least wilt incidence in trial 1 (2.08%) and trial 2 (10.94%) respectively. Wilt incidence in biocontrol treatments was significantly less compared to control plot which recorded 19-28% incidence. Number of fruits per plot in biocontrol treatments ranged between 47-52 in trial1 and 20-25 in trial 2. Higher number of fruits was observed in TA23 (trial 1) and Pf3 (trial 2) treatments

Table 2. Effect of application of talc formulation of *Trichoderma* on wilt and yield in watermelon during 2012-13

	Trial 1		Trial 2		Trial 3	
	% wilt	No. of fruits/ plot	% wilt	No. of fruits/ plot	% wilt	No. of fruits/ plot
Tv1	6.26	44.50	4.88	57.50	2.08	75.50
TA23	9.69	42.50	6.45	53.25	3.46	74.00
Pf2	5.35	46.25	3.88	59.50	1.39	76.50
Pf3	7.66	42.50	6.03	55.00	2.39	73.00
Carbendazim	11.71	40.00	9.14	51.50	3.75	72.00
Control	34.36	26.75	27.95	39.75	21.33	57.50
CD (0.05)	5.78	6.19	10.17	5.50	9.48	5.94

Table 3. Effect of application of talc formulation of *Trichoderma* on wilt and yield in watermelon during 2013-14

	Trial 1		Trial 2	
	% wilt	No. of fruits/ plot	% wilt	No. of fruits/ plot
Tv1	6.25	47.5	12.50	22.25
TA23	5.21	52.25	14.06	20.50
Pf2	2.08	47.25	12.50	25.00
Pf3	4.17	47.5	10.94	25.25
Carbendazim	6.25	45.75	20.31	17.50
Control	19.79	37.25	28.13	15.50
CD (0.05)	10.15	NS	9.62	6.59

(Table 3). Per cent reduction of wilt was 59-70% and per cent increase in number of fruits was 24-27% based on consolidated data of both the trials during 2013-14.

Data from all the five trials were combined and the pooled mean analysis indicated that, the wilt incidence in biocontrol treated plots was less than 8 per cent (5.04 to 7.78%), whereas in control it was 26.31 per cent. The number of fruits from an average 32 sq.m biocontrol treated plots ranged from 48.50 to 50.90 and it was only 35.35 in the control plot. Per cent reduction of wilt in biocontrol treated plots was 70-80 per cent and per cent increase in number of fruits was 27-30 per cent based on consolidated data of all the five trials (Table 4).

DISCUSSION

The fungus that causes Fusarium wilt in watermelon is very specific and cannot infect even closely related plants

such as cucumber and muskmelon. *F. oxysporum* f.sp. *niveum* forms resilient spores that can remain viable in the soil for many years. That means Fusarium wilt may appear in a field that may not have had a suitable host for many seasons and hence crop rotation does not eliminate the problem. Soil solarization has not provided reliable results (Cebolla *et al.*, 2000) and use of MeBr was discontinued as it is harmful to the environment (Song *et al.*, 2004). Grafting on resistant rootstocks (Miguel *et al.*, 2004) and growing of resistant varieties provides some degree of protection from Fusarium wilt, but currently commercially acceptable cultivars with adequate resistance to *F. oxysporum* f.sp. *niveum* is not available. Application of fungicides viz. prothioconazole, acibenzolar-S-methyl, and thiophanate-methyl had resulted less incidence of Fusarium wilt of watermelon (Everts *et al.*, 2014). There is a need for alternative control practices for watermelon wilt and biological control is one of the potential alternatives.

Application of conidial suspension of *Penicillium oxalicum* reduced the incidence of Fusarium wilt in watermelon and melon (De Cal *et al.*, 2009). In our field experiments, application of *Trichoderma* reduced the incidence of wilt and the difference among the isolates was not significant (Table 2 and 3). All the biocontrol treatments are at par with the chemical fungicide. Wu *et al.* (2009) reported that a bio-organic fertilizer containing *Paenibacillus polymyxa* and *Trichoderma harzianum* reduced Fusarium wilt of watermelon. Role of elevated activities of defense related enzymes as a result of systemic acquired resistance was attributed as mechanism of action for this disease suppression activity. Though we have not studied the mechanism of disease

Table 4. Effect of application of talc formulation of *Trichoderma* on wilt and yield in watermelon -pooled data of two years comprising five trials

Treatments	% wilt	No. of fruits/ plot (32 sq.m)	No. of fruits/ha	*Total income (Rs)	% reduction of wilt	% increase in no. of fruits
Tv1	6.39	49.45	15453	463594	75.70	28.51
TA23	7.78	48.50	15156	454688	70.45	27.11
Pf2	5.04	50.90	15906	477188	80.84	30.55
Pf3	6.24	48.65	15203	456094	76.30	27.34
Carbendazim	10.23	45.35	14172	425156	61.10	22.05
Control	26.31	35.35	11047	331406	0.00	0.00

*An average price of Rs. 30/ fruit is considered for income calculation

control, we observed higher hyperparasitism and lysis zones due to secretion of antibiotics in the *in vitro* assays. *Trichoderma* is known to be an aggressive parasite on host fungi and secrete many antimicrobial compounds (Harman *et al.*, 2004). There are also several reports indicating that *Fusarium* disease control can be improved using combinations of biocontrol organisms (Rajendran *et al.*, 2014).

Seed treatment is an attractive method for applying biocontrol agents, as other methods require larger amounts of propagules. However, the survival of the biocontrol agent in the soil after the application is essential for the efficient control of the pathogen. Hence it would be advisable to apply the biocontrol agents during the initial stages of growth and follow it up one more time if necessary. Higher disease reduction in the biocontrol treatments in this study may be due to the higher population applied in the rhizosphere soil as seedlings were drenched. Change in the microbial communities in rhizosphere soil was attributed in suppression of *Fusarium* wilt in watermelon where it was intercropped with aerobic rice (Ren *et al.*, 2008). Despite the evidence that some disease reductions can be achieved with biological products, commercial use remains very low (Fravel *et al.*, 2005). Improving stability of the biological organisms in the intended environment and understanding causes of the occasional failure are areas of future research to develop effective disease management strategies.

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