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Research Article

Survey, identification and management of Fusarium wilt of banana in Tamirabarani tract of Southern districts of Tamil Nadu

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ABSTRACT: In Tamil Nadu state, Thoothukudi district ranked second in banana production and especially the Tamirabarani tract of Thoothukudi and Tirunelveli districts are known for the production of various banana cultivars like Rasthali, Andhra Kozhi, Karpooravalli, Neypoovan, Poovan, Peyan and Chakkai. Among these varieties, cultivation of Rasthali faces 50-60% yield loss due to the incidence of Fusarium wilt disease. As the farmers have been practising ratooning for 6-7 year continuously which enhances the inoculum load of the pathogen and make the plantains succumb to *Fusarium oxysporum* f. sp. *cubense* (Foc). Studies carried out on different treatments in the management of Fusarium wilt of banana, *Trichoderma* sp. Tsp1(ALG) showed a maximum reduction of mycelial growth of Foc (72.18%) over control which was on par with *Pseudomonas fluorescence* 1(Pf1). Under pot culture conditions the combination of three treatments, Tsp1(ALG)+Pf1+ neem cake excelled and showed an 80% reduction of wilt incidence. A multifaceted approach comprising the application of biocontrol agents (*Trichoderma* sp.) + *Pseudomonas* sp. 1 (TNAU) and organic amendments was found to be the best management practice in containing the wilt.

KEY WORDS: Banana, fusarium wilt, internal transcribed spacer, *Trichoderma* sp.

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INTRODUCTION

Banana is the fourth most important crop after rice, wheat and maize based on the gross value production in the world. Among many fungal, bacterial and viral diseases the Panama wilt/ Fusarium wilt is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) is the most devastating disease. The pathogen originated from Southeast Asia (Ploetz, 2006) and it was first discovered by Bancroft at Eagle Farm, near Brisbane, Australia during 1874 in Silk group (AAB) banana plants (Bancroft, 1876). The *Fusarium* wilt pathogen is reported in all banana-growing regions of the world such as Asia, Africa, Australia and Tropical America (Ploetz, 2000). In India, Panama disease is caused by *Fusarium oxysporum* f. sp. *cubense* race 1 strain, resulting in yield losses of 50-70% and several varieties such as Rasthali, Amirtapani, Karpooravalli, Monthan, Ney Poovan, and Virupakshi are affected by this race (Ghag, 2019). The fungus enters the plant system via roots and colonizes the vascular tissues, blocking the water and nutrient transport that leads to the yellowing of older leaves followed by the breaking of petioles and hanging down of leaves around the pseudostem. In severe cases, longitudinal splitting can be also observed on the pseudostem. Distinguishing internal symptom observed in corm is light yellow to dark brown vascular discolouration (Yin *et al.*, 2011). To control soil-borne pathogens, fungicidal management is necessary but not practical due to huge cost, health issues, environmental hazards and residual toxicity. Biocontrol agents suppress pathogenic organisms through competition with the pathogen and stimulate the growth of the plant so that the plants quickly outgrow the pathogenic effect (Cook, 2000).

MATERIALS AND METHODS

Survey for Fusarium wilt incidence

Infected rhizome samples were collected from different banana-growing villages such as Pakkapatty, Agaram, Kongarayakurichi, Tiruchendur, Palayamkottai and Alangulam. During the survey, the total no of plants and wilt-infected plants were taken into account to calculate the per cent disease incidence using the following formula.

$$\text{Per cent Disease Incidence} = \frac{\text{No. of wilted plants X 100}}{\text{Total no. of plants observed}}$$

Pathogenicity tests of Foc isolates

All six isolates of Foc were multiplied on sand maize medium separately for pathogenicity tests in the banana cultivar Rasthali (Silk-AAB) as per the method described by Riker and Riker (1936).

Sand and maize powder were mixed at the ratio of 19:1 (sand-1900 g and maize powder 100g) to prepare sand maize medium and autoclaved at 1.4 kg/cm^2 for 2 h for consecutive days. The sterilized sand maize medium was inoculated with actively growing Foccultures separately and incubated at room temperature (28±2ºC) for three weeks and the well-grown pathogen inoculum was used for the pathogenicity study. When the plantains attained 2nd month the sand maize inoculum was applied and severity was assessed from 5th month onwards. The severity of wilt incidence was assessed based on the 1-4 grade proposed by Mohamed *et al.* (2001).

ITS sequencing of Foc2

PCR reactions were performed in a total volume of 50 µl of each sample using Emerald Amp® GT PCR master mix using genomic DNA of Foc2 as a template. The intermediate 5.8S ribosomal gene along with ITS1 and ITS2 regions were amplified using the ITS1 and ITS4 primers with PCR conditions of initial denaturing at 94ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 30 sec. Annealing at 59ºC for 30 sec and extension at 70ºC for 2 min and final extension at 72ºC for 7 min. The reactions were carried out in Eppendorf tube master cycle gradient PCR machine. The PCR products were resolved by electrophoresis in 1% agarose gel and purified by using FavorPrep GEL/PCR purification kit and sequenced at Eurofins genomics company, Bangalore, India.

The primers used for amplification of ITS region were,

ITS 1-5’ TCCGTAGGTGAACCTGCGG 3’ (forward primer)

ITS 4-5’ TCCTCCGCTTATTGATATGC 3’ (reverse primer)

Sequencing of ITS and Identification of Foc2 by Bioinformatic analysis

The obtained DNA sequences were trimmed at 5’ and 3’ regions where the sequence chromatogram was not clear. Then DNA sequence, in which a clear chromatogram was obtained was made in Fasta format. This was used as an input sequence (Query sequence) in the nucleotide blast analysis program at NCBI database. The output data retrieved from bioinformatics were analyzed and the organism showing a major score was considered a closely related species to identify the test fungus used in this study. The sequence was submitted and got published in the NCBI domain.

Effect of biocontrol agents and endophytes on the growth of Foc under *invitro* condition by dual culture technique

Two isolates of *Trichoderma* spp. Tsp1 (ALG) and Tsp2 (PKP) and one isolate of *Pseudomonas* sp. Psp1 (KKM) were isolated from rhizosphere soil of banana from Thoothukudi and Tirunelveli districts of Tamil Nadu and another two endophytic bacteria Endo1 (KKM) and Endo2 (KKM) isolated from neem and tulsi leaves from Killikulam. The standard biocontrol agents such as Tv1 (*Trichoderma asperellum* 1), Pf1 (*Pseudomonas fluorescens*) and *Bacillus subtilis* were received from the department of Plant Pathology, Agricultural College and Research Institute, Killikulam. These antagonists were tested against Foc by dual plate technique (Dennis and Webster, 1971).

Efficacy of oil cake extracts against Foc growth

The efficacy of each oil cake extract was evaluated against Foc by poisoned food technique (Schmitz, 1930). Each oil cake extract was taken at the rate of 5ml and 10 ml and mixed with 95 and 90 ml of PDA medium separately to obtain 5% and 10% concentrations. The PDA medium along with the oil cake extract was sterilized in an autoclave. In each Petri plate, 20 ml of sterilized PDA medium was poured and allowed to solidify. Seven days old actively growing mycelial disc of Focwas taken from pure culture and inoculated at the centre of each Petri plate and incubated at room temperature. The Petri plate containing PDA medium without any extracts of oil cakes served as control. The mycelial growth (cm) of the test pathogen was measured in all treatments after incubation of 7 days.

Efficacy of fungicides against the growth of Foc

Seven different fungicides namely Carbendazim (50%WP), Propiconazole (25% EC), Tebuconazole (50% EC), Hexaconazole (5% EC), Azoxystrobin (23% EC), Copper oxychloride (50% WP), and Tebuconazole (50%) + Trifloxystrobin (25%) WG were evaluated against the *Foc* at different concentrations at 250, 500 and 1000 ppm. The required quantity of different fungicidal solutions was prepared and added to the 100 ml of sterilized PDA medium and thoroughly mixed. This poisoned PDA medium was distributed into the sterilized Petri plates @ 20 ml per plate. Seven days old actively growing mycelial disc of Foc was

cut by using a sterilized cork borer and placed at the centre of each Petri plate. The PDA medium without fungicidal solutions and inoculated with Foc served as control and all the plates were incubated at room temperature (28±2ºC) for 7 days. Per cent inhibition of mycelial growth over control was calculated by using the following formula (Yadav *et al.*, 2014).

$$I = \frac{100\ (C-T)}{C}$$

Where,

I = Per cent inhibition over control

C = Growth in control

T = Growth in treatment

Effect biocontrol agents, organic amendments and fungicides against Fusarium wilt under pot culture conditions

Best-performing fungal, and bacterial antagonists, organic amendments and fungicides were also forwarded for pot culture experiments. The pot culture experiments were conducted in the cv. Rasthali by artificial inoculation with Foc in soil. Healthy suckers were planted in polythene bags containing sterile potting mixture (Red soil: sand: FYM at 1:1:1 w/w/w) with a capacity of 8 kg of soil. The Foc2 was multiplied in sand maize medium and incorporated into the potting mixture at 300 g per bag and applied at the end of 2nd month after planting. Thirteen treatments were tested in soil. Talc-based formulations of biocontrol agents were applied at the rate of 15 g per bag on the 3rd, 5th and 7th months after planting. Basal application of neem cake in the soil at a rate of 10 g/bag and soil drenching with 0.1% Carbendazim (75% WP) concentration were given on 3rd, 5th and 7th months after planting. Plants inoculated with pathogen alone served as inoculated control. Plants without any inoculation of the pathogen served as healthy control. The severity level was assessed using a grade 4 scale (Mohamed *et al*., 2001).

RESULTS AND DISCUSSION

Survey of Fusarium wilt incidence

Six Foc isolates were confirmed based on morphology and production of microconidia, macroconidia and chlamydospores. The Fusarium wilt incidence was ranging from 27% to 78% in this surveyed area. Maximum 78% in the Pakkapatty area followed by 60% in Agaram, and less incidence (27%) in Alangulam (Table 1).

Pathogenicity of Foc isolates

The isolate of Foc2, exhibited all levels of symptoms like initial yellowing of old leaves, further discolouration of young leaves and intense yellowing of all leaves and complete destruction of plant by recording the highest disease severity grade 4 followed by Foc1 and Foc3 recording the same disease severity grade 3 (Table 2; Fig. 1). Saravanan *et al.* (2003) used Foc amended sand maize medium in Rasthali banana cultivar to create pathogenicity. They observed 88% wilt index in the inoculated control.

Table 1. Incidence of Fusarium wilt in Southern districts of Tamil Nadu

S. No.	Location	District	*Per cent disease incidence (%)
1	Pakkapatty	Tirunelveli	78 (62.04)[a]
2	Agaram	Tirunelveli	60 (50.77)[b]
3	Kongarayakurichi	Thoothukudi	52 (46.14)[c]
4	Tiruchendur	Thoothukudi	40 (39.22)[d]
5	Palayamkottai	Tirunelveli	32 (34.26)[e]
6	Alangulam	Tirunelveli	27 (31.30)[f]
CD(p=0.05)			2.81

*Mean of twenty-five plants
The treatment means are compared using Duncan's multiple range test (DMRT).
In the column, the mean followed by a common letter (s) is not significantly different (p=0.05).
Values in parentheses are arcsine transformed.

Table 2. Various Foc isolates and their disease severity level

Score	Wilt severity level						
Isolates	**Foc1**	**Foc2**	**Foc3**	**Foc4**	**Foc5**	**Foc6**	**Severity***
0	-	-	-	-	-	-	No symptoms
1	-	-	-	-	-	1	Initial yellowing of old leaves
2	-	-	-	2	2	-	Yellowing of old leaves and initial discoloration of young leaves
3	3	-	3	-	-	-	Intense yellowing of all leaves
4	-	4	-	-	-	-	Dead plant

*Mean of three plants

1. Healthy **4. Grade 3**
2. Grade 1 **5. Grade 4**
3. Grade 2

Fig. 1. Pathogenicity test for virulent isolate (Foc2).

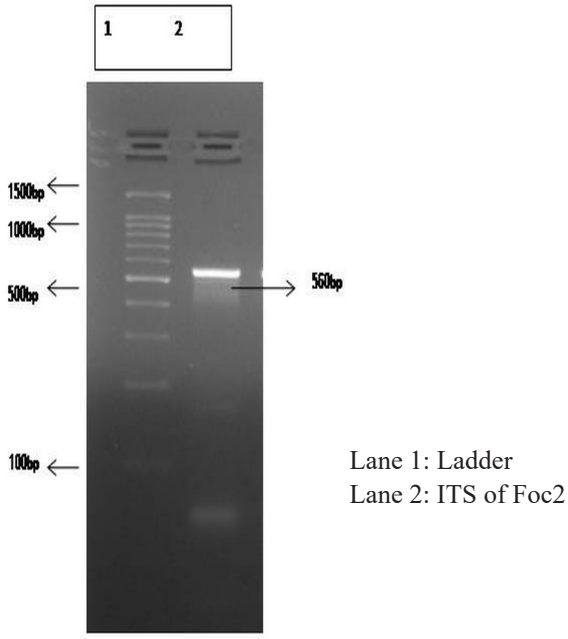


Fig. 2. Agarose gel electrophoresis of 18S rRNA gene amplicon of Foc2 isolate.

Identification and confirmation of Foc2 by molecular technique

Amplified ITS product of 560bp was sequenced. Blast searching showed matching sequences of Foc already present in the database (Accession number is **MN633389).** Our result was similar to the report given by Leong *et al*., (2009) who revealed that 13 Foc isolates were confirmed by ITS amplification using ITS 1 and ITS 4 primers. All the above isolates produced an amplicon size of 550bp (Fig. 2).

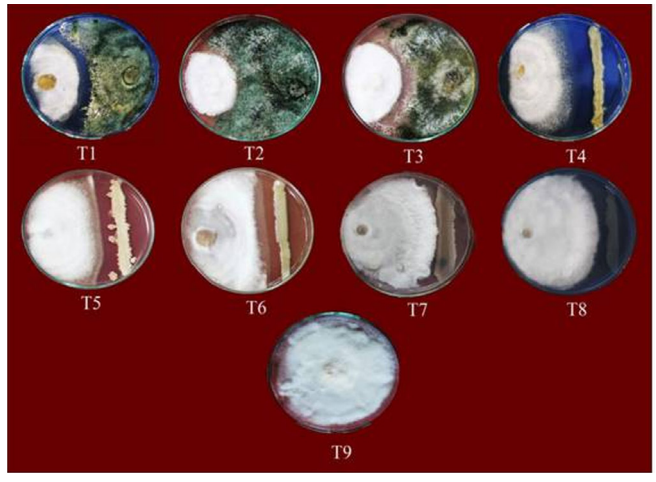
Effect of fungal, bacterial antagonists and endophytes on Foc in dual culture

Among the various biocontrol agents, *Trichoderma* sp. Tsp1 (ALG) showed a maximum reduction of mycelial growth of Foc (72.18%) over control followed by *Trichoderma asperellum* 1 and *Trichoderma* sp. Tsp2 (PKP) by recording 66.70 and 63.55% reduction respectively over control whereas, *Pseudomonas* sp.1 (TNAU), *Bacillus subtilis* and Psp1 (KKM) recorded 51.66, 44.44 and 42.25% reduction respectively over control. Endo2 (KKM) and Endo1 (KKM) were not much effective on Foc (Fig. 3).

Seven *Trichoderma* spp. isolated from various parts of Egypt were tested for their efficacy against *Fusarium oxysporum* f. sp. *Lycopersici* (Fol) in dual culture. Variation in mycoparasitism among the seven *Trichoderma* spp. against Fol was observed. The isolate T7 exhibited 67.8% inhibition of mycelial growth of Fol followed by T3 (59.33%) (Saravanan *et al*., 2003). The diversity of mycoparasitism among *Trichoderma* spp. may be due to the variation of fungal genotypes pertaining to mycelial growth and sporulation. Moreover, the environment-to-gene interaction also makes the differences in mycoparasitism. *Trichoderma* spp. produces some antibiotic substances and chitinase enzymes to degrade the pathogenic fungal cell wall.

In vitro evaluation of Oilcakes against the Foc

All the oilcake extracts at 5% concentration were not much effective in reducing the mycelial growth of Foc. The neem cake extract (10%) recorded the maximum inhibition



T1 *T. asperellum* T6 Psp1
T2 Tsp1 T7 Endo 1
T3 Tsp2 T8 Endo 2
T4 Psp 1 (TNAU) T9 Control
T5 *Bacillus subtilis*

Fig. 3. Effect of fungal, bacterial antagonists and endophytes on Foc.

Table 3. Effect of fungicides against the Foc under *in vitro* condition

T. No.	Fungicides	*Mycelial growth at different concentration (cm)			*Per cent mycelial inhibition over control at different concentration (%)		
		250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
T_1	Carbendazim (50% WP)	0.00	0.00	0.00	100 (89.71)[a]	100 (89.71)[a]	100 (89.71)[a]
T_2	Propiconazole (25% EC)	1.20	0.50	0.00	86.66 (68.61)[b]	94.44 (76.31)[b]	100 (89.71)[a]
T_3	Tebuconazole (25% EC)	1.53	1.00	0.00	83.00 (65.64)[c]	88.88 (70.52)[c]	100 (89.71)[a]
T_4	Hexaconazole (5% EC)	3.70	2.40	1.00	58.88 (59.09)[d]	73.33 (58.90)[d]	88.88 (70.52)[b]
T_5	Azoxystrobin (23 % SC)	4.60	3.80	2.50	48.88 (44.36)[e]	57.77 (49.47)[e]	72.22 (58.16)[c]
T_6	Copper oxychloride (50% WP)	7.30	6.20	5.00	18.88 (25.72)[f]	31.11 (33.89)[f]	44.44 (41.80)[d]
T_7	Tebuconazole (5%) +Trifloxystrobin (25% WG)	1.00	0.00	0.00	88.88 (70.52)[b]	100 (89.71)[a]	100 (89.71)[a]
T_8	Control	9.00	9.00	9.00	-	-	-
CD(p=0.05)		0.25	0.24	0.21	2.24	2.12	1.96

*Mean of three replications
The treatment means are compared using Duncan's multiple range test (DMRT).
Values in parentheses are arcsine transformed.

T1 Neem cake
T2 Mahua cake
T3 Gingelly cake
T4 Groundnut cake
T5 Coconut cake
T6 Control

Fig. 4. Effect of different oilcakes (10%) on Foc.

Table 4. Effect of biocontrol agents, organic amendments and fungicides on the incidence of Fusarium wilt under pot culture conditions

Treatment. No	Treatments	PDI	*Per cent disease reduction over control
T_1	Soil application of *Tv*1 @ 15 g per pot	33.33	60 (50.76)[d]
T_2	Soil application of Psp 1 (TNAU) @ 15g per pot	33.33	60 (50.76)[d]
T_3	Soil application of *Trichoderma* sp. (*T*sp1) @ 15g per pot	33.33	60 (50.76)[d]
T_4	Soil application of *Pseudomonas* sp. (*P*sp1) @ 15 g per pot	58.33	30 (33.19)[g]
T_5	Soil application of *Bacillus subtilis* @ 15 g per pot	50	40 (39.22)[f]
T_6	Combined application of Tv1(15 g) + Pf1(15) g per pot	25	69.99 (56.79)[c]
T_7	Combined application of Tv1(15 g) + sssPf1(15 g) + Neem cake (10 g) per pot	16.66	80 (63.43)[b]
T_8	Combined application of Tsp1(15 sg) + Pf1(15 g) per pot	25	69.99 (56.79)[c]
T_9	Combined application of Tsp1(15 g) + Pf1 (15 g) + Neem cake (10 g) per pot	16.66	80 (63.43)[b]
T_{10}	Soil drenching with 0.1% Carbendazim 50% WP	8.33	90 (71.56)[a]
T_{11}	Soil application of Neem cake at 10g per pot as basal	41.66	50 (45.00)[e]
T_{12}	Inoculated Control (Pathogen)	83.33	-
T_{13}	Healthy Control	0.00	-
	CD (p=0.05)	1.96	1.65

The treatment means are compared using Duncan's Multiple Range Test (DMRT).
Values in parentheses are arcsine transformed.
In the column, the mean followed by a common letter (s) is not significantly different (p=0.05).

of the pathogen (55.55% reduction over control) followed by mahua cake extract (51.33% reduction over control). Other oil cakes were not effective on the Foc at 10% concentration. The coconut cake recorded the least mycelial reduction at 10% concentration (Fig. 4). These results are in accordance with the findings of Yelmame *et al.* (2010) who reported that the neem cake extract was highly effective against mycelial growth (59.23%) of *Fusarium solani*at 10% concentration.

Our results are contradictory to the report of Dhivya *et al.* (2017) who said that neem cake extract was highly effective and recorded 80% reduction of mycelial growth of *F*. *o*. f. sp. *lycopersici*.

Efficacy of different concentrations of fungicides against the growth of Foc *in vitro*

Carbendazim ranked first and completely inhibited the mycelial growth at all concentrations by recording 100% inhibition over control. Tebuconazole+Trifloxystrobin ranked second in inhibiting completely the mycelial growth at 500ppm and 1000ppm concentrations. At 250 ppm concentration also it ranked second and recorded 88.88% reduction over control. Other fungicides namely Propiconazole and Tebuconazole exhibited 100% reduction of the Focover control at 1000 ppm followed by Hexaconazole (88.88%) and Azoxystrobin (72.22%). Copper oxychloride recorded less reduction of the pathogen at all concentrations (Table 3). Our findings are in agreement with the result of Somu *et al.* (2014) who said that Carbendazim and Propiconazole were highly effective and completely checked mycelial growth at 500 and 1000 ppm concentrations respectively on Foc.

Our results corroborate with the findings of Patra and Biswas (2016) who found that Copper oxychloride was the least effective fungicide against *F*. *o*. f. sp. *ciceri* as compared to other fungicides. Copper oxychloride (50% WP) showed 65.56% reduction of mycelial growth at 1000 ppm concentration. In our experiment also Copper oxychloride (50%WP) showed 44.44% reduction in mycelium of the test pathogen. Golakiya *et al.* (2018) reported that maximum inhibition of mycelial growth of *F*. *o*. f. sp. *ciceri* was exhibited by the combination of Carbendazim 12% + Mancozeb 63% WP followed by Tebuconazole 50% + Trifloxystrobin 25% WG (88.56%) and Azoxystrobin 11% + Tebuconazole 18.3% SC (84.22%).

Effect of biocontrol agents, organic amendments and fungicides against the incidence of Fusarium wilt under pot culture conditions

The different treatments were tested on the incidence of Foc under pot culture conditions. The infected plants drenched with 0.1% Carbendazim (75% WP) were found most effective and reduced the wilt incidence from 83.33 to 8.33% by recording 90% reduction over control. The combination of the three treatments of Tsp1+Psp 1 (TNAU)+Neem cake excelled and showed 16.66% wilt incidence and exhibited 80% reduction over control which was on par with Tv1+Psp 1(TNAU)++Neemcake (Table 4).

The individual treatments such as *Trichoderm asperellum1, Trichoderma* sp. (Tsp1) and *Pseudomonas fluorescens* (Pf1), were on par with each other recording 60% reduction of wilt incidence over control. Neem cake showed 41.66% wilt incidence and resulting 50% reduction over control. In the opinion of Saravanan *et al*. (2003) combination of neem cake extract with *P*. *fluorescens*, *T*. *viride* and *T. harzianum* significantly reduced the incidence of Fusarium wilt in banana. Soil application of *P*. *fluorescens* along with neem cake extract reduced the disease incidence (19.45 PDI) than *T. viride* with neem cake (28.37 PDI) compared to control (55.61 PDI). Application of organic amendments increased the soil microflora and antagonist which act on the pathogenic propagules by various mechanisms such as antibiosis, mycoparasitic and competition for space and nutrition.

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