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Short communication

Mass production of *Trichoderma harzianum* for managing fusarium wilt of banana

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Abstract

Trichoderma spp. isolated from rhizosphere of banana (*Musa* sp.) from different areas of Tamil Nadu, India were evaluated under in vitro condition for their antagonistic potential against *Fusarium oxysporum*, the banana fusarium wilt pathogen. *Trichoderma harzianum* isolate Th-10 was the most effective in inhibiting the mycelial growth of fusarium in vitro. Out of five different organic substrates (rice bran, rice chaffy grain, farmyard manure, banana pseudostem and dried banana leaf) tested, dried banana leaf was the best carrier material to support *T. harzianum* growth. Strain Th-10 colonized the dried banana leaves within few days and produced high density of propagules (4.6×10^{32} cfu/g of leaf). Addition of jaggery (10% w/v) to the dried banana leaves increased the multiplication of *T. harzianum* which survived for >6 months on the stored substrate. When applied as dried formulation, the population of *T. harzianum* Th-10 increased from 10^4 to 10^{13} cfu/g of soil within 60 days. In two field trials, soil application of *T. harzianum* Th-10 as dried formulation effectively controlled fusarium wilt with an efficacy comparable to that of the fungicide carbendazim.

Keywords: *Musa* sp.; *Trichoderma harzianum*; Biological control; *Fusarium oxysporum* f. sp. cubense

1. Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. cubense (E.F. Smith) Snyd. and Hans. is one of the most destructive diseases of banana worldwide (Moore et al., 2001). The pathogen is soil-borne and remains viable up to 30 years (Moore et al., 1995). Breeding banana for resistance against fusarium wilt is difficult because of the sterile and polyploid nature of the plant and the saprophytic and pathogenic habits of the pathogen (Novak, 1992). Several disease management strategies such as crop rotation with rice, application of carbendazim (0.2%) as soil drenching or injection of rhizomes with 2% carbendazim (Thangavelu et al., 2001), have limited success and the application of synthetic fungicides may result in undesirable effects on the environment. A complementary approach for managing fusarium wilt is biological control and the search for antagonistic microorganisms has allowed for several antagonistic fungi and bacteria with high activity to be identified (Locke et al., 1984; Adams, 1990; Van Peer et al., 1991; Wei et al., 1991; Vidhyasekaran et al., 1997; Mathivanan et al., 2000). *Trichoderma* spp. in particular have been reported to control soil-borne plant pathogens such as *Rhizoctonia solani* Khun., *Sclerotium rolfsii* (Sacc.) Curzi.,

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Pythium and *Fusarium* spp. (Sivan and Chet, 1986, 1987; Calvet et al., 1990; Prasad et al., 2002; Samuels, 1996; Lewis and Lumsden, 2001). Developing appropriate formulation and delivery systems is the prerequisite for implementing biological control using microbial antagonists (Lumsden and Lewis, 1989). Formulation of biological control agents depends upon biomass production and maintaining viability at the end of the process (Adekunle et al., 2001). Several commercial formulations of *Trichoderma* spp. mainly based on inert carriers are available for controlling plant diseases (Lewis and Papavizas, 1991). Multiplying *Trichoderma* spp. on easily biodegradable substrates with long shelf-life would be beneficial for field application. This paper describes the mass production of *Trichoderma harzianum* on dried banana leaves and an evaluation of its efficacy against banana fusarium wilt in greenhouse and field trials.

2. Materials and methods

Race 1 of *F. oxysporum* was isolated from rhizomes of infected banana (cv. Rasthali) using half-strength potato dextrose agar (PDA) medium as described by Thangavelu et al. (2001). A single spore culture was maintained on carnation leaf agar medium (Burgess et al., 1988) for immediate use and the culture was stored on dry filter paper at 4 °C according to Correll et al. (1986). The pathogenicity of the fungus was confirmed under pot culture conditions using banana cv. Rasthali.

Trichoderma spp. were isolated from the rhizosphere soil of banana collected from different areas of Tamil Nadu, India. One gram of rhizosphere soil was transferred to a 250 ml conical flask containing 100 ml of sterile distilled water. After thoroughly shaking for 15 min, the suspension was serially diluted in sterile distilled water. *Trichoderma* spp. were isolated using selective medium (Elad and Chet, 1983) and identified to species according to Games and Meyer (1998).

Trichoderma spp. were tested against *F. oxysporum* using dual culture technique. An 8-mm diameter mycelial disc cut from a 7 days old culture of fusarium was placed 1 cm from the edge of 9 cm Petri dishes containing PDA medium. The plates were incubated at 28 ± 2 °C for 3 days. An 8 mm diameter mycelial disc was cut from actively growing colonies of *Trichoderma* and placed in a Petri dish opposite to a fusarium mycelial disc. After 4 days, inhibition of fusarium growth was measured in terms of inhibition zone (from the edge of the fusarium mycelium to the edge of antagonist growth) and growth of fusarium mycelium (cm). Three replicates of 10 plates each were made per *Trichoderma* isolate.

Five organic substrates: farm yard manure, rice chaffy grains, dried banana leaf, banana pseudostem and rice bran were evaluated for their ability to support the growth of different isolates of *Trichoderma* spp. Of each substrate 500 g was mixed with 100 ml of 30% molasses solution (v/v), filled in polypropylene bags and sterilized twice at 121 °C for 2 h. After sterilization, the substrates were inoculated under aseptic conditions with an 8 mm mycelial disc of *T. harzianum* Th-10 obtained from a 5-days-old culture. Bags were incubated at 28 ± 2 °C for 20 days. The population of *T. harzianum* on the organic substrates was assessed 20 days after incubation by serial dilution technique using *Trichoderma* special medium (Elad and Chet, 1983).

Dried banana leaves only were selected for further studies. To find out whether jaggery increased *T. harzianum* growth, 1 kg of dried banana leaves was immersed in 1, 5 and 10% (w/v) jaggery solution for 30 min. The excess jaggery solution was drained. One set of leaf material was boiled for 10 min and dried under shade for 10 min in a tray. An 8-mm mycelial disc of *T. harzianum* Th-10 was added to it, mixed in a tray covered with polythene bags and incubated at 28 ± 2 °C. Another set of banana leaves (1 kg) was packed in polythene bags and sterilized twice at 121 °C for 2 h. The sterilized leaves were emptied onto plastic trays and an 8-mm mycelial disc of *T. harzianum* Th-10 was added before trays were covered with polythene bags and incubated at 28 ± 2 °C. Dried banana leaves treated with water served as control. The population of *T. harzianum* Th-10 on the dried banana leaves was estimated 4 days after incubation as described earlier.

Dried banana leaf (1 kg) was added 1% (w/v) jaggery solution boiled for 10 min and dried in the shade for 15 min. The material was spread in a tray and an 8 mm mycelial disc was added. The trays were covered with polythene sheets and incubated at 28 ± 2 °C. Samples were drawn at monthly interval and the population of *T. harzianum* Th-10 assessed as described earlier.

The ability of *T. harzianum* Th-10 multiplied on dried banana leaf to survive under different soil pH was tested under greenhouse condition. Soil with pH of 6.80, 7.43 and 8.52 collected from different banana growing regions of Tamil Nadu was used to fill 30 cm earthen pots. Banana plants (cv. Rasthali) derived from tissue culture were planted in the pots and the plants were grown at 32 °C and 85% rH. After 10 days, *T. harzianum* Th-10 multiplied on dried banana leaf was applied to soil at the rate of 10^4 cfu/g soil. A talc-based formulation of *T. harzianum* Th-10 was prepared according to Jeyarajan et al. (1994) and used for comparison. Three replicates per treatment were used. Rhizosphere soil samples were collected 20, 40 and 60 days after application and the population of *T. harzianum* in the samples was assessed by the serial dilution plate technique using *Trichoderma* special medium (Elad and Chet, 1983).

Two field trials were conducted at the Tamil Nadu Agricultural University, Coimbatore in 1999–2000. Suckers of banana plants (cv. Rasthali) susceptible to fusarium were obtained from a farmer's field in Thiruchirapalli, Tamil Nadu. These were planted at 1.8 m distance on and between rows on September 28, 1999 and October 2, 2000. Dried banana leaves that had been soaked in 1% of jaggery for 5 min and boiled for 10 min were inoculated with mycelial discs of *T. harzianum* Th-10. After 4 days of incubation, the banana leaves containing *T. harzianum* (4×10^{31} cfu/g material) were incorporated into the soil around the plants (7–8 cm depth) at 10 g per plant. The disease severity was recorded at the time of bunch maturing phase using a 1–5 scale (Ploetz et al., 1999). The trial was laid out in a randomized block design with 20 replicates per treatment, each trial being conducted at least twice. The population data of *T. harzianum* were $\log_{10}$ transformed before undergoing analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test (DMRT). The package used for analysis was IRRI STAT version 92-1 developed by the International Rice Research Institute Biometrics Unit, Philippines.

3. Results

Amongst 11 *Trichoderma* spp. isolated from different parts of Tamil Nadu, India, Th-10 was found to be particularly effective in inhibiting the mycelial growth of fusarium in vitro, and an inhibition zone of 1.4 cm was recorded (Table 1). The population growth of *T. harzianum* Th-10 was very high on dried banana leaf (4.6×10^{32} cfu/g material) followed by banana pseudostem (2×10^{21} cfu/g) and rice chaffy grains (8×10^{15} cfu/g) (Fig. 1). Addition of jaggery to the dried banana leaves increased the population growth of *T. harzianum* Th-10 even at 1% (4×10^{31} cfu/g) compared to control (1.9×10^5 cfu/g), 5 and 10% concentrations being not significantly higher.

Table 1
In vitro antagonistic activity of various isolates of *Trichoderma* spp. against *F. oxysporum* f. sp. *cubense*

Trichoderma spp.	Fusarium growth (cm)[a]	Inhibition zone (cm)[a]
T. viride Tv-2	5.0 cd	0.5 dc
T. viride Tv-3	4.6 b	0.3 d
T. viride Tv-1	4.1 a	1.0 b
T. harzianum Th-3	5.2 de	0.5 dc
T. harzianum Th-4	5.5 f	0.5 dc
T. viride Tv-4	6.5 g	0.4 d
T. harzianum Th-5	5.3 ef	0.3 d
T. harzianum Th-6	4.8 bc	0.3 d
T. harzianum Th-2	5.0 cd	0.7 c
T. harzianum Th-10	4.0 a	1.4 a
T. harzianum Th-1	4.2 a	1.0b
Control	7.5 h	–

[a] Average of three replicates. Mean values in a column followed by the same letter did not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test.

Survival of *T. harzianum* was studied during a 6-month storage. The initial population of *T. harzianum* Th-10 was 30×10^{32} cfu/g dried banana leaf and 13×10^{10} cfu/g of talc. In both substrates, the population of *T. harzianum* Th-10 was maintained at the same level for 1 month of storage and declined gradually afterward. After 6 months, the population had declined to 4×10^{15} cfu/g in dried banana leaves and to 1.1×10^2 cfu/g in talc-based formulation. *T. harzianum* multiplied in dried banana leaf increased rapidly with time in all soils tested. When applied as dried banana leaf formulation *T. harzianum* Th-10 increased from 10^4 to a maximum of 10^{13} cfu/g of soil after 60 days. Talc-based formulations of *T. harzianum* increased less rapidly (Table 2).

Soil application of dried banana leaf formulation of *T. harzianum* Th-10 provided 49.9%, the talc formulation 40.1%, the fungicide treatment only

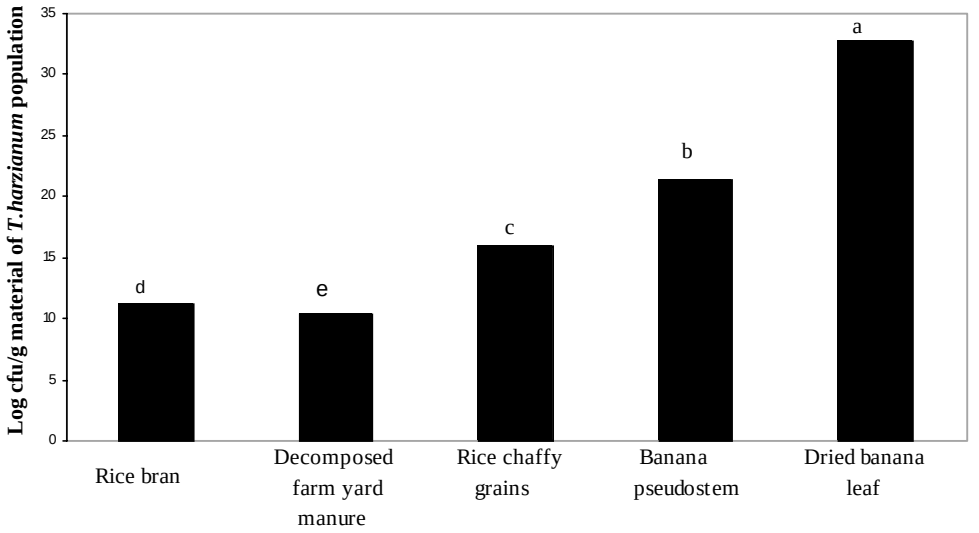

Fig. 1. Growth of *T. harzianum* Th-10 on various organic substrates. Different letters indicate statistically significant differences between treatments by Duncan's multiple range test ($P \leq 0.05$).

Table 2
Rhizosphere population of *T. harzianum* Th-10 (cfu/g of soil)[a] in different soil types after application of two different formulations

Soil pH	Days after application							
	Dried banana leaf formulation				Talc-based formulation			
	0	20	40	60	0	20	40	60
6.80	1.2×10^4 d	4×10^6 c	5×10^9 b	2.2×10^{11} a	1×10^4 d	12×10^6 c	10×10^7 a	3.8×10^7 b
7.43	5×10^4 d	1.6×10^6 c	3.5×10^{10} b	14×10^{13} a	3×10^4 c	23×10^6 b	11×10^8 a	2×10^7 b
8.52	9×10^4 d	4×10^6 c	4.1×10^9 b	10×10^{11} a	2×10^4 d	2.7×10^5 c	8×10^6 a	1.2×10^6 b

In a column, means followed by a common letter are not significantly different by Duncan's multiple range test ($P \leq 0.05$).
[a] Five Petri dishes per dilution and three replicates per treatment.

18.1% reduction in disease incidence compared to control.

4. Discussion and conclusion

The success of biological control on crop plants depends not only on effective antagonists, but also on the costs involved and the method of application. Dried banana leaf was found to be the best substrate to support the growth of *T. harzianum* Th-10 which quickly multiplied and covered the entire surface within 4 days.

Addition of 1% jaggery to banana leaves strongly stimulated growth of *T. harzianum* Th-10. During storage, only conidia were present and initial populations of 30×10^{32} cfu/g dried banana leaf decreased to 4×10^{15} cfu/g after 6 months storage at room temperature. In terms of density of propagules, Adams (1990) indicated that Trichoderma required a minimum of 10^5 cfu/g of soil to achieve effective disease control. The results of the present study indicated that the rhizosphere population of *T. harzianum* Th-10 increased several fold higher than 10^5 cfu/g of soil in just 2 months after application resulting in better disease control than with fungicide.

Lo et al. (1996) reported that application of a peat-based formulation of *T. harzianum* resulted in 100-fold increase in population compared to untreated plots, whereas in alginate formulations, *Trichoderma*

numbers did not increase (Knudsen et al., 1991). When applied as wheat bran, *T. harzianum* propagules had increased 600-fold at harvest (Ruppel et al., 1983). Mass production and delivery of *T. harzianum* using dried banana leaf (1) were cost effective, (2) had a long shelf-life, (3) supported high propagules density, (4) were easy to formulate and, (5) were readily adopted by farmers.

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