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In vitro evaluation of mutant and wild strain of *Trichoderma harzianum* against soil borne plant pathogen

■ A.A. WALUNJ*[1], P.B. ABHANG[2] AND PRIYA JOHN[2]

[1]Department of Plant Pathology, College of Agriculture, Loni, AHMEDNAGAR (M.S.) INDIA

[2]Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, NAVSARI (GUJARAT) INDIA

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***Corresponding author:**
Email: akshaya17289@gmail.com

ABSTRACT

Aqueous suspension of conidia of *Trichoderma harzianum* wild strain Th-W were placed on potato dextrose agar and expose to UV irradiation for 10, 20, 30, and 40 min at 20 cm distance from which four stable mutants of *T. harzianum i.e.*, Th-M-1, Th-M-2, Th-M-3 and Th-M-4 were obtained as it differed considerably from wild strain (Th-W) for their morphological characteristics. *In vitro* evaluation of mutant and wild strain of *T. harzianum* against three soil borne plant pathogens, *Fusarium oxysporum* f.sp. *lycopersici*, *Sclerotium rolfsii* and *Macrophomina phaseolina by* dual culture method, revealed that mutant strains overgrew all the pathogenic fungi more rapidaly than the wild strain.

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INTRODUCTION

Trichoderma harzianum has been promising biocontrol agent of many plant diseases caused by the soil-borne pathogens like fungi such as *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Alternaria* species on various crops. In addition, species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Dubey *et al*., 2007). The mechanisms of *Trichoderma* spp. antagonize phytopathogenic fungi include competition, colonization, antibiosis and direct mycoparasitism (Howell, 2003). This antagonistic potential serves as the basis for effective biological control application of *Trichoderma* spp. as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Chet, 1987).

MATERIAL AND METHODS

Development of mutant *Trichoderma harzianum* :

The mutant of *Trichoderma harzianum* was developed through UV irradiation. Conidial suspension of *Trichoderma harzianum* (Th-W) was prepared by dislodging the conidia from the ager surface by pouring sterilized water. The conidial concentration was adjusted with the help of haemocytometer to10^6ml. One ml of conidial suspension was placed on PDA plates and exposed to 30 W UV-irradiation for 10, 20, 30, and 40 min at 20 cm distance from which four stable mutants of *Trichoderma harzianum i.e.*, Th-M-1, Th-M-2, Th-M-3 and Th-M-4 were obtained. All the isolates were grown on PDA in Petri dishes. Plates were inoculated at the centre with a 5 mm mycelial disc taken from stock slants. The plates inoculated were incubated at room temperature (27 ± 2°C). Each stable isolates after four repetitions were examined properly and

regularly. Colony diameter was measured after incubation period 12, 24 and 48 (hr). Baby (1998) developed induced mutation on *T. longibrachiatum* by using ultra-violet irradiation and obtained mutant strains tolerant to bavistin. Intana *et al.* (2003) developed mutant of *T. harzianum* by using ultra-violet irradiation. Hunjan *et al.* (2004) developed two mutant strains TV34-M4 and TV34-M5 from *T. viride* by using ultra-violet irradiation. Manav and Singh (2003) developed mutant *T. harzianum* by exposing to ultra-violet irradiation for 90 min.

Antagonistic capability of mutant and wild *T. harzianum* against soil borne plant pathogen :

Sterilized PDA *i.e.* 20 ml was poured aseptically in sterilized Petriplates of 90 mm diameter. Mycelial discs (5mm) of 5 days old actively growing culture of the bioagent and the test pathogens were cut separately with the help of sterilized cork borer and placed on solidified PDA approximately 4 cm away from each other. The experiment was repeated fourth times along with their controls, where test pathogen subjected alone for growth comparison. All the inoculated plates were incubated at room temperature (27±2ºC). The radial growth of test pathogens in treated and control plates were recorded after 5 days of incubation and the per cent inhibition of mycelial growth of the pathogens was calculated by using formula of Bliss (1934). *i.e.* :

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

where, I = Inhibition per cent, C = Colony diameter in control plate, T = Colony diameter in treated plate.

RESULTS AND DISCUSSION

Radial growth rate of the wild and stable mutant of *T. harzianum* on PDA medium (Table 1) and depicted graphically in Fig. 1 showed that mutants of *T. harzianum* after 48 hr incubation showed higher radial growth as compared to 24 hr of incubation. Among the wild (Th-W) and mutant *T. harzianum* (Th-M-1, Th-M-2, Th-M-3, Th-M-4) mutant Th-M-2 recorded highly significant growth rate of 90 mm after 72 hr. These were followed by mutant isolates Th-M-3 (88.40 mm) and Th-M-4 (87.90 mm). However, after 24 hr, 48 hr and 72 hr mutant Th-M-2 showed increasing radial growth over other mutants and wild. Viswanth (2012) who recorded mutant *T. harzianum* (TM_{17}) showed highest growth rate and sporulation as compared to wild type. Henis *et al.* (1983) showed higher mycoparasitic ability of mutant against *S. rolfsii*. Mukherjee *et al*. (1997) showed higher mycoparasitic ability of mutant against *S. rolfsii*. Singh *et al.* (2002) found that *T. harzianum* inhibited the growth of *F. udum*.

Table 1 : Growth rate (mm) of wild and mutant *Trichoderma harzianum*

Sr. No.	Isolates of *T. harzianum*	*Average colony diameter (mm)		
		24 hr	48 hr	72 hr
1.	Th-W	22.00	54.00	82.00
2.	Th-M1	36.00	66.00	87.90
3.	Th-M2	40.00	70.00	90.00
4.	Th-M3	38.50	68.50	88.40
5.	Th-M4	38.00	68.00	88.00
S.E. ±			0.37	
C.D. (P = 0.05)			1.10	
C.V. (%)			2.57	

*Average of four repetitions; Th-W: wild *T. harzianum*, Th-M-1, Th-M-2, Th-M-3, Th-M-4: Mutant *T. harzianum*

Table 2 : Antagonistic capacity of *Fusarium oxysporum*, *Sclerotium rolfsii* and *Macrophomina phaseolina*

Sr. No.	*Trichoderma harzianum* isolates	% inhibition of mycelium growth (PIMG)		
		Fusarium oxysporum	*Sclerotium rolfsii*	*Macrophomina phaseolina*
1.	Th-W	65.11	65.11	62.79
2.	Th-M-1	73.25	73.25	70.93
3.	Th-M-2	75.53	76.58	75.46
4.	Th-M-3	73.83	74.34	72.09
5.	Th-M-4	73.48	73.48	71.39
6.	Control	-	-	-
S.E.±		0.42	0.42	0.38
C.D. (P=0.05)		1.25	1.27	1.15
C.V. (%)		2.47	2.37	2.18

Th-W: wild *T. harzianum*, Th-M-1, Th-M-2, Th-M-3, Th-M-4: Mutant *T. harzianum*

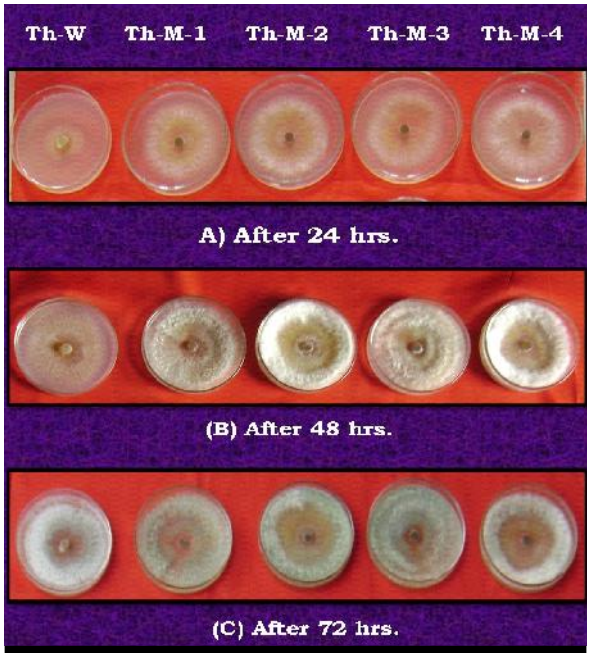

Fig. 1 : Growth rate of wild and mutant *Trichoderma harzianum*

The antagonists tested against *S. rolfsii* were more or less inhibited the growth of the pathogen. Among the wild (Th-W) and mutant of *T. harzianum* (Th-M-1, Th-M-2, Th-M-3, Th-M-4) highest per cent mycelial inhibition was observed by mutant Th-M-2 (75.00 %) with 22.00 mm average colony diameter after five days of incubation which was followed by mutant Th-M-3 (73.29 %) and Th-M-4 (72.61 %) with average colony diameters 23.50 mm and 24.10 mm, respectively. The mutants had better per cent inhibition as compared to wild *T. harzianum* Th-W (60.79 %) with average colony diameter of 34.50 mm. which was showed in Table 2 and Fig. 2. Mech (2006) reported antagonistic effect of wild and mutant *T. harzianum* against the *S. rolfsii* showing maximum reduction of radial growth of pathogen by Th-M_2 as compared to Th-W, Th-M_1 and Th-M_3.

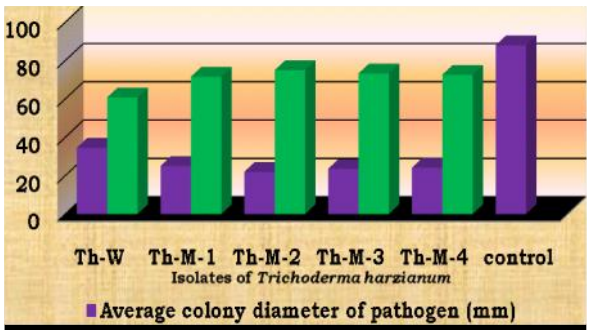

Fig. 2 : Antagonistic effect of wild and mutant *Trichoderma harzianum* against *Sclerotiaum rolfsii*

The antagonists tested against *F. oxysporum* were more or less inhibited the growth of the pathogen. Between wild *T. harzianum* (Th-W) and mutant *T. harzianum* (Th-M-1, Th-M-2, Th-M-3, Th-M-4), significantly highest mycelial inhibition was observed by isolate Th-M-2 (75.53 %) with 21.04 mm average colony diameter. which was followed by isolates Th-M-3 (73.83 %) and Th-M-4, (73.48 %) with average colony diameters 22.50 mm and 22.80 mm, respectively. While lowest per cent growth inhibition was found with isolates Th-W (65.11 %) with 30.00 mm average colony diameter of pathogen. which was showed in Table 2 and Fig. 3. Manav and Singh (2006) who tested mutant and parent strain of *T. harzianum* against three soil borne plant pathogens; *F. oxysporum* f. sp. *ciceri*, *R. solani* and *M. phoseolina* by dual culture method, revealed that mutant strain overgrew all the pathogenic fungi more rapidly than the parent strain. Balasubramanian *et al.* (2010) observed that the wild and mutant strains of *T. harzianum* showed differences in their spore colour, growth rate and pigmentation. The UV- mutant showed a puffy pale green pigmentation. Nagamani and Viswanth (2012) observed that the mutant *T. harzianum* (TM_{17}) showed highest growth rate and sporulation as compared to wild type. Johnson and Curl (1972) screened the selected mutant and parent strains of *T. harzianum* against three test pathogens, *Fusarium oxysporum* f. sp. *ciceri*, *Macrophomina phaseolina* and *Rhizoctonia solani* by dual culture method, revealed that mutant strain overgrew all the pathogenic fungi more rapidly than the parent strain.

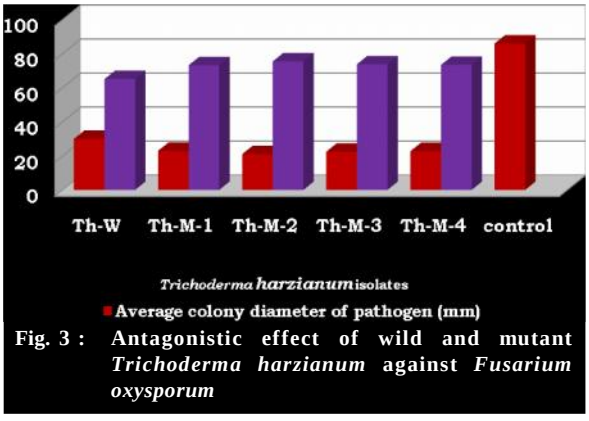

Fig. 3 : Antagonistic effect of wild and mutant *Trichoderma harzianum* against *Fusarium oxysporum*

Minimum mycelial growth and highest per cent growth inhibition were recorded in the case of *T. harzianum* isolate Th-M-2 (75.46 %) with 21.10 mm average colony diameter of the *Macrophomina phaseolina*. This was followed by isolates, Th-M-3 (72.09 %) with 24.00 mm average colony diameter of the pathogen and Th-M-4 (71.39 %) with 24.60 mm average colony diameter of pathogen. Lowest per cent growth inhibition was recorded with isolate Th-W (62.79 %) with 32.00 mm colony diameter which was showed in Table 2 and Fig. 4.

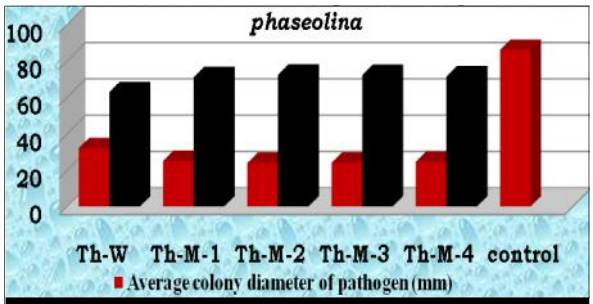

Fig. 4 : Antagonistic effect of wild and mutant *Trichoderma harzianum* against *Macrophomina phaseolina*

Patil and Kamble (2011) who reported that mutants had highest per cent reduction of mycelial growth. Similarly Ranganathswamy *et al.*, 2012; Chavan *et al.*, 2012; Lokesh *et al.*, 2012 and Sharma *et al.*, 2013 also worked in the related topic and the results coincides with the present findings.

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