

ADAPTABILITY OF UV IRRADIATED MUTANT OF *TRICHODERMA HARZIANUM* TO CARBENDAZIM

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ABSTRACT

Trichoderma harzianum, a potential bioagent used against different seed and soil borne plant pathogens is highly susceptible to the most widely used fungicide carbendazim. UV irradiated mutants of *T. harzianum* was developed by exposing *T. harzianum* to 30 W UV-irradiation for 10, 20, 30, and 40 min at 20 cm distance. Different concentrations of carbendazim (10, 20, 30, 50, 100 ig/ml) were tested for sensitivity against wild and mutants of *T. harzianum* (Th-W, Th-M-1, Th-M-2, Th-M-3, Th-M-4). Mutant of *T. harzianum* Th-M-2 and Th-M-3 showed minimum growth inhibition at 10 ig/ml concentration of carbendazim. Mutants *T. harzianum* (M-1, M-2, M-3, M-4) showed better inhibition as compared to wild *T. harzianum* (Th-W) at any concentration of the fungicide carbendazim.

INTRODUCTION

Biological control is defined as the reduction of the inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man (Baker and Cook's, 1974).

Trichoderma harzianum is highly susceptible to the fungicide carbendazim that is often used for seed treatment for controlling several internally seed borne pathogens. Hence, seed treatment with application of *Trichoderma* and carbendazim at the same time becomes a hindrance in seed treatment. However, it would be a pragmatic approach if the usage of both carbendazim and *T. harzianum* are integrated where in advantages of both methods would be efficient for management of crop diseases. Mutants of *T. viride* (TvM₁) and *T. harzianum* (ThM1) showed high compatibility with the agrochemicals i.e., fungicides, insecticides, herbicides (Madhavi et al., 2008).

Trichoderma proved as worldwide accepted biological control of plant diseases particularly for seed and soil borne diseases. Farmers started eco-friendly plant disease management or biological based integrated disease management. Hence there is a need to improve capability of *Trichoderma* spp. by using physical and chemical mutagens such as UV rays and gamma rays for tolerance to fungicides.

MATERIALS AND METHODS

Development of mutant *Trichoderma harzianum*

The mutant of *T. harzianum* was developed through UV irradiation. The conidial suspension of *T. harzianum* (Th-W) was prepared by dislodging the conidia from the agar surface

by pouring sterilized water. The conidial concentration was adjusted with the help of haemocytometer to 10⁶m Counted the conidia in the middle square which consists of 25 groups of 16 small squares, each group 0.2 mm square (Aneja K. R. 2002). 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 *T. harzianum* i.e., Th-M-1, Th-M-2, Th-M-3 and Th-M-4 were obtained, respectively. Developed mutants of *T. harzianum* by using 30W ultra-violet irradiation by using different periods i.e., 0, 10, 20, 30, and 40 min (Hassan and Kareem, 2011). Developed mutant strains of *Trichoderma* spp by using ultra-violet irradiation (Nagamani and Viswanth 2012). All the mutant isolates were grown on PDA in Petri dishes by placing 5 mm mycelial disc at the centre of Petri dish. The inoculated plates were incubated at room temperature (27 ± 2°C) and four repetitions of each isolated were maintained. Each stable isolates after four repetitions were examined properly and regularly. Colony diameter was measured after incubation period 12, 24 and 48 (hr). 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 (Balasubramanian et al., 2010). The mutant *T. harzianum* (TM₁₇) showed highest growth rate and sporulation as compared to wild type (Nagamani and Viswanth, 2012).

Adaptibility of mutant *T. harzianum* against carbendazim

The PDA aliquots of 25 ml containing the chemical were poured aseptically in sterilized Petri plate and allowed to solidify. Medium without test chemical served as control. Mycelial disc of 5 mm diameter was cut from 5 days old culture

of wild (Th-W) and mutants of *T. harzianum* (Th-M-1, Th-M-2, Th-M-3, Th-M-4) and transferred aseptically at the centre of each plate having aliquots of test chemical. Simultaneously, plates with wild (Th-W) and mutants of *T. harzianum* (Th-M-1, Th-M-2, Th-M-3, Th-M-4) on PDA (without chemical) were taken as control. Each treatment was replicated four times. The inoculated Petri plates were incubated at room temperature and the colony diameter was measured after 5 days incubation. Per cent inhibition of the mycelial growth was calculated by using the formula given by Bliss (1934) i.e., $I = (C-T/C) \times 100$ Where, I = Inhibition per cent, C = Colony diameter in control plate, T = Colony diameter in treated plate. Mutants of *T. harzianum* had high resistance to the fungicide benomyl (De melo *et al.* (2010). Mutant strains of *Trichoderma* spp. by Ultra-Violet irradiation. Among seven isolated, TM₁₇ recorded tolerance upto 1.3 per cent carbendazim, while the wild type failed to grow even upto 0.01 per cent carbendazim (Nagamani and Viswanth, 2012).

RESULTS AND DISCUSSION

Growth rate of wild and mutant *T. harzianum*

The wild and mutant strains of *T. harzianum* differed considerably with respect to their colony characters, growth rate and sporulation. Greenish aerial fluffy colony type was observed in the mutant strains as compared to light green aerial of the wild strain. After 24 hr, 48 hr and 72 hr mutant Th-

M-2 showed increasing growth rate over other mutants and wild. All the mutant strains showed high sporulation as compared to wild strain (Table 1). The mutant *T. harzianum* (Th38 M-7) showed an increase in colony diameter, dry mycelial weight and sporulation as compared to the parent strain (Th38) (Manav and Singh 2006). Mutant *T. harzianum* (TM₁₇) showed highest growth rate and sporulation as compared to wild type (Nagamani and Viswanth 2012).

Mutant *T. harzianum* adaptability against carbendazim:-

The mutant strains grew on medium containing carbendazim up to 30 µg/mL concentration. Whereas the parent strain failed to grow even at any of concentration of carbendazim. Mutant of *T. harzianum* Th-M-2 and Th-M-3 showed minimum growth inhibition at 10 µg/mL concentration of carbendazim. Increase in concentration of carbendazim up to 30 µg/ml, there was increase in inhibition of mycelial growth of isolate of mutant *T. harzianum* (Th-M-2). *T. harzianum* mutants (M-1, M-2, M-3, M-4) showed complete inhibition at 50 and 100 µg/ml concentration of carbendazim (Table 2 and Fig. 1).

Here, an attempt was made in developing mutants of *T. harzianum* using UV radiation. In the present study as wild strain of *T. harzianum* was found very much sensitive to fungicide carbendazim and did not show any kind of growth even at very low concentration (10 µg/mL) of fungicide. Similarly Nagamani and Viswanath (2012) developed mutant strains of *Trichoderma* spp. by UV-irradiation. The mutants had

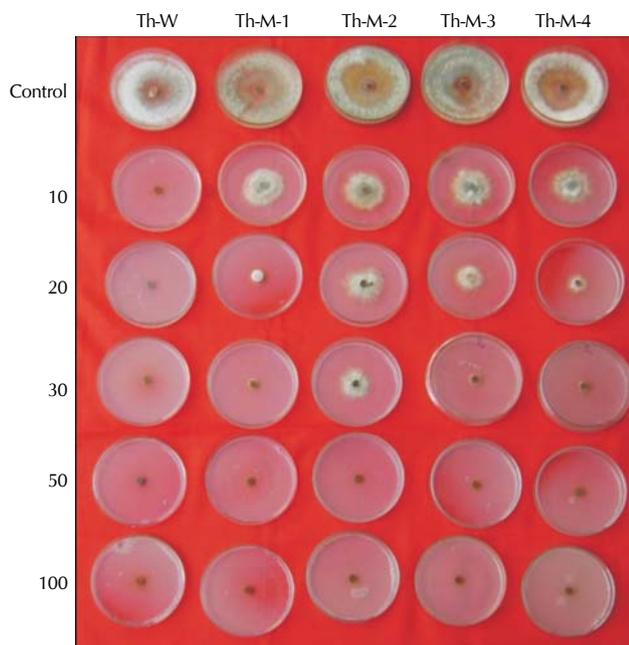
Table 1: Comparison of wild and mutant *Trichoderma harzianum* for the colony morphology and sporulation on Potato Dextrose Agar (PDA)

Sr.No.	Isolates	Morphological characteristics
1.	Th-W	Initially colony with light green aerial mycelium. Highly sporulating i.e. 7.68×10^6 conidia/ml. Light yellow pigmentation. Size of conidia $3.0-3.2 \times 2.2-2.6 \mu\text{m}$. size of conidiophores $8.5-9.2 \times 1.5-2.8 \mu\text{m}$.
2.	Th-M-1	Initially colony with whitish mycelium later turn greenish. Aerial mycelium growth at margin. High sporulation i.e. 7.72×10^6 conidia/ml. Faster growth rate and yellow pigmentation. Size of conidia $1.3-2.3 \times 1.0-1.5 \mu\text{m}$. size of conidiophores $10.6-11.5 \times 1.1-1.5 \mu\text{m}$.
3.	Th-M-2	Initially colony with whitish mycelium later turn greenish. Fluffy aerial mycelium growth at margin. Profuse sporulation after 5 days i.e. 9.78×10^6 conidia/ml. Faster growth rate and dark yellow pigmentation. Size of conidia $1.2-2.0 \times 1.0-1.4 \mu\text{m}$. size of conidiophores $11.5-12.5 \times 1.5-1.8 \mu\text{m}$.
4.	Th-M-3	Initially colony with whitish mycelium later turn dark greenish. Less fluffy aerial mycelium growth at margin. Sporulation i.e. 8.64×10^6 conidia/ml. Faster growth rate and yellow pigmentation. Size of conidia $1.0-1.4 \times 1.0-1.2 \mu\text{m}$. size of conidiophores $10.5-12.2 \times 1.2-1.4 \mu\text{m}$.
5.	Th-M-4	Initially colony with whitish mycelium later turn greenish. Highly fluffy aerial mycelium growth at margin. Sporulation i.e. 7.94×10^6 conidia/ml. Faster growth rate and light yellow pigmentation. Size of conidia $1.0-1.2 \times 1.0-1.6 \mu\text{m}$. size of conidiophores $9.2-9.8 \times 1.0-1.2 \mu\text{m}$.

Table 2: Adaptability of wild and mutant *Trichoderma harzianum* with carbendazim at different concentrations

Sr. No.	Isolates <i>Trichoderma harzianum</i>	10 µg/ml		20 µg/ml		30 µg/ml		50 µg/ml		100 µg/ml	
		* Average colony diameter (mm)	% inhibition over control	* Average colony diameter (mm)	% inhibition over control	* Average colony diameter (mm)	% inhibition over control	* Average colony diameter (mm)	% inhibition over control	* Average colony diameter (mm)	% inhibition over control
1	Th-W	0.71(0.00)	100	0.71(0.00)	100	0.71(0.00)	100	0.00	100	0.00	100
2	Th-M1	6.36(40.00)	55.05	4.95(24.00)	73.03	0.71(0.00)	100	0.00	100	0.00	100
3	Th-M2	6.75(45.00)	49.43	5.96(35.00)	60.67	5.34(28.00)	68.53	0.00	100	0.00	100
4	Th-M3	6.75(45.00)	49.43	5.34(28.00)	68.53	0.71(0.00)	100	0.00	100	0.00	100
5	Th-M4	6.28(39.00)	56.17	4.74(22.00)	75.28	0.71(0.00)	100	0.00	100	0.00	100
6.	Control	9.46(89.00)	-	9.46(89.00)	-	9.46(89.00)	-	89	-	89	-
S.Em.	0.03	-	0.03	-	0.02	-	-	-	-	-	-
C.D. @ 5%	0.09	-	0.11	-	0.07	-	-	-	-	-	-
C.V.%	1.10	-	1.49	-	1.81	-	-	-	-	-	-

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



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

Figure 1: Tolerance of wild and mutant *Trichoderma harzianum* with carbendazim at different concentrations

difference in phenotypic characters *i. e.*, mycelium growth, pigmentation, and sporulation (Selvakumar *et al.*, 2000). Mech *et al.*, 2006 tested wild and mutant *T. harzianum* in potato dextrose agar amended medium with different concentration viz., 0.01, 0.05, 0.1 and 1.0 per cent respectively. It was found that TM-3 mutant could tolerate upto 0.1 per cent of carbendazim while wild *T. harzianum* (Th-W) failed to grow at the same concentration. It is novel work because an attempt has been made in developing mutants of *T. harzianum* that are more adapted to fungicides as compared to their wild strains.

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