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ORIGINAL ARTICLE

UV-induced mutation in *Trichoderma harzianum* and effect of the putative mutants on plant pathogens

Fasna Beegam K and Susanta Banik*
Dept. of Plant Pathology, SASRD, Nagaland University, Medziphema campus, Nagaland-797106
*Corresponding author: susanta@nagalanduniversity.ac.in

ABSTRACT
To induce mutation in T. harzianum spore suspension (10^5/ml) was prepared from five days old culture and spread on Petri plates containing PDA medium. These Petriplates with the lid removed were exposed to ultraviolet irradiation for 5, 10,20,30,40 min. from a distance of 30 cm. Compared to wild strain which covered the whole plate in 6 days, it took only 4 days for the putative mutants. Fast growth was shown by all the mutants created. The highest growth was shown by mutants T. harzianum/20 min., T. harzianum/30 min. and T. harzianum/40 min. compared to parent or wild strain. In vitro evaluation on radial growth (mm) and sporulation rate of wild strain and mutants after four DOI showed that abundant sporulation was recorded by mutants T. harzianum/20 min. When all the UV putative mutants were tested against plant pathogens, the mutant of T. harzianum obtained after 20 minutes of UV exposure recorded highest inhibition of R. solani (85.18% versus 68.14% by parental strain), F. oxysporum (65.18% versus 56.29% by parental strain) and S. rolfsii (69.63% versus 59.26% by parental strain).
Keywords: UV Induced, Mutation, Radial Growth, Trichoderma harzianum

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INTRODUCTION

Biological control has been looked upon as a capable alternate method for plant disease management under the persistent circumstances of climate variation, food security and commercialization in agriculture [1]. *Trichoderma* is a natural bio control agent and it is well known for suppression of several plant pathogens. Since its discovery in 1930s [2], research on *Trichoderma* has yielded many successful bioformulations in recent time that are effective against plant diseases in field condition. The important mechanisms in control of plant pathogens are mycoparasitism, competition, induced r
esistance of plant host and antibiosis [3, 4]. It has been used successfully against seed-borne diseases, soil-borne diseases, storage rots and diseases in the phyllosphere[3, 5, 6]. Antagonistic ability of this fungus genus is mainly determined by extracellular enzymes produced by *Trichoderma*[7]. Owing to its properties of antagonism of plant pathogens, plant growth promotion, induction of systemic and localized resistance in plants and capability to survive in different environments *Trichoderma* has been regarded as one of the most successful fungal biocontrol agents for past few decades [8].*Trichoderma* spp. are highly sensitive to the fluctuations in environmental conditions, inconsistency in their performance and incompatibility of *Trichoderma* spp. with many agro-chemicals [9], though they perform well in the management of plant diseases. Addressing these issues, strain improvement in *Trichoderma* is very necessary which can be done by several means which includes molecular approaches like genetic modification and recombination techniques [10], mutation, transformation and protoplast fusion [11, 12] development of compatible consortia and use of adjuvants, spreaders and stickers in the formulation. *Trichoderma* spp. are highly sensitive to the fluctuations in environmental conditions, inconsistency in their performance and incompatibility of *Trichoderma* spp. with many agro-chemicals [9], though they perform well in the management of plant diseases. Addressing these issues, strain improvement in *Trichoderma* is very necessary which can be done by several means.

Therefore, induced mutation of *Trichoderma* spp. appears to be a way to obtain the promising strains of *Trichoderma* spp. to improve their disease control capability using ultra violet (UV) rays [13]. Mutation of *Trichoderma* spp. by different mutagens has been reported to bring about changes in morphological features like colony diameter, sporulation, dry mycelial weight and enzymes like β-1,3glucanase, β-1,4 glucanasse, cellulase and antibiotics like trichodermin, gliotoxin and viridian. Certain mutants of *Trichoderma* spp. have been found to be a better biocontrol agent (BCA) against phytopathogens as compared to their parent strains [14, 15]. Selection of such beneficial mutants of *Trichoderma* may be better option in plant disease management.In view of the above, the present investigation was undertaken with the aim of inducing mutation and studying the putative mutants against plant pathogens in vitro.

MATERIAL AND METHODS

UV mutagenesis

The method described by Hamad *et al*. [16] was used with slight modifications. Native strain of *T. harzianum* was sub cultured on PDA medium. Spore suspension was adjusted to 10^5spores/ml with the help of haemocytometer, from five days old culture was prepared and transferred to the sterilized Petri plates under aseptic condition in laminar chamber. These Petriplates with the lid removed were exposed to ultraviolet irradiation for 5, 10,20,30,40 min. from a distance of 30 cm in the laminar chamber. After irradiation, the plates were covered and incubated at 28±1^{0}C. The experiments were conducted in Complete Randomised Design(CRD) and three replications were maintained for each treatment.

The putative mutants were grown in pure culture by single spore isolation. Morphological study and cultural characterisation were done by mainly observing the colony diameter, sporulation and colour of the culture. All these features were compared with wild strain to ensure the persistence of the characteristics in the mutants.

Efficacy of putative mutants of *T. harzianum* against different fungal pathogens

Efficacy of mutants and parent were tested for their antagonistic potential(mycoparasitism) by dual culture technique against plant pathogens. Activity of mutants of *T.harzianum* was evaluated against different soil-borne pathogens *viz.*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum* under *in vitro* condition by adoption of dual culture technique [17]. The experiment was laid out in Completely Randomised Design (CRD) with three replications. Twenty ml sterilized PDA was aseptically poured in sterilized Petriplates and allowed to solidify .Mycelial discs(5mm) taken from the actively growing colonies of the test pathogens(7 days old culture) were placed simultaneously on the PDA seeded Petriplates opposite to each other, 1 cm apart from the periphery. Each treatment was replicated thrice. The inoculated Petriplates were incubated at27±2°C. First observation was taken just after contact of pathogen and antagonist and radial growth of the test pathogen was measured.

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

Where, I= Percent inhibition of pathogens by mutants

C= Radial growth in control (mm)

T= Radial growth in the treatment (mm)

Statistical analysis and interpretation

The data was subjected to analysis by Fisher's method of analysis of variance. Significance of variance among the data was calculated out by calculating the F value and comparing it with the tabulated value of F [18].The treatment means were also compared among themselves by calculating Critical difference.

RESULTS AND DISCUSSION

Induction of mutation in *T. harzianum* by UV radiation

To induce mutation in *T. harzianum*spore suspension (10^5/ml) was prepared from five days old culture and spread on Petri plates containing PDA medium. These Petriplates with the lid removed were exposed to ultraviolet irradiation for 5, 10,20,30,40 min. from a distance of 30 cm.

As per the results mentioned in Table 1 and Table 2, fast growth was shown by all the putative mutants created. Growth was very less in parental strain compared to UV-induced mutants. Wild strain showed 78.60 mm colony diameter after 4 DOI with poor sporulation compared to the putative UV mutants which showed colony diameter of 90.00 mm with good or abundant sporulation 96 hours after inoculation.

Many of the successful endeavours had been made to improve the potential of *Trichoderma* species by exposing the spores to physical mutagen, UV rays and chemical mutagen EMS [19-25].

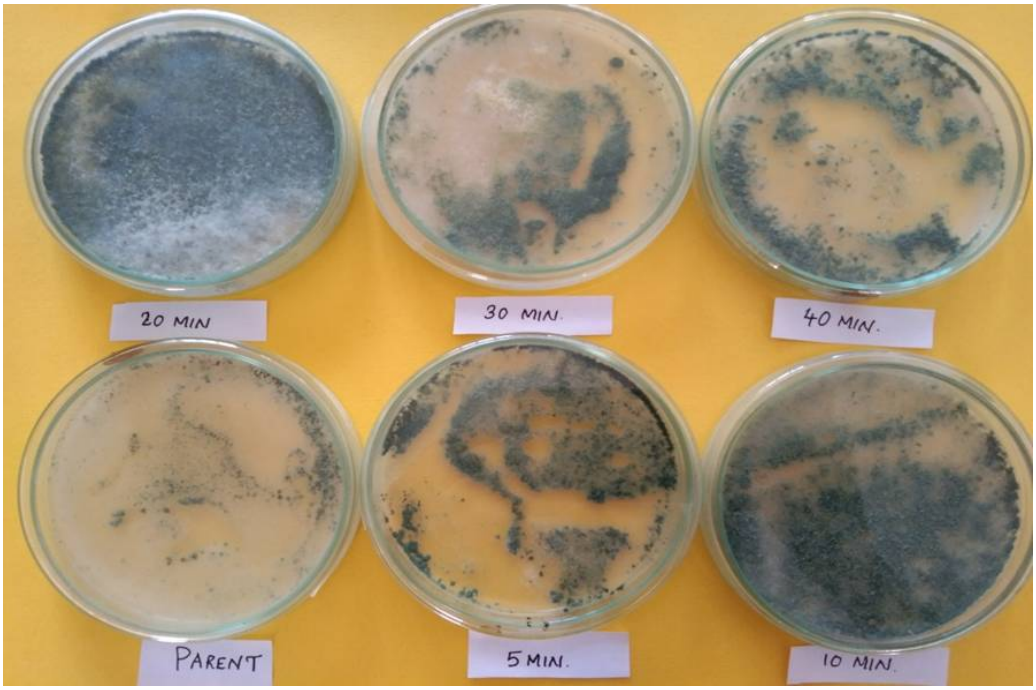

Plate 1. Growth differences between wild and UV-irradiated isolates of*Trichoderma harzianum*

Comparison of putative mutants of *T. harzianum* with its wild type

The mutants were grown in pure culture by single spore isolation. Morphological study and cultural characterisation were done by mainly observing the colony diameter, sporulation and colour of the culture. All these features were compared with wild strain to ensure the persistence of the characteristics in the mutants.

In vitro evaluation on colony diameter of parent strain and UV mutants is presented in Table 1. The data presented in Table 1 clearly depicted that UV irradiation has resulted in significant increase in mycelial growth of *Trichoderma harzianum* compared to control in all the days of observations. However, the highest radial growth of *T. harzianum* was recorded 90 mm with 20 minutes of exposure to UV-radiation compared to 78.60mm in control. UV-irradiation to *T. harzianum* for more time like 30 minutes and 40 minutes didn't result in higher growth than 20 minutes of exposure (96 hours after inoculation). UV-irradiation of *T. harzianum* for five minutes and 10 minutes recorded 86.00 mm and 89.30mm mycelial growth respectively that are statistically at par with each other and also with that of 20 minutes of exposure to UV light 96 hours post-inoculation.

The findings of the present investigation are in agreement with Abdollah *et al*. [26]and Sreenivasalu *et al.* [27]. Abdollah *et al*. [26] observed that the morphological characteristics like colonies shape, color, sporulation and mycelia growth rate could be changed by UV-irradiation of different duration. Sreenivasalu *et al.*[27] also reported that mutagenesis generated altered characteristics compared to its wild. Number of obtained mutants showed morphological changes in growth properties such as colony appearance, colony color, sporulation rate and pigmentation.

Putative mutants and the parents were tested *in vitro* against different phytopathogens mainly *Rhizoctonia solani, Fusarium oxysporum and Sclerotium rolfsii* to study their antagonistic potential.

Effect of antagonistic activity of wild strain and UV mutants against *Rhizoctonia solani*

All the UV putative mutants along with the parental strain were tested against the pathogen. Highest mycelial inhibition percentage was shown by *T. harzianum*/ 20 minute of 85.18%. Least inhibition percentage was recorded by the parental strain of 68.14%. Percent mycelial inhibition shown by mutants were in the range of 74.07 to 85.18 % (Table 3, Plate 2).

The findings are in conformity with the results obtained by various workers [13, 27, 28] who tested the *Trichoderma* species mutants against *R. solani* and observed successful inhibition of this pathogen by dual culture.

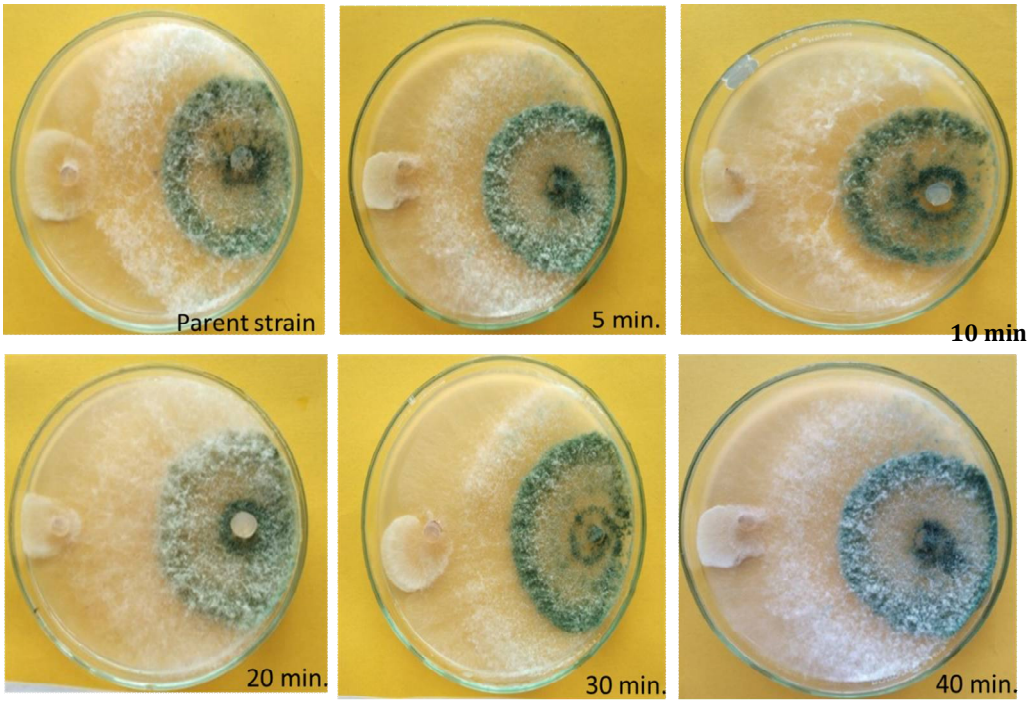

Plate 2: Effect of antagonistic activity of wild and UV mutants against *R. solani*

Effect of antagonistic activity of wild and UV mutants against *Fusarium oxysporum*

Results showed that *in vitro* inoculation of *T. harzianum*/ 20 min UV mutant exhibited maximum mycelial inhibition of *F. oxysporum* up to 65.18% followed by other UV mutants under study i.e. *T. harzianum*/ 40 min UV, *T. harzianum*/ 30 min UV, *T. harzianum*/ 5 min UV, *T. harzianum*/ 10 min UV over the control *in vitro*. Least mycelial inhibition percent exhibited by parent *T. harzianum*up to 56.29%. The pattern of parasitization was almost similar among the parent and mutants (Table 3, Plate 3).

The findings are in agreement with Ahmed and Baker [29] who reported that the mutants of *T. harzianum* are more antagonistic to plant pathogenic fungi than their wild parental counterpart.

Papavizas and Lewis [30] tested soil-borne plant-pathogenic fungi *(Fusarium oxysporum*and *Sclerotium rolfsii*) and UV-induced mutants by dual culture technique, observed complete inhibition of these pathogens by UV- induced mutants while the wild strain did not.

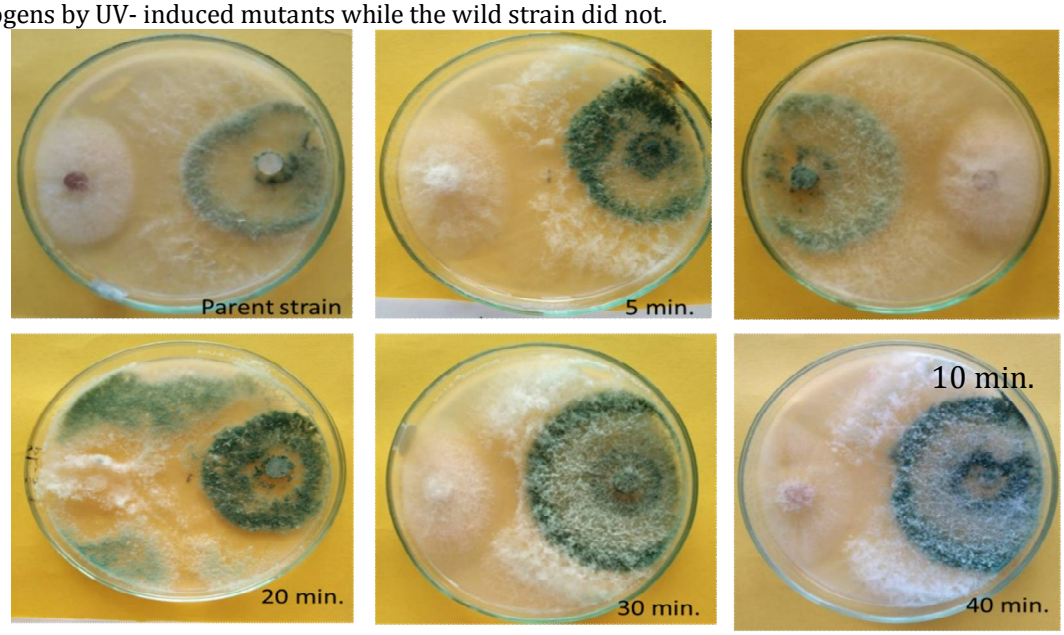

Plate 3. Effect of antagonistic activity of wild and UV mutants against *F. oxysporum*

Effect of antagonistic activity of wild and UV mutants against *Sclerotium rolfsii*

The UV mutants developed under different durations of UV of *T. harzianum* along with their parent were evaluated for antagonistic action against the pathogen *S. rolfsii*. The mutant *T. harzianum*/ 20 min UV gave maximum mycelial inhibition of 69.63 %. This was followed by *T. harzianum*/ 40 min UV with 64.44% followed by percent inhibition of 63.7% by *T. harzianum*/ 30 min UV and followed by *T. harzianum*/ 5 min UV with 60 % and followed by parent *T. harzianum*with 59.26% (Table 3, Plate 4).

The present findings are in agreement with Rajappan*et al*. [31] who reported the use of mutant strain of *T. viride* (induced mutation by UV radiation) to control *S. rolfsii* and this mutant strain provided higher efficacy than its wild type strain.

Alfiky[32]selected five *Trichoderma* mutants which were either statistically equal to or significantly better than the parent when tested against *S. rolfsii* and *R. solani,* with mutant scoring the highest growth inhibition at 76.6% and 78.3%, respectively. Similarly, all five selected *Trichoderma* mutants were either equal to or significantly better than their original wild type when challenged with the pathogens. Mutant scored 67.4% growth inhibition against *S. rolfsii* as the highest performing mutant, and mutant performed the best against *R. solani*, with a growth inhibition value of 56.3% [32].

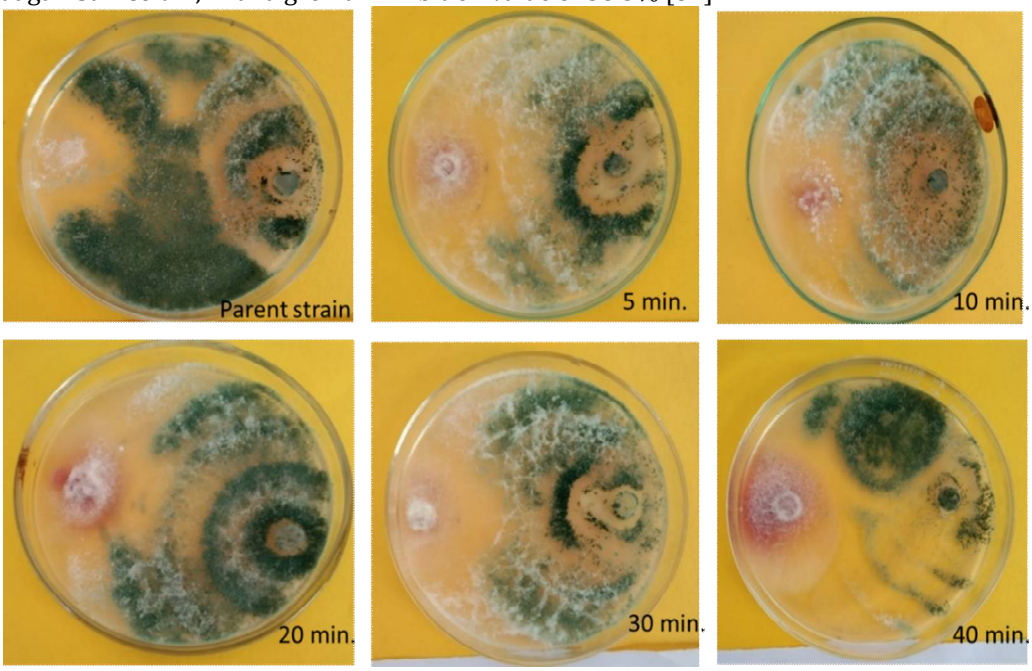

Plate 4. Effect of antagonistic activity of wild and UV mutants against *S. rolfsii*

Table 1: *In vitro* evaluation on colony diameter (cm) of parent strain and UV putative mutants

TREATMENTS	24 Hours	48 Hours	72 Hours	96 Hours
T_0Control): Parental strain of *Trichoderma harzianum*	0.00	1.13	4.60	7.87
T_1 *Trichoderma harzianum*/ 5 min UV	1.07	4.20	6.47	8.60
T_2 *Trichodermaharzianum*/10 min UV	1.33	4.33	7.20	8.93
T_3*Trichoderma harzianum*/20min UV	1.00	3.80	6.47	9.00
T_4*Trichoderma harzianum*/30 min UV	1.20	3.87	7.53	9.00
T_5 *Trichoderma harzianum*/40 min UV	1.47	4.47	6.87	9.00
SEm±	0.12	0.23	0.23	0.19
CD (p=0.05)	0.36	0.72	0.72	0.59

Table 2: Radial growth (mm) and sporulation of wild strain and putative mutants of *T. harzianum* four days after inoculation

Treatments	Radial growth(mm) on PDA plates four days after inoculation	Sporulation after four DOI
T_0 (Control)-Parental Strain of Th	78.70	Poor
T_1 .Th/5 min UV	86.00	Good
T_2-Th/10 min UV	89.30	Moderate
T_3-Th/20 min UV	90.00	Abundant
T_4 -Th/30 min UV	90.00	Moderate
T_5 -Th/40 min UV	90.00	Moderate

Table 3: Antagonistic activity of putative UV mutants against plant pathogens *in vitro*.

Treatments	Mycelial inhibition percentage over control		
	R. solani	*F. oxysporum*	*S. rolfsii*
T_0: Parent *T. harzianum*	68.14 (55.64)	56.29 (48.61)	59.26 (50.40)
T_1: *T harzianum*/ 5 min UV	74.07 (59.42)	57.77 (49.47)	60.00 (50.80)
T_2:*T.harzianum*/ 10 min UV	75.53 (60.45)	56.59 (48.78)	59.26 (50.35)
T_3:*T.harzianum*/ 20 min UV	85.18 (67.40)	65.18 (53.86)	69.63 (56.58)
T_4: *T. harzianum*/ 30 min UV	78.52 (62.39)	57.77 (49.47)	63.70 (52.95)
T_5: *T. harzianum*/ 40 min UV	77.03 (61.37)	59.25 (50.33)	64.44 (53.43)
SEm±	1.20	0.83	2.14
CD@5%	3.71	2.57	6.59

Figures in parentheses are arc sine transformed values

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COMPETING INTEREST

The authors have declared that no competing interest exists.

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