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Antagonistic Property of the Stable *Trichoderma* Mutants against *Fusarium oxysporum* f.sp. *Lentis* and *Rhizoctonia solani*

Tulipa De, Ayon Roy

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ABSTRACT

Antagonistic *Trichoderma* species can be considered as promising biocontrol agents. Expected mechanisms of antagonism acted by *Trichoderma* sp. include antibiosis, competition, mycoparasitism, hyphal interactions and enzyme secretion. An experiment was designed at Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya during 2019-2020. Two best strains of *Trichoderma* i.e., UBT-18 (*T. harzianum*) and T21 (*T. asperellum*) were used. 'Bangle method' was used for determining antagonistic potential of UV mutated stable *Trichoderma* isolates as well as their respective parental strains against two major soil borne pathogens viz., *Fusarium oxysporum* f.sp. *lentis* and *Rhizoctonia solani*. The present study showed that T1strain of T21 was most effective in its inhibition ability against both the pathogens but UBT-18 could not give better performance due to its slow growth.

Tulipa De*[1], Ayon Roy[2]
[1]PhD Scholar, [2]Professor
Department of Plant Pathology,
Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar 736165, West Bengal, India

Email: tulipade2015@gmail.com
*Corresponding author

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INTRODUCTION

Among the widely used Biocontrol agents, *Trichoderma* spp. have appeared as the most potential agents for their unique biocontrol activities and for having a wide array of beneficial plant interactions (Nawrocka and Malolepsza 2013). The balance between spore formation and hyphal growth is critical to the development of *Trichoderma* both in the soil and rhizosphere (Steyaert *et al*. 2010). Pathogens that can be checked by *Trichoderma* include *Fusarium*, *Rhizoctonia, Pythium, Phytophthora, Sclerotinia*, and *Verticillium*. Antagonistic *Trichoderma* species can be considered as promising biocontrol agents. Expected mechanisms of antagonism acted by *Trichoderma* sp. include antibiosis, competition, mycoparasitism, hyphal interactions and enzyme secretion.

During the past few decades, genetic improvement of *Trichoderma* spp. by induced mutation using physical and chemical mutagens have been attempted successfully to ameliorate the efficacy of native strains (Walnuj and Jhon 2013). The transition from vegetative growth to conidial phase can be triggered in *Trichoderma* by the application of UV-blue light, low pH, nutrient stress, or mechanical injury to the mycelium. Certain mutants of *Trichoderma* spp. have been to have better rhizosphere competence compared to their parent strains. Selection of such beneficial mutants may be a better avenue for the management of plant pathogens.

MATERIALS AND METHODS

The experiments were conducted at Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya during 2019-2020. The details of experimental procedures adopted, materials used and techniques followed during the course of present investigation are described below.

Media used

1. Potato Dextrose Agar (PDA) - was used with compositions as suggested by Riker and Riker (1936).
2. Potato Dextrose Broth -It is helpful for sporulation of test pathogen and in *Trichoderma* mass multiplication.
3. *Trichoderma* specific medium (TSM) – was used having components as described by Elad *et al*. (1981) and later modified by Saha and Pan (1997). The medium was used for selective isolation of *Trichoderma* from soil and for isolation of mutated *Trichoderma* isolates after exposure to different doses of UV radiation. The viability of mutated isolates was also examined on this medium and then studied through generation study.

Native *Trichoderma* strains used

Two best strains of *Trichoderma* i.e., UBT-18 and T21 were used. These strains were isolated from soil of Cooch Behar (vegetable plots, tea plantation).

Fungal pathogens used

Two major soil borne pathogens viz., *F. oxysporum* f.sp. *lentis* and *R. solani* were isolated from diseased plants of lentil and rice, respectively on PDA for studying the antagonistic effect of mutated *Trichoderma* strains against them.

Antagonistic potential determination of *Trichoderma* using 'Bangle method'

The 'Bangle method'(Singh *et al*. 2004) was used for determining antagonistic potential of UV mutated stable *Trichoderma* isolates as well as their respective parental strains against the test pathogens. In this method a pathogen disc (8-9 mm diameter) having mycelia was cut by using cork borer from the periphery of the colony and inoculated centrally in a petri plate containing 20 ml sterilized PDA. Around this pathogen disc, a sterilized bangle dipped in conidial suspension of mutated *Trichoderma* was placed. Requisite replications (3-4) of each treatment of *Trichoderma* strains were maintained. In control plates, only free growth of the pathogens was allowed or sterilized bangles were placed without *Trichoderma* suspensions. Both control plates and antagonist inoculated petri plates were incubated in BOD at 28±1° C. Inhibition by *Trichoderma* on growth of pathogen with respect to control was calculated to get the inhibition percentage using the formula given below.

% of inhibition={(Growth of pathogen in control plate–growth of pathogen in

Trichoderma treated plate)/growth of pathogen in control plate} ×100

RESULTS AND DISCUSSION

Dual culture done using the Bangle method as described in and results are presented in Table 1, Fig.1 and Table 2, Fig. 2. The biocontrol efficacy was tested against two test pathogens. It is evident from Table 1 that the treatment T9 of UBT-18 (*T. harzianum*) exhibits higher percentage inhibition of growth over control (47.22%) compared to wild UBT-18 (40.98%) incase of *Fusarium oxysporum* f.sp. *lentis*. The lowest percent inhibition of growth over controlis shown by T2 (41.67). Therefore, treatment T9 exhibits 6.24% increase of inhibition overwild isolate. On the other hand, UBT-18 does not show better inhibition against *Rhizoctonia solani* where the wild UBT-18 shows 68.89% inhibition of growth over control but the maximum inhibition percentage was seen in case of T8 reaching only 37.78%. T5 shows the lowest percent inhibition of growth over control (20.11%). In this case, treatment T8 shows 31.11% decrease of inhibition over wild isolate. From the Table 2 it has been clearly visible that treatment T1 of T-21 (*T. asperellum*) exhibits higher percent inhibition of growth of *Fusarium oxysporum* f.sp. *lentis* over control (72.78%) compared to the wild isolate showing only 37.31% inhibition. T1 shows 35.47% increase of inhibition over wild isolate. The lowest inhibition

Table 1. Antagonistic potential of *T. harzianum* (UBT-18) against soil borne plant pathogens by Dual culture method.

Fungal strain/mutant *T. harzianum*(UBT-18)	*Fusarium oxysporum* f.sp.*lentis*		*Rhizoctonia solani*	
	% inhibition of growth over control	% increase or decrease of inhibition over wild isolate	% inhibition of growth over control	% increase or decrease of inhibition over wild isolate
Wild UBT 18	40.98		68.89	
T2	41.67	0.69	22.89	(-) 46.00
T4	46.00	5.02	23.78	(-) 45.11
T5	44.44	3.46	20.11	(-) 48.78
T6	42.22	3.24	22.44	(-) 46.45
T7	44.44	3.46	22.67	(-) 46.22
T8	45.33	4.35	37.78	(-) 31.11
T9	47.22	6.24	32.22	(-) 36.67
SEm±	1.14		3.59	
CD(P=0.05)	NS		10.54	

Fig.1. Dual culture by 'Bangle method' of mutated UBT-18 *Trichoderma* strain against pathogens.

Table 2. Antagonistic potential of *T. asperellum* (T-21) against soil borne plant pathogens by Dual culture method.

Fungal strain /mutant *T. asperellum* (T-21)	*Fusarium oxysporum* f.sp.*lentis*		*Rhizoctonia solani*	
	% inhibition of growth over control	% increase or decrease of inhibition over wild isolate	% inhibition of growth over control	% increase or decrease of inhibition over wild isolate
Wild T-21	37.31		38.43	
T1	72.78	35.47	70.44	32.01
T2	68.33	31.02	43.56	5.13
T3	70.56	33.25	45.78	7.35
T4	62.11	24.80	52.22	13.79
T5	66.67	29.36	33.56	(-) 4.87
SEm±	0.66		2.65	
CD(P=0.05)	2.00		8.02	

percentage of growth over control was shown by treatment T4 (62.11%) but still it has 24.80% increase of inhibition over wild type. This explains that all mutants of T-21 were more efficient in controlling *Fusarium oxysporum* f.sp.*lentis*. In case of the pathogen *Rhizoctonia solani* also, treatment T1 shows higher percent inhibition of growth over control (70.44%) compared to the wild isolate (38.43%). Here, T1 has 32.01% increase of inhibition over wild isolate. But T5 shows only 33.56% inhibition of growth over control exhibiting 4.87% decrease of inhibition over wild isolate.

In summary, T1 strain of T21 shows highest inhibition ability against both the test pathogen.UBT-18 could not give better performance due to its slow growth. Sufficient competitive ability is not present in bio control agents to replace a pathogen which is already established. Therefore, co-inoculation of pathogens was done at the same time with the antagonist to get better efficacies. Mutagenesis is an approach that induces the genetic variations of targeted organisms. Induced changes could either be random or targeted depending on the selected mutagenesis technique. Polyketide synthase is a multi-domain enzyme that is responsible for the production of the secondary metabolite polyketides. Hertweck (2009) showed a five-fold increase of polyketide synthasein the mutant strain compared to the parental strain. The

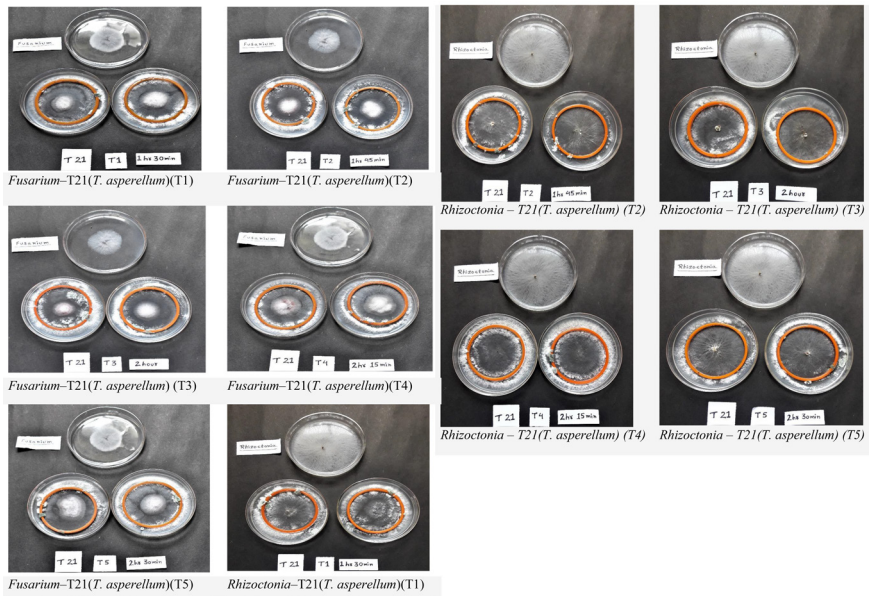

Fig. 2. Dual culture by 'Bangle method' of mutated T21 *Trichoderma* strain against pathogens.

investigation of Zhang *et al*. (2013) relayed that the mutant *T. harzianum* T-E5 manifested 30.2% exalted indole acetic acid yield compared to its wild kind. This rise enhanced plant-root colonization and plant biomass. It was reported that a UV-induced single point mutation localized to a regulatory domain in a UV-derived *T. reesei* mutant, had significantly increased xylanase expression and protein secretion. The observations of Wagh *et al*. 2015, Alfiky 2019 stated the possible mechanisms of T1 isolate of T21 against *Fusarium oxysporum* f. sp.*lentis*.

CONCLUSION

The antagonistic potential of stable mutated *Trichoderma* isolates as well their respective parental strains against the soil borne fungal pathogens indicated that treatment T9 of UBT-18 (*T. harzianum*) exhibited higher percentage inhibition of growth over control compared to the wild UBT-18 in case of pathogen *Fusarium oxysporum* f.sp. *lentis*. On the other hand, treatment T8 of UBT-18 was able to inhibit growth over control which was less compared to the wild one in case of *Rhizoctonia solani*. In case of *Fusarium oxysporum* f.sp. *lentis*, treatment T1 of T21 (*T. asperellum*) exhibit higher percent inhibition of growth over control compared to the wild isolate. All the mutants of T21 were more efficient in controlling *Fusarium oxysporum* f.sp. *lentis* compared to the wild one. In case of pathogen *Rhizoctonia solani* also, treatment T1 showed higher percentage of inhibition of growth over control compared to the wild isolate. In conclusion, T1 strain of T21was most effective in its inhibition ability against both the pathogens but UBT-18 could not give better performance due to its slow growth.

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