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Induction of Antagonistic Potential In Trichoderma Viride By Using Chemical Mutagen

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Abstract:
Total 112 mutants were obtained from wild *Trichoderma viride* after treatment with Sodium azide mutagen at different time intervals and by using various concentrations. Among these mutants, Tv_{SA-56}showed highest inhibition percentage **(66.66%)** of *Pythium aphanidermatum* causing rhizome rot of turmeric. Whereas wild *Trichoderma viride* showed **58.88%** inhibition of *Pythium aphanidermatum*, which was lower than Tv_{SA-56}.Therefore, chemical mutagens can be used to induce antagonistic potential in *Trichoderma viride* for prevention of *Pythium aphanidermatum*and to reduce disease incidence in Turmeric.
Key Words: *Trichoderma viride, Pythium aphanidermatum,* Antagonistic potential, Chemical mutagen.

Introduction:

Turmeric (*Curcuma longa* L.) is an ancient, aromatic herbaceous plant belongs to Zingiberaceae. It is an important spice of India and its rhizomes are commercially important and are used for colouring, as a dye, spice and condiment and used in various ayurvedic medicinal preparations.In 1280, **Marco Polo** first discovered turmeric in China and referred it as **'Poor man's Saffron'**(Lal, 2012).He characterized turmeric as a vegetable having properties similar that of saffron (Shaikh, 2013). Vogel and Pelletier (2012) reported yellow coloured component from Turmeric and named it as 'Curcumin' which was found to be mixture of resin and turmeric oil. (Gupta et al., 2012).Turmeric rhizomes are affected by various fungal pathogens, *Pythium aphanidermatum* causing rhizome rot is most dominant one. It is one the highly destructive pathogen responsible for great yield loss. Water lodged conditions in field favors conditions for disease development and results in increased disease incidence (Hindustan times, 2018). Along with applications of various agrochemicals different biological control agents are also used for disease management. *Trichoderma* species are most dominantly used fungal biocontrol agent against large number of fungal plant pathogens such as,*Rhizoctoniasolani, Rhizoctonia bataticola*(Vasudevaand Chakravarthi,1954). For obtaining better results researchers are focusing on different techniques used for strain improvement of biocontrol agents. Induced mutation is one of them. Strain improvement is done for various aspects which may include increase in enzyme production such as cellulases, xylanases, chitinases, increase in production of antibiotics and antagonistic potential of antagonist used. (Joes et al., 2005; Normansell, 1982; Nakkeeran et al., 2005).Therefore, in present investigation efforts have been made to induce antagonistic potential in *Trichoderma viride*against *Pythium aphanidermatum* causing rhizome rot of turmeric by application of chemical mutagen Sodium azide.

Material And Methods:

Isolation of *Trichoderma* species:

Trichoderma viride was isolated from soil samples collected from turmeric rhizospheres of different districts of Maharashtra state. Collected soil samples were screened for isolation of *Trichoderma* species by using Soil plate method (Warcup, 1947). Isolated *Trichoderma* species were identified by using key of *Trichoderma* given by Bissette (1991).

Isolation of *Pythium aphanidermatum*:

*Pythium aphanidermatum*was isolated from diseased rhizomes of turmeric collected from different districts of Maharashtra. Collected turmeric rhizomes were washed by using tap water, blotted by using sterile blotting paper. Then surface sterilized with 70 % alcohol and washed 2-3 times with sterile distilled water. These washes turmeric pieces cut into small pieces and inoculated onCzapek Dox Agar medium.Identification of *Pythium aphanidermatum* was done by using *Pythium* monograph given by Van der PlaatsNiterink (1981).

Mutagenesis of *Trichoderma viride* culture:

Preparation of Mycelial suspension:

Seven days old culture of *Trichoderma viride* was used for preparation of mycelial suspension. Sterile distilled water is added in the pure culture of *T. viride* and rubbed by using sterile glass rod. This mycelial suspension is transferred to a beaker and used for chemical mutagenesis by using Sodium azide.

Treatment with Sodium azide:

Mycelial suspension is treated with various concentrations of Sodium azide such as 1, 0.5, 0.1, 0.01, 0.001 ppm solutions and at the time interval of 5 and 10 minutes. Treated mycelial suspension was inoculated on petri plates containing Czapek Dox agar medium. Inoculated petri plates incubated for 2-3 days and each colony was separately re-inoculated on petri plate with CDA culture medium. These re-inoculated petri plates incubated for 7 days at 28±2°c in BOD Incubator and used for evaluation of their antagonistic potential

Determination of antagonistic potential of Mutants:

Sodium azide mutants obtained from the treatment were screened for their antagonistic potential against *Pythium aphanidermatum*using dual culture technique(Morton and Stroube, 1955; Skidmore and Dickinson, 1976). 8mm disc of Chemical mutants of *Trichoderma viride* and *Pythium aphanidermatum* were inoculated on polar ends of CDA culture media containing petri plates. These inoculated plates incubated for 7 days at 27±2°c in BOD Incubator. Petri plate inoculated with wild *Trichoderma viride* and pathogen considered as control. Readings were recorded on the 7th day of incubation period. Percent inhibition was calculated by using Vincet's formula (1947):

$$\text{Inhibition Percentage (\%)} = \frac{C - T}{C} \times 100$$

Where,

I = Percent Growth Inhibition (%)
C = Colony Diameter in Control plate
T = Colony Diameter in treatment plate

Results And Discussion:

Wild *Trichoderma viride* treated with different concentrations of Sodium azide at the time interval of 5 and 10 minutes resulted into 112 SA induced mutants of *Trichoderma viride*. All mutants of showed antagonistic activity against *Pythium aphanidermatum*. Highest antagonistic activity was observed in 0.1 ppm concentration (22.3%) which was followed by 0.5 ppm (21.4%), 1 ppm (20.5%) and 0.01 ppm (18.7%). Whereas lowest mutation frequency was observed in 0.001 ppm solution which was 16.9%.

Table1: Induction of antagonistic potential in *Trichoderma viride* with treatment with different concentration of Sodium azide:

No.	**ncentration of Sodium azide**	**ne interval**	**mber of Mutant Colonies**	**Frequency percentage (%)**
1	1 ppm	inutes	11	20.53
		minutes	12	
2	0.5 ppm	inutes	12	21.42
		minutes	12	
3	0.1 ppm	inutes	13	**22.3**
		minutes	12	
4	0.01 ppm	inutes	10	18.7
		minutes	11	
5	0.001 ppm	inutes	09	16.9
		minutes	10	
Total number of SA mutant colonies			**112**	

Obtained SA- mutants and wild *Trichoderma viride*were screened for antagonistic efficacy against *Pythium aphanidermatum*turmeric causing rhizome rot. Among 112 SA- mutants obtained, mutant no. 56 i. e. T_{vsa56}showed highest antagonism with inhibition percentage 66.66%. Out of 112Mutants, 40.17% SA-mutants showed more antagonistic potential than Wild *Trichoderma viride*having 58.80 inhibition

percentage of *Pythium aphanidermatum*. Therefore, SA induced mutants of *Trichoderma viride* found to be more antagonitstic to *Pythium aphanidermatum*. Induction of mutation in *Trichoderma* species for maximum inhibition of fungal pathogens is considered as most important method in strain improvement of biocontrol agents. After obtainment mutants, their bio-efficacy is tested against fungal pathogens by using different methods such as- field trials, dual culture technique etc. Many researchers have been worked on chemical mutagenesis of fungi for strain improvement. Shafique (2011) used Ethyl Methane Sulfonate (EMS) for chemical mutagenesis of *Trichoderma viride* for maximum production of cellulase. Kumar et al., (2015) used combined application of UV treatment and chemical mutagen Ethyl Methane Sulfonate (EMS) for high production of ß-glucosidase, xylanase, cellulases in *Aspergillus terreus* D-34. Nakkeeran et al., (2005) conducted experiment on chemical mutagenesis of *T. viride* by treating with Nitroguanidine and obtained mutants screened for antagonistic potentiality against *Rhizoctonia solani* causing cotton root rot. Results revealed that seed treatment with MG-6 mutant recorded lowest disease incidence and performed better than parent *Trichoderma viride*. Singh et al., (2016) tested antagonistic potentiality of *Trichoderma harzianum* and *T. atroviride* mutants, produced by treatment with N'-methyl-N'-nitro-N'-guanidine against *Sclerotium rolfsii*. According to them when both parent and mutant strains of *Trichoderma harzianum* and *T. atroviride*applied for field trial experiments, mutant isolates suppressed pathogen *Sclerotium rolfsii*significantly and reduces disease incidence when incorporated in the soil. Patil (2020) treated *Trichoderma asperellum* with Ethyl Methane Sulfonate (EMS) and 5- Bromouracil (5-BU) and concluded that mutants obtained were more superior in antagonistic activity as compared to wild *Trichoderma asperellum with* highest inhibition percentage against *Rhizoctonia solani* causing blight in Blackgram.

Conclusion:

Chemical mutation by using Sodium azide can be used for strain improvement in *Trichoderma viride*for increased antagonistic activity against *Pythium aphanidermatum*causing Turmeric rhizome rot.

Table 2: Study of antagonistic potential of *Trichoderma viride*varients (T_{wild} and T_{mutant}–Sodium azide treated) against *Pythiumaphanidermatum*:

. No.	*T. viride*mutant	Inhibition percentage (%)	. No.	*viride*mutant	Inhibition percentage (%)	r. No.	*viride*mutant	Inhibition percentage (%)
1.	T_{vsa1}	57.7	41.	T_{vsa41}	50.5	81.	T_{vsa81}	60.0
2.	T_{vsa2}	47.7	42.	T_{vsa42}	51.1	82.	T_{vsa82}	55.5
3.	T_{vsa3}	55.5	43.	T_{vsa43}	50.5	83.	T_{vsa83}	64.4
4.	T_{vsa4}	50.5	44.	T_{vsa44}	61.1	84.	T_{vsa84}	54.4
5.	T_{vsa5}	54.4	45.	T_{vsa45}	64.4	85.	T_{vsa85}	52.2
6.	T_{vsa6}	56.6	46.	T_{vsa46}	54.4	86.	T_{vsa86}	55.5
7.	T_{vsa7}	46.6	47.	T_{vsa47}	60.0	87.	T_{vsa87}	54.4
8.	T_{vsa8}	57.7	48.	T_{vsa48}	55.5	88.	T_{vsa88}	61.1
9.	T_{vsa9}	62.2	49.	T_{vsa49}	53.3	89.	T_{vsa89}	58.5
10.	T_{vsa10}	50.0	50.	T_{vsa50}	60.0	90.	T_{vsa90}	63.3
11.	T_{vsa11}	61.1	51.	T_{vsa51}	57.7	91.	T_{vsa91}	57.7
12.	T_{vsa12}	61.1	52.	T_{vsa52}	53.3	92.	T_{vsa92}	56.6
13.	T_{vsa13}	55.5	53.	T_{vsa53}	50.5	93.	T_{vsa93}	64.4
14.	T_{vsa14}	60.0	54.	T_{vsa54}	55.5	94.	T_{vsa94}	57.7
15.	T_{vsa15}	55.5	55.	T_{vsa55}	54.4	95.	T_{vsa95}	63.3
16.	T_{vsa16}	61.1	56.	**T_{vsa56}**	**66.6**	96.	T_{vsa96}	60.0
17.	T_{vsa17}	50.5	57.	T_{vsa57}	58.8	97.	T_{vsa97}	60.0
18.	T_{vsa18}	47.7	58.	T_{vsa58}	57.7	98.	T_{vsa98}	55.5
19.	T_{vsa19}	46.6	59.	T_{vsa59}	60.0	99.	T_{vsa99}	61.1
20.	T_{vsa20}	56.6	60.	T_{vsa60}	56.6	100.	T_{vsa100}	55.5
21.	T_{vsa21}	55.5	61.	T_{vsa61}	56.6	101.	T_{vsa101}	54.4
22.	T_{vsa22}	55.5	62.	T_{vsa62}	53.3	102.	T_{vsa102}	61.1
23.	T_{vsa23}	51.1	63.	T_{vsa63}	57.7	103.	T_{vsa103}	57.7

24.	T_{vsa24}	61.1	64.	T_{vsa64}	55.5	104.	T_{vsa104}	61.1
25.	T_{vsa25}	61.1	65.	T_{vsa65}	62.2	105.	T_{vsa105}	62.2
26.	T_{vsa26}	64.4	66.	T_{vsa66}	62.2	106.	T_{vsa106}	61.1
27.	T_{vsa27}	57.7	67.	T_{vsa67}	58.8	107.	T_{vsa107}	57.7
28.	T_{vsa28}	62.2	68.	T_{vsa68}	50.0	108.	T_{vsa108}	55.5
29.	T_{vsa29}	57.7	69.	T_{vsa69}	60.0	109.	T_{vsa109}	55.5
30.	T_{vsa30}	56.6	70.	T_{vsa70}	64.4	110.	T_{vsa110}	57.7
31.	T_{vsa31}	61.1	71.	T_{vsa71}	61.1	111	T_{vsa111}	57.7
32.	T_{vsa32}	54.4	72.	T_{vsa72}	60.0	112.	T_{vsa112}	61.1
33.	T_{vsa33}	64.4	73.	T_{vsa73}	62.2			
34.	T_{vsa34}	50.5	74.	T_{vsa74}	55.5			
35.	T_{vsa35}	62.2	75.	T_{vsa75}	57.7			
36.	T_{vsa36}	63.3	76.	T_{vsa76}	61.1			
37.	T_{vsa37}	55.5	77.	T_{vsa77}	55.5			
38.	T_{vsa38}	53.3	78.	T_{vsa78}	63.3			
39.	T_{vsa39}	60.0	79.	T_{vsa79}	62.2			
40.	T_{vsa40}	61.1	80.	T_{vsa80}	55.5			
Control: 58.80								
Total number of SA Mutant isolates: 112								

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