AUCTORES
Globalize your Research

Journal of Clinical Research and Reports

Meshu Shewarega Foyate*

Open Access

Research Article

Improvement in Biocontrol Ability of Trichoderma Through Chemical Mutation and Gamma Irradiation for The Control of Soil Borne Pathogens

Meshu Shewarega Foyate*

College of Agriculture and Natural Resource, Department of Plant Science, Wolkite University

***Corresponding Author:** Meshu Shewarega Foyate, College of Agriculture and Natural Resource, Department of Plant Science, Wolkite University.

Received date: January 11, 2024; Accepted date: January 22, 2024; Published date: January 31, 2024

Citation: Meshu S. Foyate (2024), Improvement in Biocontrol Ability of Trichoderma Through Chemical Mutation and Gamma Irradiation for The Control of Soil Borne Pathogens, *J Clinical Research and Reports*, 15(1); **DOI:10.31579/2690-1919/347**

Abstract

Biocontrol agent like Trichoderma viride was acclaimed as effective, eco-friendly and cheap, nullifying the ill effect of chemicals, These biocontrol agents are identified to act against on array of important soil borne plant pathogens viz.,Sclerotium rolfsii, Rhizoctonia bataticola and Fusarium oxysporum f. sp. ciceri etc." Improvement in biocontrol ability of Trichoderma viride through mutation" was carried at Department of Plant Pathology, during 2019-2020.

The antagonistic ability of a biocontrol agent was determined by its physiological state so that change in physiological or genetically conditions could alter the antagonism. Hence the genetic modification using mutagenesis offers the potential for producing improved bio-protection is likely to enhance their biocontrol capabilities against soil borne pathogen. Mutagenesis by chemical i.e. Ethyl Methyl Sulphonate (EMS) @ 100, 150, 200 and 250 µl/ml and time interval at 30, 45 and 60 minutes. The influence of gamma irradiation on improving production of antifungal metabolites and biological proficiency of the biocontrol agent against soil borne pathogen. Trichoderma viride were irradiated in different dose of gamma radiation (cobalt-60) @ 0, 20, 30, 40 and 50 k-rad. for induced mutation.

Sixteen Trichoderma viride mutants were obtained after chemical mutagenesis and gamma irradiation and tested for their antagonistic activities in vitro.

Morphological character of efficient mutants was tested up to six generation to check their stability. Among these, four stable mutants viz., TVME 2b, TVME 2c, TVME 3c and TVGM1 were proved as effective antagonists against Sclerotium rolfsii, Rhizoctonia bataticola and Fusarium oxysporum f.sp. ciceri basis of their maximum bioefficiency.

Mutants TVME2b, TVME2c, TVME3c and TVGM1 exhibited the highest inhibition to mycelial growth of Sclerotium rolfsii with an inhibition percentage 64.76, 64.22, 63.08 and 62.93% respectively. Rhizoctonia bataticola was inhibited to the extent of 63.47, 60.87, 61.93 and 61.02% by relative mutants, while Fusarium oxysporum f. sp. ciceri was restricted and recorded 63.50, 62.88, 62.03 and 60.74% respectively. The highest chitinase enzyme units/mg of protein i.e. 0.64, 0.63, 0.63 and 0.62 was exerted in TVME2b, TVME2c, TVME3c and TVGM1 having respectively. However, TVME1c contains 0.37 enzyme units; it showed maximum ability against pathogens. hence present study proved that mutagenesis is the efficient tool for improving bioefficiency of Trichoderma viride, a biocontrol agent.

Keywords: trichoderma viride; soil borne pathogens; mutation; ethyl methyl sulphonate

Introduction

1.1 Background

Many fungal genera have been playing a major role in the root disease complex causing seed decay, damping off, root rot, seedling blight, collar rot, crown rot, foot rot and wilt. These are the problematic diseases throughout the world, each having wide host range covering all group of plants. Lewis and Papavizas (1991) recorded that about 90% of the 2000 major diseases of 31 principle crops in United State are caused by soil borne pathogens. In the exact estimation in India, it can be safely assumed that more the rapidly growing human population needs an increase in agricultural production. However, the emergence of plant diseases raised

the difficulty of this challenge (Boyd et al., 2012). Plant diseases had greatly reduced the production of many major crops, including potato, rice, barley, wheat, and soybean (Chakraborty et al., 1999). Many fungal plant pathogens were reported to cause plant diseases, and greatly reduce the production of economically important crops. For instance, Fusarium oxysporum f. sp. cubense, a soilborne fungus, was shown to cause Panama disease in banana. On the other hand, the more than 50% crop losses are due to soil inhabiting microorganisms (Elad, 1982). In severe cases, the potato yield loss is due to Fusarium dry rot could be as high as 60% ((Al-Mughrabi 2010).Plant diseases affect plants in the field as well as post-harvested crops. Apart from reducing crop yields, some of the species like Fusarium.

Chemical control for these soil borne pathogens is arduous, uneconomical and not advisable owing to risk of ground water pollution, death of non-target beneficial flora and fauna and evolution of fungicide resistant pathogen variants Moreover, many components of pesticides are recalcitrant and tend to persist in the environment for long period of time (Hai et al., 2012). Besides that, it is not cost effective to use chemical pesticides in the long run. Biological control of these pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Keeping these in view research on biological control of this group of pathogens is being carried out worldwide.

There are many biological control agents which have been reported as an efficient alternative to reduce the use of fungicides. However, in order to continue management of new strain /races of soil borne fungal pathogens being evolved in the nature, there is necessity to evolve new effective biocontrol agents. Amongst many effective bio control agents, *Trichoderma* is one of them whose species have been reported to be inhibitory to many soil borne pathogens (Harman et al., 2004). Bio control agent like *Trichoderma* spp. are acclaimed as effective, eco-friendly and cheap, nullifying the ill effect of chemicals. Therefore, of late, these biocontrol agents are identified to act against on array of important soil borne plant pathogens causing serious disease of crops. Therefore, considering the cost of chemical pesticides and hazardous involved, biological control of plant disease appears to be an effective and eco-friendly approach being practice world over (Dominguesa et al. 2000).

Trichoderma is a well-known biological control agent (BCA) because of its ability to reduce the population of soil borne pathogens. It is also a soil borne fungus and show significant activity against a wide range of plant pathogenic fungi (Elad et al., 1982 and Migheli et al., 1998). It has been used with success against a broad such as fungi and other organisms (including airborne and soil borne), bacteria, nematode, protozoa, and even viruses (Howell., 2009) Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f.sp.*ciceri is* causing wilt of chickpea. Bioagents significantly reduced the wilt incidence, and increased seed germination and plant growth parameters as compared to chemical fungicides (Shabir-U-Rehman et al., 2014) They act against plant pathogens in several ways, either by mycoparasitism, antibiotic-mediated suppression, lytic enzymes and other by-products production, competition for nutrient, or induction of host resistance (Pal and Gardener 2011).

The mycoparasitism, antibiosis, competition for nutrients of space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration for inorganic nutrients and inactivation of the pathogen's enzymes were important mechanisms of *Trichoderma* spp. against plant pathogens (Harman, 2000).

1.2 Importance of Study

Genetic modification using mutagenesis offers the potential for producing improved bio-protection is likely to enhance their biocontrol capabilities against different soil borne pathogen. One attractive approach would be to select the stable and safe transgenic fungi with different genes coding for cell wall degrading enzymes (chitinase and glucanase and protease) which express co-ordinately to combat the phytopathogen. Chitin, glucan and proteins are the main structural components of fungal cell wall. In plants, induction of chitinase, glucanase and other hydrolytic enzymes is one of the coordinated, often complex and multifaceted defense mechanisms triggered in response to phytopathogen attack.

Mutagenesis or protoplast fusion of biocontrol agents was applied to improve the antifungal production and antagonistic potential over a broad spectrum of phytopathogens, survival, longevity and activity. (Haggag et al., 2010). It is necessary to mutagenesis of the microorganisms including those used as bio control agents were applied to improve of the antifungal production and antagonistic potential over a broad spectrum of phytopathogens, survival, longevity and activity (Ximena et al., 2007

1.3 Objectives of the Study

- Induction of mutation in *Trichoderma viride.*
- To assess the antagonistic effect of *Trichoderma viride* mutants against predominant soil borne pathogen.

1.4 Scope and Limitations

Trichoderma has been widely studied for their biocontrol ability, but Trichoderma have some limitations in their use as biocontrol agents in agriculture is due to the unpredictable efficiency which is affected by biotic and abiotic factors in soil. Hence it is essential to increase their bio control efficacy against targeted pathogen and to made it tolerant to environmental parameters, mutagenesis is the tool for improving genetic makeup of Bio agents.

Materials and Methods

The present investigation on "Improvement in bio control ability of Trichoderma viride through mutation" was carried out during 2019/2020 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Ethyl methyl Sulphonate (EMS), Ethanol, Crab shell chitin, Sodium azide and others such as formaldehyde, Streptomycin Sulphate, mercuric chloride etc. Pure culture of Trichoderma viride culture was collected from the Department of Plant Pathology Dr. PDKV, Akola. Pure cultures of soil born pathogen viz., Rhizoctonia bataticola, Sclerotium rolfsii, and Fusarium oxysporum f. sp. ciceri were collected from Department of Plant Pathology, Dr. P.D.K.V, Akola.

Chemical Mutagenesis

Induction of Chemical mutagenesis was carried according to procedure of Chandra et al. (2009) and Durand et al. (1988): Conidiophores of 8 days old culture of *T. viride* were used for mutagenesis. Spore suspension of *T. viride* was treated with Ethyl Methyl Sulphonate (EMS) @ 100 µl/ml, 150 µl/ml, 200 µl/ml and 250µl/ml incubate at 28^0C in orbital shaker for 30 minutes. Then kept in centrifuge machine at 5000 rpm to remove the chemical traces, centrifuge it for three times and then washed with distilled water. Suspension was spread on to the surface PDA medium and incubated at 28^0C for 72^0 hour.

Same procedure was done for 45 minutes and 60 minutes. In each treatment maintained three replications. After incubation colonies were transferred on fresh PDA medium and grown up to six generations to check the stability of *Trichoderma viride* mutants.

Mutation induced by Gamma Radiation.

Induction of mutation by gamma radiation was carried according to the procedure of Gadgil et al. (1995), Migheli et al. (1998) and Rey et al. (2000) at Bhabha Atomic Research Centre, Mumbai. The 10 days sporulate culture of *Trichoderma viride* was irradiated with cobalt – 60 gamma radiation @ 41.6 gray/min. The applied doses level were 20 k-rad,30 k-rad,40 k-rad and 50 k-rad. After irradiation culture were

transferred on fresh PDA medium and grown up to six generation to check the stability of *Trichoderma viride* mutants.

Dual culture Technique

Antagonistic activity of *Trichoderma viride* mother culture as well as mutants were assayed against *Rhizoctonia bataticola, Sclerotium rolfsii, Fusarium oxysporum* f.sp. *udum* and *Fusarium oxysporum* f. sp *solani* by using dual culture inoculation technique described by Vincent, (1927), Mandal et al., (1999) in Petri plates. Five mm disc from the periphery of actively growing pathogen on PDA was placed in centre of 90 mm diameter Petri plates containing PDA. Four discs of each actively growing mutants of *Trichoderma viride* were placed at equidistance on all four sides 30 mm apart from centre disc of pathogenic fungus. The plates were incubated at ambient condition under alternate dark and light cycle up to 7 days.

$$\text{Per cent Growth inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Mycelial growth (mm) in control plate.

T = Mycelial growth (mm) in treatment plate.

Results And Discussion:

This chapter deals with the results obtained on the basis of studies carried out and the fact along with the discussion hereunder.

Morphological characteristics of *Trichoderma viride* mutants at sixth generation

		Colony diameter (mm) at 7 DAI	Colony growth type	Colony colour	Pigmentation	Phialides	Conidia shape	Sporulation	Chitinase enzyme content (enzyme units/mg of protein)
1.	TVME1a	90.00	Subarial and disperse	Milky white to Greenish yellow	Yellow colour	Branched	Ellipsoidal	+	0.50
2.	TVME1b	89.67	Subarial and disperse	Light grey to light yellow	Yellow colour	Frequently paired	Ellipsoidal	++	0.49
3.	TVME1c	87.50	Subarial	Light grey to Greenish yellow	Yellow colour	Group simple	Ellipsoidal	+++	0.37
4.	TVME2a	90.00	Flat and Superficial	Light grey to light yellow	Amber colour	Branched	Ellipsoidal	++	0.58
5.	TVME2b	87.90	Flat and Superficial	Milky white to Greenish yellow	Yellow colour	Frequently paired	Oblong to Ellipsoidal	++	0.60
6	TVME2c	89.33	Subarial and disperse	Milky white to Greenish yellow	Yellow colour	Branched	Ellipsoidal	++	0.64
7.	TVME3a	89.67	Flat and Superficial	Light grey to light yellow	Amber colour	Branched	Oblong to Ellipsoidal	++	0.58
8.	TVME3b	90.00	Flat and Superficial	Light grey to light yellow	Yellow colour	Branched	Oblong to Ellipsoidal	++	0.61
9.	TVME3c	87.90	Subarial and disperse	Milky white to Greenish yellow	Amber colour	Group simple	Ellipsoidal	+++	0.63
10.	TVME4a	89.67	Subarial and disperse	Light grey to light yellow	Yellow colour	Branched	Ellipsoidal	++	0.51
11.	TVME4b	90.00	Subarial and disperse	Light grey to light yellow	Yellow colour	Group simple	Ellipsoidal	+	0.61
12.	TVME4c	90.00	Flat and Superficial	Milky white to Greenish yellow	Yellow colour	Branched	Ellipsoidal	++	0.63
13.	TVGM1	90.00	Subarial and disperse	Green	Amber colour	Branched	Ellipsoidal	+++	0.62
14.	TVGM2	90.00	Flat and Superficial	Green	Yellow colour	Group simple	Oblong to Ellipsoidal	+++	0.58
15.	TVGM3	87.90	Subarial and disperse	Green	Yellow colour	Branched	Ellipsoidal	+	0.49
16.	TVGM4	87.00	Flat and Superficial	Milky white to Greenish yellow	Dirty yellow colour	Group simple	Ellipsoidal	++	0.45
17.	Mother culture	88	Subarial and disperse	Milky white to Greenish yellow	Yellow colour	Group simple	Globose to obvoid	++	0.39

Efficacy of *Trichoderma viride* mother culture and mutants against *Sclerotium rolfsii* (per cent growth inhibition) at 7 DAI

Statistically significant differences were obtained among the *T. viride* mutants over control (mother culture). The superior treatment was T_5

(TVME2b) mutant exhibited the highest inhibition to mycelial growth of *Sclerotium rolfsii* with an percentage 64.76%.which was at par with T_6 (TVME2c),T_9 (TVME3c) and T_{13} (TVGM1) i.e. 64.22, 63.08 and 62.93 % respectively. whereas T_{10} (TVME4a) showed lowest inhibition i.e. 52.56% against growth of *Sclerotium rolfsii*. Data presented in table 3.

Treatments	Code Name	Mean Radial Growth (mm)	Percent Growth Inhibition (%)
T1	TVME 1a	34.75	59.88
T2	TVME 1b	37.51	56.70
T3	TVME 1c	36.42	57.95
T4	TVME 2a	35.73	58.75
T5	TVME 2b	30.52	64.76
T6	TVME 2c	30.59	64.22
T7	TVME 3a	35.99	58.45
T8	TVME 3b	30.59	64.22
T9	TVME 3c	31.98	63.08
T10	TVME 4a	41.09	52.56
T11	TVME 4b	34.61	60.04
T12	TVME 4c	34.61	60.04
T13	TVGM1	32.11	62.93
T14	TVGM2	33.12	61.76
T15	TVGM3	34.62	60.03
T16	TVGM4	40.00	53.82
T17	*T. viride* mother culture	40.29	53.49
	Control	86.63	-
	'F' test	Sig.	-
	SE(m)±	0.61	-
	CD (P=0.01)	2.371	-

Table 3. Efficacy of Trichoderma viride mother culture and mutants against Sclerotium rolfsii (per cent growth inhibition) at 7 DAI

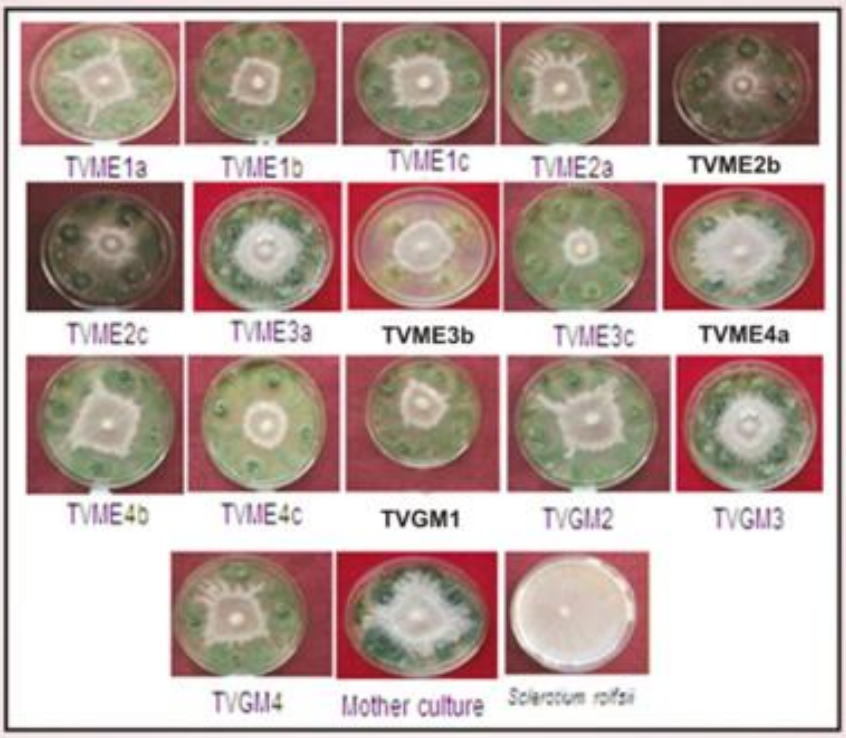

Plate 8 Efficacy of *Trichoderma viride* mutants against *Sclerotium rolfsii* in dual culture technique

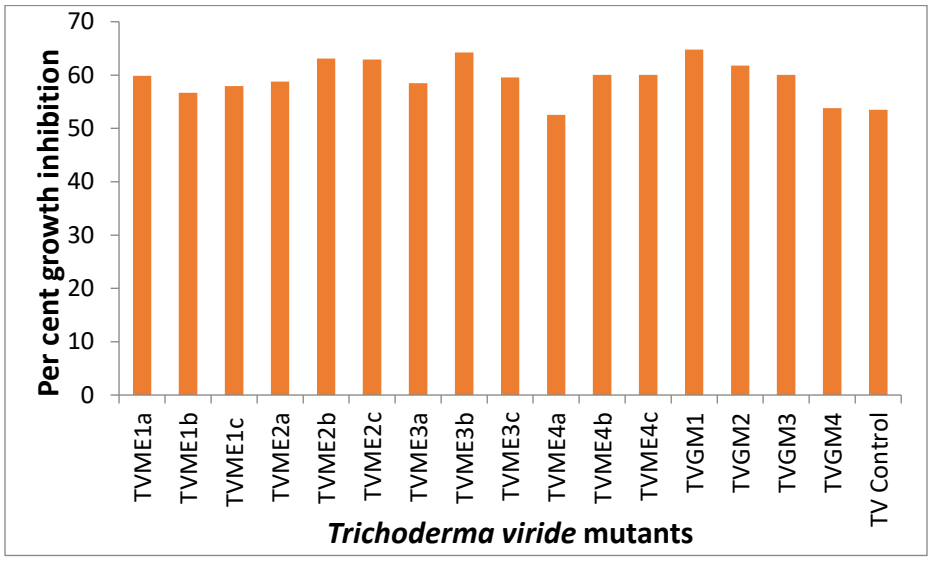

Figure 1: Efficacy of Trichoderma viride mother culture and mutants against Sclerotium rolfsii at 7DAI

Induced mutation is one of the most widely used tools to get variants which might have improved biocontrol properties like survival ability, antagonistic and biocontrol potential and ability to colonize plant parts (Baker, 1991). Current findings are in agreement with Papavizas and Lewis (1982) who evaluated the UV induced biotypes of *T. viride* differed considerably from WT 6 in appearance, growth habit and fungitoxic metabolite production and also effectively arrested the growth of *Rhizoctonia solani* of cotton and radish and *Sclerotium lepillorum* of onion. Chaudhary (2009) also evaluated biopesticides Niprot (*Trichoderma viride*) for calibration of dosage extent and suppression of collar rot caused by *S. rolfsii* and observed that Niprot was able to suppress the disease. Mohamed et al. (2010) adopted UV treatment for mutagenesis of *Trichoderma viride* to enhance three effective hydrolytic enzymes viz., chitinase, β-1, 3- galactoronase and cellulases which having biocontrol abilities against *S.rolfsii*. In the present investigation efforts have been made to generate efficient mutants of *T. viride* having more antagonistic potential against soil borne pathogens and enzymic activities compared to mother culture.

4. Efficacy of *Trichoderma viride* mother culture and mutants against *Rhizoctonia bataticola* (per cent growth inhibition) at 7 DAI.

All the *Trichoderma viride* mutants were significant in inhibiting the radial mycelial growth of *R. bataticola*. The efficient treatment was T8 (TVME 3b) i.e. 64.19% which was at par with T5 (TVME2b) and T_7 (TVME3a) i.e. 63.47 and 62.94%. The lowest per cent growth inhibition of *Rhizoctonia bataticola* was found by T16 (TVME4c) i.e. 45.42%. Data are presented in table 4.

Treatments	Code Name	Mean Radial Growth (mm)	Percent Growth Inhibition (%)
T1	TVME 1a	41.00	52.87
T2	TVME 1b	36.27	58.31
T3	TVME 1c	35.43	50.57
T4	TVME 2a	35.87	58.77
T5	TVME 2b	31.78	63.47
T6	TVME 2c	34.04	60.87
T7	TVME 3a	32.24	62.94
T8	TVME 3b	31.15	64.19
T9	TVME 3c	33.12	61.93
T10	TVME 4a	36.18	58.41
T11	TVME 4b	36.66	57.86
T12	TVME 4c	44.00	49.42
T13	TVGM1	33.91	61.02
T14	TVGM2	35.33	59.39
T15	TVGM3	42.00	51.72
T16	TVGM4	47.48	45.42
T17	*T.viride* mother culture	43	50.57
	Control	87	-
	'F' test	Sig	-
	SE(m)±	0.57	-
	CD (P=0.01)	1.656	-

PGI - Per cent growth inhibition

Patil and Kamble (2011) obtained five *T. koningii mutants* by UV treatments with different time variables. Among these five mutants *T.koningii* - 2 showed maximum antagonistic activity against *R. bataticola* causing charcoal rot *in vitro*.

Table 4. Efficacy of Trichoderma viride mother culture and mutants against *Rhizoctonia bataticola* (Percent growth inhibition) at 7DAI

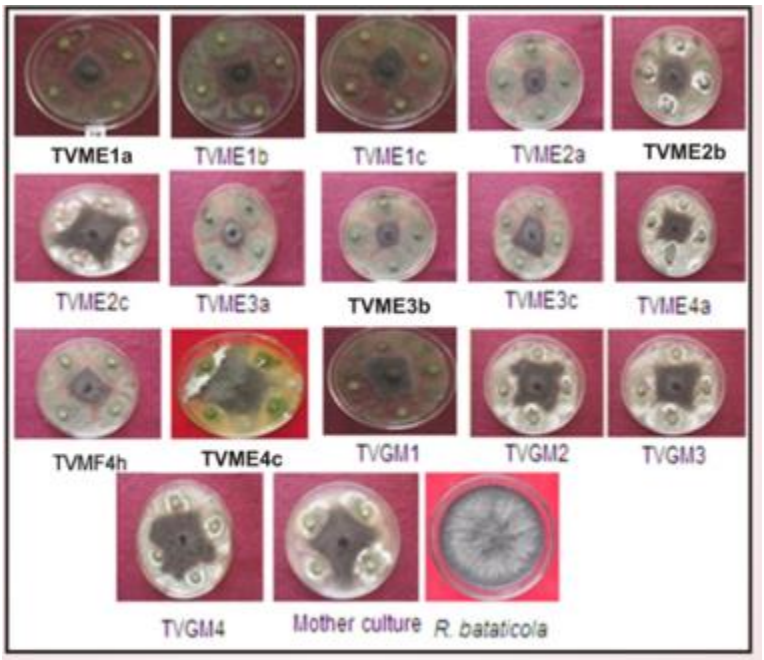

Plate 9. Efficacy of *Trichoderma viride* mutants against *Rhizoctonia bataticola* in dual culture technique

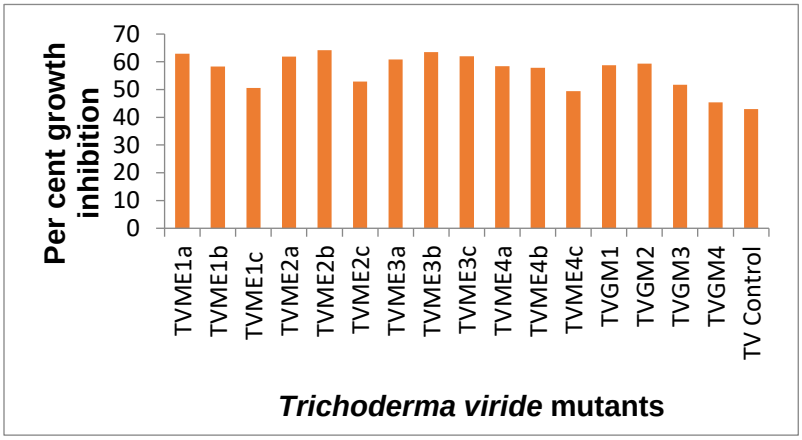

Figure 2: Efficacy of Trichoderma viride mother culture and mutants against Rhizoctonia bataticola at 7 DAI

Efficacy of *Trichoderma viride* mother culture and mutants against *Fusarium oxysporum* f. sp.*ciceri* (per cent growth inhibition) at 7 DAI

All the *Trichoderma viride* mutants were significant in inhibiting the radial mycelial growth of *Fusarium oxysporum* f.sp.*ciceri* the efficient treatment was T5 (TVME 2b) i.e. 63.50% which was at par with T6 (TVME2c) T_9(TVME3c) and T_{13} (TVGM1) i.e. 62.88 and 62.03 and 60.74%. The lowest per cent growth inhibition of *Fusarium oxysporum* f.sp.*ciceri was* found by T16 (TVGM4) i.e. 47.77 %. Data are presented in table 5.

Treatments	Mean Radial Growth (mm)	Percent Growth Inhibition (%)
(T1)TVME 1a	37.23	56.19
(T2)TVME 1b	39.00	54.11
(T3)TVME 1c	36.60	56.93
(T4)TVME 2a	41.09	51.65
(T5)TVME 2b	31.02	63.50
(T6)TVME 2c	31.54	62.88
(T7)TVME 3a	38.00	55.28
(T8)TVME 3b	39.29	53.77
(T9)TVME 3c	32.27	62.03
(T10)TVME 4a	41.45	51.22
(T11)TVME 4b	37.29	56.12
(T12)TVME 4c	34.42	59.50
(T13)TVGM1	33.46	60.74

(T14)TVGM2	41.28	51.42
(T15)TVGM3	41.47	51.20
(T16)TVGM4	44.39	47.77
(T17)*T.viride* mother culture	41.14	51.59
Control	84.99	-
'F' test	Sig	-
SE(m)±	0.60	-
CD (P=0.01)	2.33	-

Table 5: Efficacy of Trichoderma viride mother culture and mutants against Fusarium oxysporum f. sp. ciceri (per cent growth inhibition) at 7 DAI

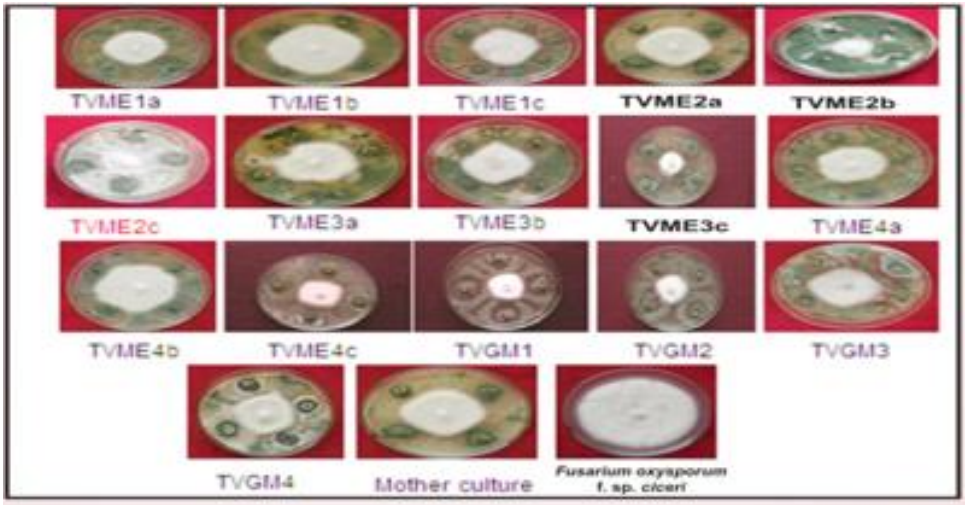

Plate 10. Efficacy of *Trichoderma viride* mutants against *fusarium oxysporum* f. sp. *ciceri* in dual culture technique

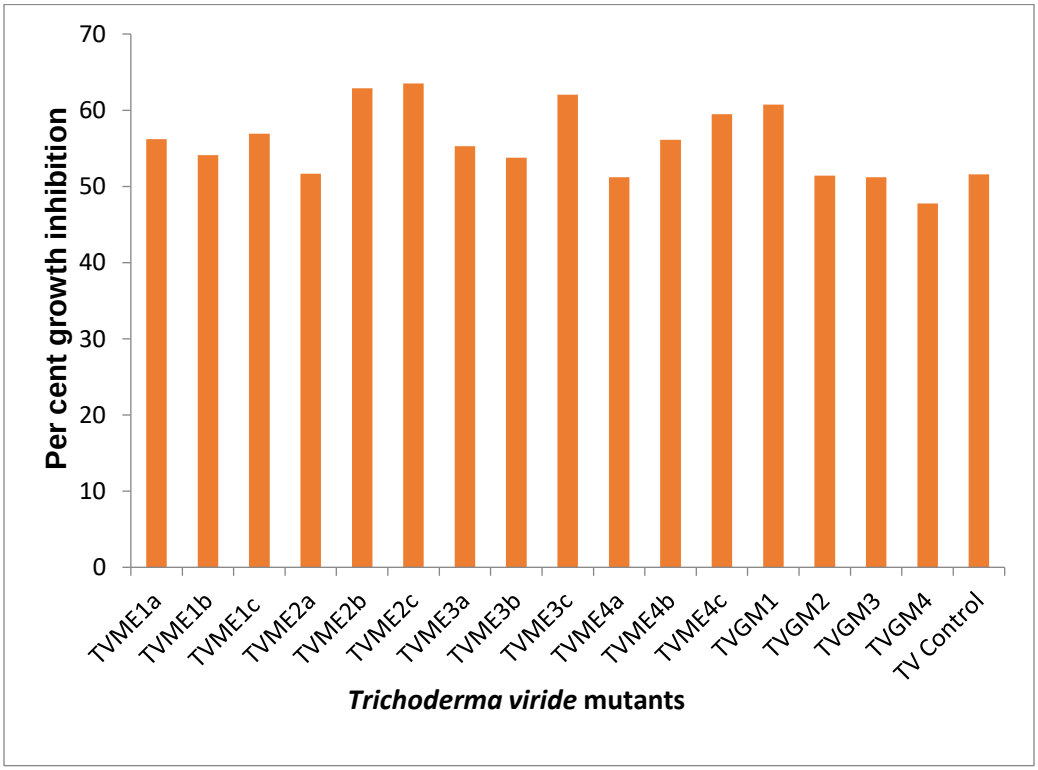

Figure 3: Efficacy of Trichoderma viride mother culture and mutants against Fusarium oxysporum f. sp. ciceri at 7DAI

Inhibition of *Fusarium udum* by *Trichoderma* was also reported by Papavizas (1985).Padamodaya and Reddy (1996) screened ten isolates of *Trichoderma* spp. For arresting the growth *of Fusarium oxysporum in vitro* and reported that *T. viride* was highly inhibitory in dual culture. Present investigations are in agreement with Mohamed et al. (2006) who obtained two stable salt tolerant mutants having great biological proficiency against *Fusarium oxysporum* the causal agent of tomato wilt disease.

Conclusions and recommendation

The present work aimed to apply the mutagenesis and irradiation techniques for genetically improvement of the bioagents *Trichoderma viride* to enhance their biocontrol abilities against predominant soil borne fungal pathogens *viz., Fusarium oxysporum* f. sp. *ciceri, Sclerotium rolfsii* and *Rhizoctonia bataticola*. These are important soil borne fungal pathogens affecting wide range of hosts and have a worldwide distribution on numerous field crops and vegetables.

The present investigation entitled "Improvement in biocontrol ability of *Trichoderma viride* through mutation*"* was carried out during 2019-20 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi vidyapeeth, Akola. The objectives of the studies were

1. Induction of mutation in *Trichoderma viride.*
2. To asses the antagonistic effect of *Trichoderma viride* against predominant soil borne pathogen.

Mutagenesis and irradiation was found to be the novel strategy for developing *Trichoderma* mutants with enhanced biocontrol abilities. *Trichoderma viride* mother culture obtained from Department of Plant Pathology, PGI, Dr. PDKV Akola and it was subjected to chemical mutagenesis i.e. Ethyl methane Sulphonate (EMS) @ 100,150,200 and 250 µl/ml for time interval at 30,45 and 60 minutes. The sporulate culture of *Trichoderma viride* irradiated at (cobalt-60) 0,20,30,40 and 50 k-rad, It was done at BARC, Mumbai. Total sixteen mutants were obtained from chemical mutagenesis and gamma irradiation. Mother culture of *Trichoderma viride* and its mutants were tested for their antagonistic potential against soil borne plant pathogens, viz., *Sclerotium rolfsii, Rhizoctonia bataticola, Fusarium oxysporium* f.sp.*ciceri.*by dual culture technique.

Morphological character of efficient mutants of *Trichoderma viride* were tested upto sixth generation to check their stability. *Trichoderma viride* mutants exhibited morphological variation such as growth rate, colony diameter, colony type, colony colour, pigmentation, phialides, and sporulation. Mutants were differed in their biocontrol potential against soil borne pathogen as well as chitinase enzyme content.

Among the mutants most of them were found effective against *Slerotium rolfsii, Rhizoctonia bataticola, Fusarium oxysporium* f.sp. *ciceri,* than mother culture, in dual culture technique. The mutant TVME 3b, TVGM1, TVME2b and TVME2c exhibited maximum antagonistic activity against *Slerotium rolfsii* which showed, 65.10, 64.59, 64.47 and 62.37 per cent growth inhibition respectively. In case of *Rhizoctonia bataticola,* the mutants TVME 2b, TVME3b, TVME 1a and TVME2a exhibited maximum per cent growth inhibition i.e. 62.43,62.40,61.64 and 61.56 per cent respectively.*Fusarium oxysporium* f.sp.*ciceri* was also highly inhibited by mutants TVME 2b, TVME2c, TVME 3c and TVGM1 and it showed maximum per cent growth inhibition i.e. 61.68,60.60,59.41 and 58.90 per cent respectively.

The mutants were assayed for estimation of chitinase enzyme and the mutants TVME 2b, TVME 2cand TVME 3c and TVGM1 found to possesses highest chitinase enzyme units/mg of protein i.e. 0.64, 0.63, 0.63 and 0.62 respectively.

Conclusions

- The morphological character tested upto sixth generation to check their stability.There was existence of some morphological variation in mutants and mother culture of *Trichoderma viride*. Mutants were differed in their biocontrol potential against tested plant pathogen as well as chitinase enzyme units.
- The mutants TVME 2b, TVME 2c, TVME 3c andTVGM1 were found effective against *Slerotium rolfsii*.
- The mutants TVME 3b, TVME 2b, TVME 3a and TVME 3c were found effective against *Rhizoctonia bataticola*.
- The mutants TVME 2b, TVME2c, TVME 3c and TVGM1 were found effective against *Fusarium oxysporium* f.sp. *ciceri.*
- TVME 2b, TVME 2c and TVME 3c and TVGM1 found to possesses highest chitinase enzyme units/mg of protein i.e. 0.64, 0.63, 0.63 and 0.62 respectively

The ability of high quantum of chitinase with higher growth rate and sporulation was directly related to maximum inhibition ability of mutants.

References:

1. Al-Mughrabi, K. I., (2010) Biological control of Fusarium dry rot and other potato tuber diseases using Pseudomonas fluorescens and Enterobacter cloacae. Biological Control, 53, pp. 280-284.
2. Baker, R. (1991) Induction of Rhizosphere competence of benomyl tolerant mutants of Trichoderma spp. Can. J. Microbiol. 34:694-696.
3. Baker, R. and Paulitz T. C. (1996). Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens In: Principles and Practice of Managing Soil borne Plant Pathogens. Hall, R. (ed.).The American Phytopathol.Soc.St. Paul, MN.50-79.
4. Chakraborty, M. R., S. Dutta, S. Ojha and N. C.Chatterjee. 2004. Antagonistic potential of biocontrol agents against Botrydioplodia theobromes causing Die-back of Bottle Brush. Acta Botanica Hungarica 46(3-4):279-286.
5. Chaudhary M. 2009. Bioinsensitive management of collar rot affecting tropical sugar beet with biopesticides NIPROT (Trichoderma viride) and Su-Mona (Pseudomonas fluroscencens). 99 (6):522.
6. DubeyS.C.and B. Patel. 2000. Determination of tolerance in Thantophorus cucumer,Trichoderma spp, Gliocladium virens and Rhizobium spp. to fungicide.Indian Phytopath. 54(1): 98-101.
7. Elad, Y., A. Kalfon and I. Chet.1982a.Control of Rhizoctonia solani in cotton by seed coating with Trichoderma Spores.Plant and Soil. 66: 279-282.
8. Elad, Y., I. Chet and Y. Hennis.1982b. Degradation of plant pathogenic fungi by Trichoderma harzianum.Can. J. Micrbiol. 28: 719-725.
9. Elad,Y., I. Chet, P.Bhoyle and Y.Hennis.(1983). Parasitism of Trichoderma spp on Rhizoctonia solani and Sclerotium rolfsii scanning electron microscopy and fluorescence microscopy. Phytopathology.73: 85-88.
10. Elad, Y., I. Chet. (1983) Improved selective media for isolation of Trichoderma spp or Fusarium spp. Phtoparasitica.11 (1) : 55-58.
11. Garcia, I., J. M. Lora, J. de la Cruz, T. Benitez, A.Llobell and J. A. Pintor-Toro,(1994). Cloning and characterization of a chitinase (chit42) cDNA from the mycoparasitic fungus Trichoderma harzianum. Curr.Genet.27 :83–89.
12. Haggag, W.M. and Mohamed, H.A. (2002). Enhancement of antifungal metabolite production from gamma-ray induced mutants of some Trichoderma species for control of onion white rot disease. Pl. Pathol. Bull. 11: 45-56.
13. Hai, F. I. et al., (2012). Pesticide removal by a mixed culture of bacteria and white-rot fungi. Journal of the Taiwan Institute of Chemical Engineers, 43, pp. 459-462
14. Harman, G.E. (2000). Myths and dogmas of biocontrol changes in perceptions derived from research on Trichoderma harzianum T-22. Plant Dis. 84: 377-393.
15. Harman, G.E., Y.L.Wang and Y.Shen.(1999). Bioassay of Trichoderma harzianum strains for control of rice sheath blight. Phytopathology 89 (6): 586.
16. Lewis, J. A. and G. C. Papavizas. (1991). Biocontrol of plant diseases: the Approach for tomorrow.Crop Protection, 10: 95-105.
17. Mandal, S., K. D. Srivastava; R. Agrawal and D. V. Singh. (1999). Mycoparasitic action of some fungi on blotch pathogen

(Drechslera sorokmiana) of wheat.Indian Phytopath. 52 (5): 39-43

18. Migheli, Q., L. Gonzalez-Candelas, L. Dealessi, A. Camponogara and Ramon-vidal, D. (1998). Transformants of Trichoderma longibrachiatum over expression the β-1,4-endoglucanase gene egl1 show enhanced biocontrol of pythium ultimum on cucumber. Phytopathol. 88: 673-677
19. Mohamed, H. A. A., M. Haggag Wafaa and A. G. Attallah (2010).Genetic enhancement of Trichoderma virideto overproduce different hydrolytic enzymes and their biocontrol potentiality against root rot and white mold diseases in plants. Agric. Biol. J. N. Am 1 (3) : 273-284.
20. Padmodaya, B. and H. R. Reddy. (1996) .Screening of Trichoderma spp.Against Fusarium oxysporum f. sp. lycopersici causing wilt in tomato.Indian. J. Mycol. Pl. Pathol. 26 (3):266-270.
21. Pal, K. K. and Gardener, B. M., 2011. Biological Control of Plant Pathogens [Online].
22. Papavizas, G C., J. A. Lewis and T. H. Abd-El-Moity.(1982). Evaluation of new biotypes of Trichoderma harzianum for tolerance to benomyl and enhanced biocontrol capabilities.Phytopathology 72: 126- 132.
23. Patil, V. B and S. S. Kamble. (2011). The influence of Ultraviolet light on Antagonistic activity of Trichoderma koningii against Macrophomina phaseolina causing charcoal rot of sweet potato. Int. J. of Academic Res 3 (1):702-704.
24. Rey, M.,Delgado J.J.,Rincon A.M.,Limon C.M.,Benitez T.,Perez E.A.,Cantoral,F.J. (2000).Improvement of Trichoderma strains for biocontrol.Micologia industrial Y Micopatogia,17:531-536
25. Shabir-U-Rehman, W. A. Dar, S. A. Ganie, Javid A. Bhat, Gh.Hassan Mir, Rubina Lawrence,Sumati Narayan and Pardeep Kumar Singh (2013) Comparative efficacy of Trihoderma viride and Trichoderma harzianum against Fusarium oxysporium f sp.ciceris causing wilt of chickpea.Vol.7(50),pp.5731-5736
26. Vincent, J. M. (1927). Distortion of fungal hyphae in the presence of certain InhibitorsNature.159:350.
27. Ximena, A., A. Luz María and A. Antonio. (2007). New strains obtained after UV treatment and protoplast fusion of native Trichoderma harzianum: their biocontrol activity on Pyrenochaeta lycopersici. Electron. J. Biotechnol. 10(4) : 604-617.

DOI:10.31579/2690-1919/347