Environment and Ecology 37 (1A) : 216—220, January—March 2019
Website: environmentandecology.com ISSN 0970-0420

# Evaluation of the Regeneration Capacity of Mutants in *Trichoderma* spp. on Different Culture Media

**Chandarappa B. P., Basavarajappa M. P., Parijatha V. N.**

Received 2 August 2018 ; Accepted 8 September 2018 ; Published on 29 September 2018

**Abstract** To evaluate the regeneration capacity of *Trichoderma* spp. on different culture media namely, potato dextrose agar, Czapak media and *Trichoderma* selective media. After inoculation of spore suspension in different culture media, the colony forming units (cfu) was recorded after mutation at different interval of time. The maximum regenerated colony forming units (cfu) observed in potato dextrose agar (PDA) and lowest in *Trichoderma* selective media (TSM). Potato dextrose media was considering as a suitable media for regeneration of mutant spores.

**Keywords** Colony forming units, *Trichoderma* spp., Culture media, Regenaration.

## Introduction

Biological control involves the use of biological organisms to control pathogens or diseases. Most of the *Trichoderma* spp. members are promising biological control agents (bioprotectants) against most of plant diseases. *Trichoderma* are free-living fungi and common in soil and root ecosystems. They are opportunistic, a virulent, plant symbionts, as well as being parasites of other fungi (Harman et al. 2004). These filamentous fungi are very wide spread in nature, with high population densities in soils and plant litters. They are saprophytic, quickly growing and easy to culture and they can produce large amount of conidia with long shelf life. Among the different bio-control agents so far identified, species of *Trichoderma* are the most effective in reducing disease incidence of various crops. Biomass used for biological control must be inexpensive to produce. It should be capable of beingdried with retention of a high level of germinable propagules, be insensitive to environmental fluctuations (e.g., temperature and humidity) and possess a long shelf life.

In recent years, there is a new direction towards improving bio control activities of bio-agents, among them *Trichoderma* having wide range of advantages and applicability. So that, there is a need of produce superior strains of *Trichoderma* which could be used as an effective biocontrol agent. There are several different processes available for producing improved bioprotectants, namely mutagenesis, the use of recombinant DNA and protoplast fusion. In this study, advance biotechnological tools i.e., mutagenesis was used. The studies were conducted for the use of various culture media for growth performances of *T. harzianum* (Elad et al. 1981, Harman et al. 1990, 1991). So, it is important to search suitable and cheap media for regeneration of *Trichoderma* spp. after mutation process. The present investigation was undertaken to evaluate the regenerated capacity of *Trichoderma* spp. on different culture media.

## Materials and Methods

The mutation was carried out by using four *Trich-*

Chandarappa B. P.*
Department of Plant Pathology, College of Agriculture, Vijayapur 586101, India (University of Agricultural Sciences, Dharwad 580005, India)

Basavarajappa M. P.
Associate Professor, University of Horticultural Science,Bagalkot, Karnataka, India

Parijatha V. N.
Department of Entomology, SHUATS, Allahabad 211007, India
e-mail: chandrunayakagri@yahoo.com, basump@rediffmail.com
*Corresponding author

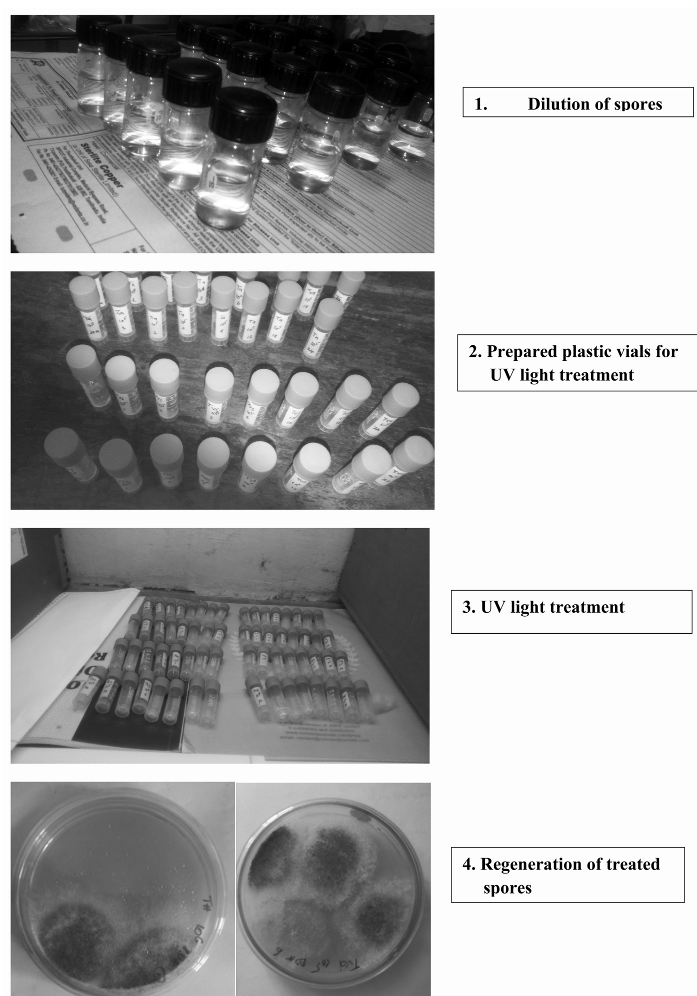


**Fig. 1.** UV light mutation steps.

*oderma* species viz., *Trichoderma harzianum*, *Trichoderma virens* and *Trichoderma viride* isolates were collected from NBAIR (National Bureau of Agricultural Insects Resources) Bengaluru and *Trichoderma asperellum* from IIOR (Indian Institute of Oil Research) Hyderabad and those bio-agents was sub cultured on PDA slants and allowed to grow at 28±1°C for 10 days and such slants were preserved in a refrigerator.

Production of *Trichoderma* mutants through UV radiation

UV irradiation of *Trichoderma* isolates: The parent strains, *T. harzianum* (*PTh*), *T. viride* (*PTvd*), *Trichoderma virens* (*PTvs*) and *Trichoderma asperellum* (*PTas*) were grown on PDA slants at 30°C to induce sporulation. One week after sub-culturing conidial suspension was prepared by dislodging the conidia from the agar surface with a sterile needle to a sterile plastic vial and by pouring sterilized physiological saline (0.85% NaCl) containing 0.1% Tween-80 to disperse spore clumps. The prepared conidial suspension was divided to two portions in two sterilized small plastic vials for each treatment (time interval of exposure to UV light). Conidial concentrations were adjusted to $\approx 1 \times 10^6$ / ml. The first plastic vial was used as control. The subsequent plastic vial were

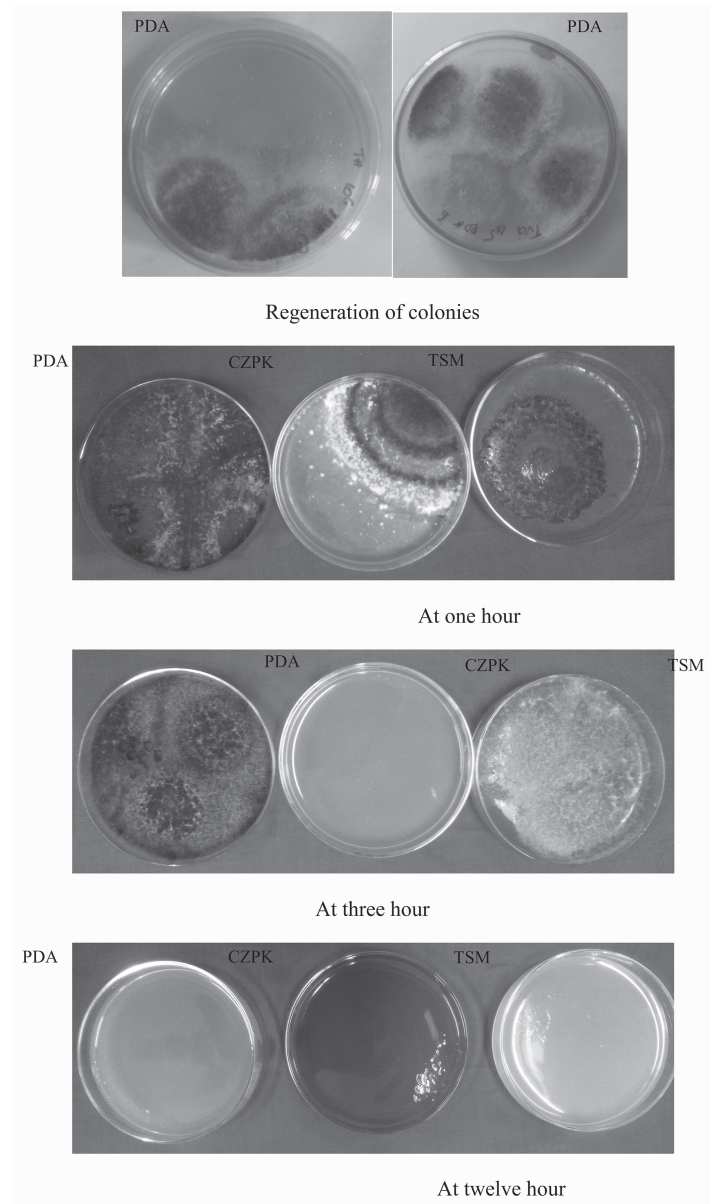


**Fig. 2.** Regenerated of colonies on different hours.

treated with 500 µg/ml sodium nitrate ($NaNO_2$) and was irradiated for different time intervals i.e. 30 min, 1, 2, 3, 6, 12, 18 and 24 h under ultraviolet lamp (GERMICIDAL LAMP (VL-G), UV tube T-15C 15W 254 nm) where the distance between the vial and the lamp adjusted to 30 cm. These arrangements were made in separate isolated place carefully.

Regeneration and isolation of mutants

Potato dextrose media, Czapak media and *Trichoderma* selective media were used as a regeneration media. 20 ml of PDA was poured in 90 mm sterilized petri plates leave it for solidification. Like that other media also poured and solidified. After irradiation under

**Table 1.** Colony forming units after mutation with different media. cfu=colony forming units.

| Mutation time | *Trichoderma* spp. | No. of cfu without mutation PDA | CZPK | TSM | No. of cfu after mutation PDA | CZPK | TSM |
|---|---|---|---|---|---|---|---|
| 30 Minutes | *Trichoderma harzianum* | 3 | 2 | 3 | 3 | 1 | 1 |
| | *Trichoderma virens* | 3 | 1 | 0 | 1 | 0 | 0 |
| | *Trichoderma viride* | 4 | 0 | 1 | 3 | 0 | 0 |
| | *Trichoderma asperellum* | 3 | 0 | 0 | 1 | 0 | 0 |
| 1 hour | *Trichoderma harzianum* | 2 | 0 | 0 | 2 | 1 | 0 |
| | *Trichoderma virens* | 2 | 1 | 0 | 3 | 0 | 0 |
| | *Trichoderma viride* | 3 | 0 | 0 | 3 | 0 | 0 |
| | *Trichoderma asperellum* | 2 | 0 | 1 | 2 | 0 | 1 |
| 2 hours | *Trichoderma harzianum* | 2 | 0 | 0 | 2 | 1 | 0 |
| | *Trichoderma virens* | 1 | 0 | 2 | 2 | 0 | 2 |
| | *Trichoderma viride* | 2 | 1 | 0 | 3 | 0 | 0 |
| | *Trochoderma asperellum* | 1 | 0 | 0 | 2 | 0 | 0 |
| 3 hours | *Trichoderma harzianum* | 2 | 0 | 0 | 1 | 2 | 0 |
| | *Trichoderma virens* | 2 | 1 | 0 | 2 | 0 | 1 |
| | *Trichoderma viride* | 3 | 0 | 0 | 2 | 0 | 3 |
| | *Trichoderma asperellum* | 1 | 0 | 0 | 2 | 2 | 0 |
| 6 hours | *Trichoderma harzianum* | 1 | 0 | 1 | 2 | 0 | 0 |
| | *Trichoderma virens* | 1 | 0 | 0 | 1 | 1 | 0 |
| | *Trichoderma viride* | 2 | 0 | 0 | 2 | 1 | 2 |
| | *Trichoderma asperellum* | 1 | 0 | 0 | 0 | 0 | 0 |
| 12 hours | *Trichoderma harzianum* | 1 | 0 | 0 | 1 | 1 | 0 |
| | *Trichoderma virens* | 1 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma viride* | 2 | 1 | 1 | 0 | 0 | 0 |
| | *Trichoderma asperellum* | 1 | 0 | 0 | 0 | 0 | 0 |
| 18 hours | *Trichoderma harzianum* | 1 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma virens* | 1 | 1 | 0 | 0 | 0 | 0 |
| | *Trichoderma viride* | 2 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma asperellum* | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 hours | *Trichoderma harzianum* | 1 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma virens* | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma viride* | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Trichoderma asperellum* | 0 | 0 | 0 | 0 | 0 | 0 |

UV light, the conidial suspension was incubated at 30$^{o}$C for 45–60 min in dark. After incubation period 0.1 ml was poured on the solidified PDA, TSM and Czapek mineral medium supplemented with 0.1% Triton X-100 to restrict the growth of the fungal colonies and without mutated were also poured in different media as a control. Each media was replicated in thrice. These plates were incubated at 30$^{o}$C for six days until the fungal colonies were observed. The many colonies were appeared on media. Those were evaluated as number of colony forming units in different media. They were isolated separately and identified as mutants.

## Results and Discussion

The much reputed *Trichoderma* has been identified as a potential biocontrol agent against many phytopathogenic fungi and many species of *Trichoderma* are potential biocontrol agents against a wide range of soil-borne plant pathogenic fungi (Smith et al. 1990, Harman and Hayes 1993, Elad 2000).

Mutagenic treatments: Induced mutation with ultraviolet (UV) light at specific wave length of 254 nm to four *Trichoderma* spp. (*T. harzianum* (*PTh*), *T. viride* (*PTvd*), *Trichoderma virens* (*PTvs*) and *Trichoderma asperellum* (*PTas*) as represented in Fig. 1. Similarly, UV radiation used for induced mutation in *Trichoderma* spp. by Faull et al. (1994), Elakkiya and Muralikrishnan (2014). The conidial concentrations were adjusted and induced mutation as mentioned in material and methods. An aliquot of radiated sus-

pension was spread over a surface of solidified PDA media for regeneration of colonies (Intana 2003).

After exposing *Trichoderma* species to different time intervals ; each one was cultured on different media like PDA, Czapack and TSM (*Trichoderma* selective media) and incubated for six days, the regenerated colonies counted from each of exposure interval in each isolates. Even though TSM (*Trichoderma* selective media) was used for regeneration, the maximum number of regenerated colonies was observed in PDA (Fig. 2). So that, among these three media, both parental and mutant colonies regeneration was more in PDA and PDA was found to be best suitable media for the regeneration of colonies (Table 1). At each exposure time, out of number of colonies forming units was observed. In *Trichoderma harzianum*, after 12 h didn't get any cfu's, in *T. virens* and *T. viride*, there was no regeneration after six hours explosion to UV lightand in case of *T. asperellum,* there was no cfu's after three hours.

These results are in accordance with the findings of Abbasi et al. (2014) who observed that mutation was induced in *Trichoderma harzianum* through gamma radiation and 24 mutants were selected. Similar, studies were also carried out by Hassan Abdel Latif and Haggag (2010) and reported that after subjecting to mutagenesis and they obtained three mutants in *T. koningii* and four mutants in *T. reesei*.

## Conclusion

For regeneration of colonies used different cultured media like PDA, Czapack and TSM. Even though TSM (*Trichoderma* selective media) was used for regeneration, the maximum number of regenerated colonies was observed in PDA. So that, among these three media, both parental and mutant colonies regeneration was more in PDA and PDA was found to be best suitable media for the regeneration of colonies.

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