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# INVITRO SCREENING OF NATIVE *TRICHODERMA* ISOLATES AGAINST *SCLEROTIUM ROLFSII* CAUSING COLLAR ROT OF GROUND NUT

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**ABSTRACT**
Screening of forty four isolates of *Trichoderma* (Tri-1 to Tri-44) against *Sclerotium rolfsii* was done through dual culture technique for their efficacy to reduce mycelial growth and formation of sclerotial bodies. Among the 44 tested isolates 10 isolates *viz.,* Tri-8, Tri- 13, Tri- 15, Tri- 16, Tri- 19, Tri-23, Tri-27, Tri- 29, Tri- 41 and Tri- 44 were found be efficient in reducing both mycelial growth and formation of sclerotial bodies by the pathogens. Further, the effective isolates were tested for production of volatile metabolites. Isolates Tri-13 (*T. viride*) and Tri-29 (*T. viride*) were found to reduce the growth of *S. rolfsii* through volatile metabolites compare to other tested isolates and control.

**KEY WORDS**: Trichoderma, Sclerotial bodies, *Sclerotium rolfsii.*

## INTRODUCTION

Collar rot disease is caused by *Sclerotium rolfsii* being one of the most important diseases of crops. It has very wide host range and not easily controlled by chemical means (Sharma *et al.,* 2002). Pathogen will produce mustard seed like sclerotia, which are very resistant to degradation in soil and serve as inoculum for the next season and also help in spreading of the disease to other plants. In the past few years, management of disease by using antagonists which help in reducing the biomagnification. This is influenced by an idea of using indigenous isolates inspite of using the alian strains, so that they can adopt and multiply easily when applied externally. Hence the present investigation was undertaken to get an efficient strain of *Trichoderma* for effective management of collar rot in raichur soils.

## MATERIALS AND METHODS

Rhizosphere soil was collected from disease suppressive area of different crops from Raichur district. Soil sample was serially diluted and plated on Trichoderma selective media and incubated for 7 to 10 days. Actively growing colony of *Trichoderma* spp. was selected and plated on potato dextrose agar. About 44 isolates of *Trichoderma* were obtained form different soil types of different crops rhizospere. The morphological characteristics of fungus such as mycelial colour, shape of conidia, conidiophores, formation of phialides and chlamydospores were studied under microscope. Further the identification was confirmed by sending them National Centre for Fungal Taxonomy (NCFT), Inderpuri, New Delhi. These isolates were evaluated against *Sclerotium rolfsii*, and then the best performing ten isolates were further evaluated for volatile compounds production which inhibits the mycelial growth.

**Dual culture technique**

Fifteen ml of PDA medium was poured into sterile petri plates and allowed for solidification. Five mm discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, *Trichoderma* was placed on the other side *i.e.,* at an angle of 180$^{o.}$ Plates with no antagonists served as control for the pathogen. The plates were incubated at 28 ± 1$^{o}$ C for seven days. Each treatment was replicated thrice. The extent of antagonistic activity by *Trichoderma* isolates *i.e*., growth after contact with fungal plant pathogens was recorded after incubation period by measuring growth of fungal plant pathogens in dual culture plate and in control plate. The per cent inhibition of fungal plant pathogens was calculated using formula as suggested by Vincent (1927).

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

Where,
I = Per cent inhibition
C = Growth of fungal plant pathogens in control (mm)
T = Growth of fungal plant pathogens in dual culture plate (mm)

**Test for production of volatile metabolites**

Selected isolates from dual plate culture were tested for the production of volatile metabolites as inhibitory compounds against the growth of pathogens. Isolates were tested for production of inhibitory volatile compounds by "inverted plate technique" adopted by Dennis and Webster (1971). About 5 mm agar discs were cut from actively growing margin of the *Trichoderma* isolates and laid on the centre of the petri plate containing PDA. 5mm mycelial disc of pathogen (*S. rolfsii*) was inoculated at centre of separate PDA plates of same diameter. Subsequently, the upper lids of both the pathogen-inoculated and *Trichoderma*-inoculated plates were removed the lid containing pathogen was inverted over the plate containing *Trichoderma.* Then both plates were sealed at the junction with the paraffin. One control plate was maintained with pathogen plate inverted and sealed over the uninoculated medium. Each treatment was replicated thrice. All the plates were incubated at the 25 ± 1$^{o}$C for seven days. Observations on radial growth of

pathogen were taken; per cent inhibition was calculated by comparing with the control plate as described earlier.

## RESULTS AND DISCUSSION

Bio efficacy of all forty four *Trichoderma* isolates was studied against *Sclerotium rolfsii* causing collar rot of groundnut presented in the table 1. Among the forty four isolates, Tri-8 (*Trichoderma piluliferum*) had shown maximum percent inhibition of mycelial growth (71.02%) of *Sclerotium rolfsii.* Isolate Tri-33 had shown minimum percent inhibition of mycelial growth (43.30%). Isolates Tri- 23 (69.26%), Tri-13 (68.98%), Tri-27 (67.20%), Tri-16 (66.76%), Tri-19 (66.02%), Tri- 41 (65.83%), Tri-15 (65.37%) and Tri-44 (64.63%) were found to next best inhibitors of mycelial growth, however all other isolates were capable of reducing growth of pathogen (Plate 1).

Isolates Tri-12 (99.21%), Tri-13 (99.21%), Tri-15 (99.21%), and Tri-44 (99.21%) were found to be most efficient in reducing the number of sclerotial bodies in plate when compared to the control plate whereas isolate Tri-43 (30.71%) was less effective reducing the number of sclerotial bodies**.**

**TABLE 1.** *In vitro* evaluation of 44 *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot/ stem rot of groundnut

| Sl. No. | Isolate | Identification | Inhibition of mycelial growth over control (%) | No. of sclerotial bodies formed in dual plate | Reduction in sclerotial bodies over control plate (%) |
|---|---|---|---|---|---|
| 1. | Tri-1 | *Trichoderma viride* | 61.30 (51.53) | 19.00 | 85.04 (67.25) |
| 2. | Tri-2 | *Trichoderma piluliferum* | 52.34 (46.34) | 26.00 | 79.53 (63.10) |
| 3. | Tri-3 | *Trichoderma* sp. | 54.78 (47.74) | 17.00 | 86.61 (68.54) |
| 4. | Tri-4 | *Trichoderma* sp. | 44.48 (41.83) | 23.00 | 81.89 (64.82) |
| 5. | Tri-5 | *Trichoderma virense* | 54.54 (47.60) | 5.00 | 96.06 (78.59) |
| 6. | Tri-6 | *Trichoderma viride* | 60.46 (51.04) | 26.00 | 79.53 (63.10) |
| 7. | Tri-7 | *Trichoderma virense* | 62.86 (52.45) | 30.00 | 76.38 (60.92) |
| 8. | Tri-8 | *Trichoderma piluliferum* | 71.02 (57.43) | 27.00 | 78.74 (62.55) |
| 9. | Tri-9 | *Trichoderma harzianum* | 52.59 (46.49) | 25.00 | 80.31 (63.66) |
| 10. | Tri-10 | *Trichoderma* sp. | 55.46 (48.13) | 33.00 | 74.02 (59.35) |
| 11. | Tri-11 | *Trichoderma viride* | 61.78 (51.81) | 44.00 | 65.35 (53.94) |
| 12. | Tri-12 | *Trichoderma virense* | 64.35 (53.36) | 1.00 | 99.21 (85.90) |
| 13. | Tri-13 | *Trichoderma viride* | 68.98 (56.15) | 1.00 | 99.21 (85.90) |
| 14. | Tri-14 | *Trichoderma* sp. | 64.22 (53.26) | 28.00 | 77.95 (62.00) |
| 15. | Tri-15 | *Trichoderma virense* | 65.37 (53.95) | 1.00 | 99.21 (85.90) |
| 16. | Tri-16 | *Trichoderma hamatum* | 66.76 (54.79) | 3.00 | 97.64 (81.24) |
| 17. | Tri-17 | *Trichoderma virense* | 62.04 (51.97) | 55.00 | 56.69 (48.85) |
| 18. | Tri-18 | *Trichoderma* sp. | 54.91 (47.82) | 47.00 | 62.99 (52.53) |
| 19. | Tri-19 | *Trichoderma piluliferum* | 66.02 (54.35) | 9.00 | 92.91 (74.58) |
| 20. | Tri-20 | *Trichoderma viride* | 62.28 (52.11) | 28.00 | 77.95 (62.00) |
| 21. | Tri-21 | *Trichoderma* sp. | 49.62 (44.78) | 16.00 | 87.40 (69.22) |
| 22. | Tri-22 | *Trichoderma* sp. | 62.16 (52.04) | 41.00 | 67.72 (55.38) |
| 23. | Tri-23 | *Trichoderma virense* | 69.26 (56.33) | 10.00 | 92.13 (73.72) |
| 24. | Tri-24 | *Trichoderma viride* | 63.38 (52.76) | 52.00 | 59.06 (50.22) |
| 25. | Tri-25 | *Trichoderma viride* | 62.96 (52.51) | 36.67 | 71.13 (57.50) |
| 26. | Tri-26 | *Trichoderma viride* | 62.22 (52.08) | 32.00 | 74.80 (59.87) |
| 27. | Tri-27 | *Trichoderma viride* | 67.20 (55.07) | 12.33 | 90.29 (71.86) |
| 28. | Tri-28 | *Trichoderma* sp. | 43.33 (41.17) | 25.00 | 80.31 (63.66) |
| 29. | Tri-29 | *Trichoderma viride* | 67.68 (55.36) | 8.00 | 93.70 (75.48) |
| 30. | Tri-30 | *Trichoderma virense* | 63.69 (52.95) | 30.00 | 76.38 (60.92) |
| 31. | Tri-31 | *Trichoderma harzianum* | 64.20 (53.25) | 27.00 | 78.74 (62.55) |
| 32. | Tri-32 | *Trichoderma* sp. | 62.59 (52.29) | 26.00 | 79.53 (63.10) |
| 33. | Tri-33 | *Trichoderma* sp. | 43.30 (41.15) | 37.00 | 70.87 (57.33) |
| 34. | Tri-34 | *Trichoderma* sp. | 54.35 (47.50) | 40.00 | 68.50 (55.86) |
| 35. | Tri-35 | *Trichoderma* sp. | 52.73 (46.57) | 42.00 | 66.93 (54.90) |
| 36. | Tri-36 | *Trichoderma hamatum* | 53.89 (47.23) | 26.00 | 79.53 (63.10) |
| 37. | Tri-37 | *Trichoderma* sp. | 42.46 (40.66) | 49.00 | 61.42 (51.60) |
| 38. | Tri-38 | *Trichoderma* sp. | 62.56 (52.27) | 48.00 | 62.20 (52.06) |
| 39. | Tri-39 | *Trichoderma virense* | 54.54 (47.60) | 50.00 | 60.63 (51.14) |
| 40. | Tri-40 | *Trichoderma piluliferum* | 50.16 (45.09) | 66.00 | 48.03 (43.87) |
| 41. | Tri-41 | *Trichoderma harzianum* | 65.83 (54.23) | 11.00 | 91.34 (72.90) |
| 42. | Tri-42 | *Trichoderma virense* | 62.89 (52.47) | 23.00 | 81.89 (64.82) |
| 43. | Tri-43 | *Trichoderma viride* | 62.22 (52.08) | 88.00 | 30.71 (33.65) |
| 44. | Tri-44 | *Trichoderma harzianum* | 64.63 (53.51) | 1.00 | 99.21 (85.90) |
| | Control | *Sclerotium rolfsii* | 0.00 (0.00) | 126 | 0.00 (0.00) |
| . | S.Em ± | - | 0.48 | 0.61 | 0.75 |
| | CD (0.01) | - | 1.78 | 2.28 | 2.80 |

Figures in parenthesis are arc sign transformed values

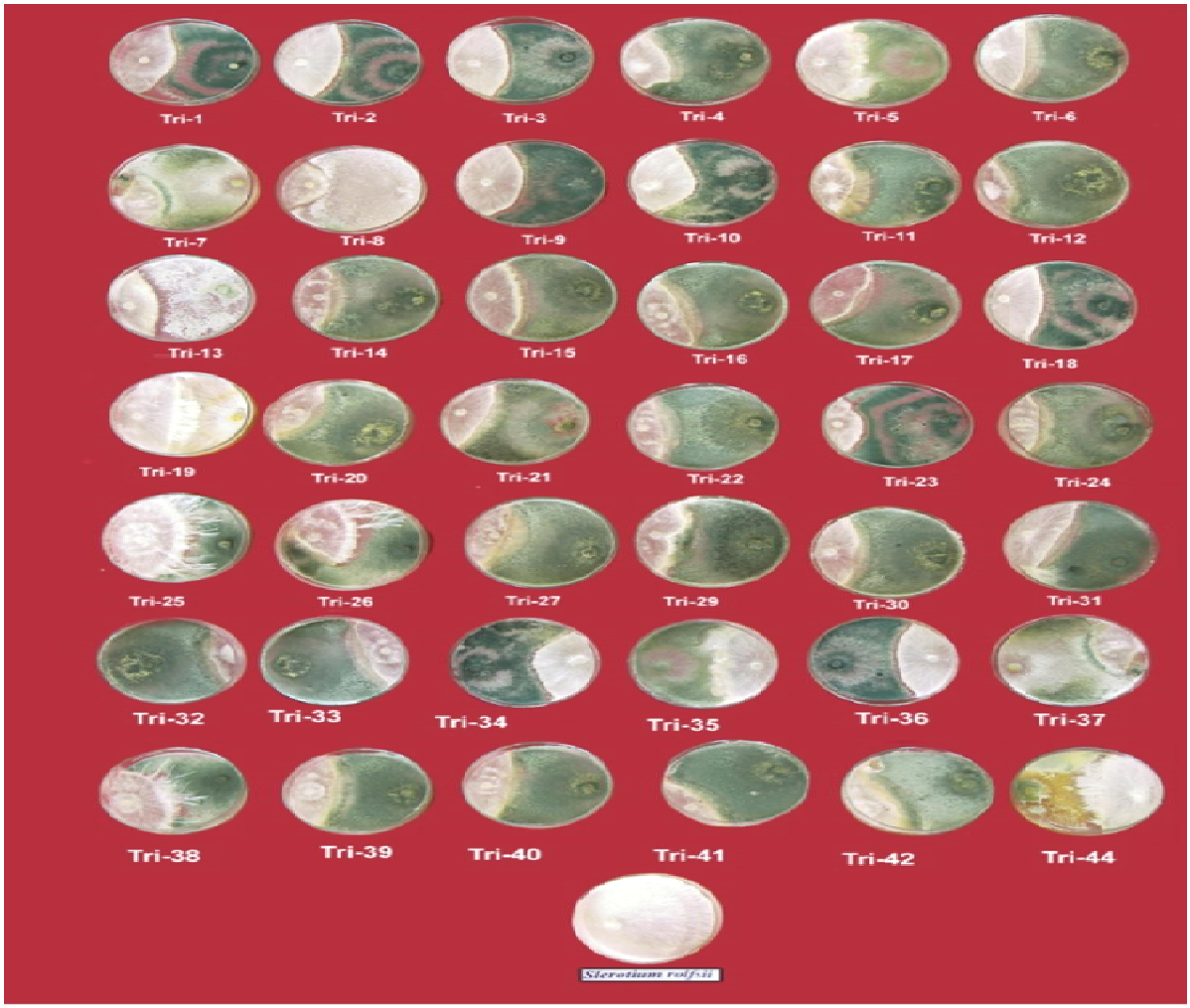


**Plate 1. *In-vitro* evaluation of *Trichoderma* isolates against *Sclerotium rolfsii***

*T. viride* showed maximum inhibition in the mycelial growth of *S. rolfsii* as observed by Kapoor (2008). The antagonistic activity of *T. harzianum* observed in the present study was similar to the findings of Mukhopadhyay (1987). The similar results were also observed by Singh (1991), Prasad *et al.* (1999), and Mukherjee and Tripathi (2000). As of our knowledge goes presently, the evidences regarding the biocontrol efficacy of *Trichoderma piluliferum* are very less or nil. This is one of the new additions to vast diversity of *Trichoderma* with respect to biocontrol activity from these findings.

Comparatively best performing isolates (the isolates *viz;* Tri-8, Tri-13, Tri-15, Tri-16, Tri-19, Tri-23, Tri-27, Tri-29, Tri- 41 and Tri- 44 were found be efficient in reducing growth of pathogen, as well as sclerotial bodies) were tested for the production of volatile compounds.

**TABLE 2.** Effect of volatile compounds produced by *Trichoderma* spp. on *Sclerotium rolfsii*

| Isolate | Identification | *Sclerotium rolfsii* | |
|---|---|---|---|
| | | Pathogen growth (mm) | Inhibition (%) |
| Tri-8 | *T. piluliferum* | 57.60 | 36.00 (36.87) |
| Tri-13 | *T. viride* | 27.66 | 69.33 (56.38) |
| Tri-15 | *T. virense* | 70.90 | 21.22 (27.43) |
| Tri-16 | *T. hamatum* | 64.44 | 28.41 (32.18) |
| Tri-19 | *T. piluliferum* | 73.11 | 18.81 (25.70) |
| Tri-23 | *T. virense* | 71.20 | 20.89 (27.20) |
| Tri-27 | *T. viride* | 70.91 | 21.22 (27.43) |
| Tri-29 | *T. viride* | 43.56 | 51.52 (45.87) |
| Tri-41 | *T. harzianum* | 54.60 | 39.37 (38.86) |
| Tri-44 | *T. harzianum* | 70.34 | 21.87 (27.88) |
| | S.Em± | | 0.84 |
| | C.D.(0.01) | | 3.42 |

Figures in parenthesis are arcsine transformed values

**Effect of volatile compounds produced by *Trichoderma* spp. on *Sclerotium rolfsii***

Among the ten different *Trichoderma* isolates screened for the production of volatile compounds and their effect on the growth and per cent inhibition (Table 2) of *S. rolfsii*. The growth of *S. rolfsii* ranged between 27.66 mm to 73.11mm with highest per cent inhibition of 69.33 by Tri-13 that is *T. viride* and lowest per cent inhibition by Tri-19 (18.81). The observation revealed that all the ten selected isolates of *Trichoderma* spp. produced volatile compounds that inhibit *Sclerotium rolfsii*. *T. viride* showed maximum inhibition of *Sclerotium rolfsii* (69.33%). The inhibitory effects found here were mainly attributed to the antibiosis by volatile metabolites and culture filtrates (Dennis and Webster, 1971 and Mukhopadhyay, 1987). The inhibitory activity of *T. harzianum, T. viride* and *T. virense* against soil borne fungal pathogens found here were similar to findings of Mukharjee and Tripathi (2000) who reported production of volatile and non-volatile substances of bio-control agents.

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