Isolation and characterization of *Trichoderma asperellum* for antagonistic activity against different soil-borne plant pathogens

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Abstract

Rhizospheric soil samples were collected from Akola, Pune, Solapur, Amravati, Sangli and Nagpur and abbreviated as Tv1, Tv2, Tv3, Tv4, Tv5 and Tv6, respectively for the isolation of *Trichoderma asperellum* on *Trichoderma* Selective Medium (TSM) by serial dilution and pour plate technique. Morphological characteristic viz., radial growth and colony characters of these isolates were studied on PDA. The maximum radial growth (mm) was recorded in Tv1 and Tv4 i.e. 90.00 mm collected from Akola and Amravati districts from cotton and soybean crop, respectively whereas, minimum was 87.50 mm observed in Tv3 of Solapur district from Jowar crop. The antagonistic efficacy of *Trichoderma asperellum* against the soil born plant pathogens *viz.*, *Fusarium solani*, *Sclerotium rolfsii* and *Rhizoctonia bataticola* were tested. The maximum per cent inhibition (82.44%) and minimum radial growth (15.80 mm) of *Fusarium solani* was observed in isolate Tv5. In case of *Sclerotium rolfsii* the minimum radial growth (17mm) with maximum per cent inhibition (81.11%) was recorded in isolate Tv3 collected from Solapur district. Antagonism between *Trichoderma asperellum* and test pathogen *Rhizoctonia bataticola* showed maximum growth inhibition (80.33%) in Tv3 isolate.

Keywords: *Trichoderma asperellum*, isolation, soil-borne pathogen, location, antagonism

Introduction

The *Trichoderma* spp. serves as a potential alternative to chemical control measure and growing pathogen resistance crop cultivars. *Trichoderma* is easily identified in culture media, which produces large number of characteristics small, green or white conidia, from phialides growing pathogen resistance crop cultivars. *Trichoderma* are also known to produce different antibiotic substances e.g. Gliotoxin, Gliovirin, Viridin, and Trichoviridin. *Trichoderma* have also been known to inhibit the growth of pathogenic fungi by modifying the rhizosphere. Moreover, infestation of *Trichoderma* in the rhizosphere helps plant to promote nutrient / fertilizer uptake, seed germination and photosynthetic rates (Asad et al. 2014) [3]. 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 biocontrol agents, *Trichoderma* is one of them whose species have been reported to be inhibitory to many soil borne pathogens (Harman *et al.*, 2004) [1].

**Material and Methods**

**Selection of sites for sampling and soil sample collection**

Soil samples were collected from different districts of Maharashtra. The approachable locations of different districts were selected and visited for soil sampling (Table No.1). Generally healthy plants were selected from standing crop of that location and rhizospheric soil was collected. For rhizospheric soil, plant was gently and carefully uprooted, soil tightly adhering the root was collected, such five samples were collected randomly from the crop field, mixed and 1/4th part was used as composite rhizospheric soil sample of the region.

**Table 1:** Soil samples collected from different districts of Maharashtra

| Sr. No. | Location | Crop associated |
|---------|----------|-----------------|
| 1.      | Akola    | Cotton          |
| 2.      | Pune     | Maize           |
| 3.      | Solapur  | Jowar           |
| 4.      | Amravati | Soybean         |
| 5.      | Sangli   | Brinjal         |
| 6.      | Nagpur   | Pigeon pea      |

**Isolation of *Trichoderma asperellum* from rhizospheric soil by serial dilution method**

The *Trichoderma asperellum* was isolated from the soil collected from the different locations by serial dilution technique. The *Trichoderma* selective medium was used for the isolation of *Trichoderma asperellum*. 1 ml of soil suspension from dilutions (10^{-3} and 10^{-5}) was aseptically added to sterile petriplates containing twenty ml of *Trichoderma selectives* medium and incubated 37°C for 3 days. After incubation, well separated individual colonies with yellow green and whitish green pigments were marked. The individual colonies were picked up with sterile loop and transferred to Potato Dextrose agar media plates and the pure cultures so obtained were stored in a refrigerator at 40 °C for further use. (Arunugam K. *et al*. 2013) [1].

*Trichoderma* selective medium (TSM) (Elad *et al.*, 1981; Mukherjee, 1991) [6, 11] was used for isolation of *Trichoderma asperellum*. The ingredients are as follows:

**Table 2:** Ingredients of *Trichoderma* Selective Medium (TSM)

| Chemicals               | Quantity |
|-------------------------|----------|
| MgSO_{4}.7H_{2}O         | 0.2 g    |
| K_{2}HPO_{4}             | 0.9 g    |
| KCl                      | 0.15 g   |
| NH_{4}NO_{3}             | 1.0 g    |
| Glucose                  | 3.0 g    |
| Chloramphenicol          | 0.20 g   |
| Apron 35SD               | 0.015 g  |
| Captan                   | 0.2 g    |
| Rose Bengal              | 0.15 g   |
| Agar-agar                | 20.0 g   |
| Distilled water          | To make volume 1 Litr. |

**Purification of Trichoderma asperellum cultures**

*Trichoderma asperellum* isolates were purified by single spore culture. The spores of the isolates were inoculated into a Petri dish seeded with PDA medium. Sub- culturing was done from the growing front of the single new colony. Small amount of spores were taken on the tip of a sterilized inoculating needle and streaked on PDA poured Petri dishes. This process was repeated by taking inoculum from edge of colonies growing in the freshly streaked Petri plate, and again streaking it in PDA plates. Colony arising from single spore was picked up and inoculated on a fresh plate. This culture was used for further studies.

**Study of morphological characteristics and growth rate of Trichoderma asperellum isolates**

The morphological characteristics and growth rates of the 6 isolates of *Trichoderma asperellum* were determined on Potato dextrose agar (PDA) medium. A 5 mm diameter plug was cut from the actively growing edge of a fresh colony (before the start of conidial production) of the isolates, using a sterile cork borer. The disc was placed in a 90 mm Petri dish, containing 20 ml of PDA medium, approximately 1.5 cm from the edge of the Petri dish with the mycelial surface facing downwards. Three replications were maintained for each isolate. The Petri dishes were incubated in darkness at 28±10 °C. The colonies were examined at 24 h intervals and colony radius was measured from the edge of the inoculum plug after 7 days. The following observations on growth rate and cultural characters of the isolates were recorded:

1. Colony diameter on PDA after 7 days
2. Colony growth type
3. Colony colour
4. Pigmentation in the colony

**Collection of pure cultures of soil inhibiting plant pathogens**

Pure cultures of soil born plant pathogen *viz.*, *Fusarium solani*, *Sclerotium rolfsii* and *Rhizoctonia bataticola*, were collected from Department of Plant Pathology, Dr. P.D.K.V. Akola, which previously known pathogenic nature.

**Dual culture Technique**

Antagonistic activity of *Trichoderma* isolates were assayed against *Rhizoctonia bataticola*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp *solani* by using dual culture inoculation technique described by Vincent, (1927) [19]. Mandal *et al.*, (1999) [18] 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. Three discs of each actively growing isolates of *Trichoderma asperellum* were placed at equidistance on all four sides 30

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**Table 3:** Location and code of *Trichoderma asperellum* isolates

| Sr. No. | Location | Crop associated | Code name |
|---------|----------|-----------------|-----------|
| 1.      | Akola    | Cotton          | Tv1       |
| 2.      | Pune     | Maize           | Tv2       |
| 3.      | Solapur  | Jowar           | Tv3       |
| 4.      | Amravati | Soybean         | Tv4       |
| 5.      | Sangli   | Brinjal         | Tv5       |
| 6.      | Nagpur   | Pigeon pea      | Tv6       |
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. Simultaneously the pathogenic fungus disc (5mm) was incubated on PDA Petri plates alone and incubated under similar condition for same period. Plates were observed every day for noting the behaviour at the point of intermating of two cultures under stereoscopic microscope. On seventh days after incubation, the growth of pathogenic test fungus was measured and per cent growth inhibition was calculated using the following formula.

\[
\text{Per cent Growth inhibition} = \left(\frac{C - T}{C}\right) \times 100
\]

Where
- \(C =\) Mycelial growth (mm) in control plate.
- \(T =\) Mycelial growth (mm) in treatment plate.

### Table 4: Antagonistic efficacy of *Trichoderma asperellum* isolates against *Fusarium solani* in dual culture technique

| Treatment No. | Code name | Description |
|---------------|-----------|-------------|
| T1            | Tv1       | *Trichoderma asperellum* (Tv1) + *Fusarium solani* |
| T2            | Tv2       | *Trichoderma asperellum* (Tv2) + *Fusarium solani* |
| T3            | Tv3       | *Trichoderma asperellum* (Tv3) + *Fusarium solani* |
| T4            | Tv4       | *Trichoderma asperellum* (Tv4) + *Fusarium solani* |
| T5            | Tv5       | *Trichoderma asperellum* (Tv5) + *Fusarium solani* |
| T6            | Tv6       | *Trichoderma asperellum* (Tv6) + *Fusarium solani* |
| T7            | Control   | Fusarium solani culture |

### Table 5: Antagonistic efficacy of *Trichoderma asperellum* isolates against *Sclerotium rolfsii* in dual culture technique

| Treatment No. | Code name | Description |
|---------------|-----------|-------------|
| T1            | Tv1       | *Trichoderma asperellum* (Tv1) + *Sclerotium rolfsii* |
| T2            | Tv2       | *Trichoderma asperellum* (Tv2) + *Sclerotium rolfsii* |
| T3            | Tv3       | *Trichoderma asperellum* (Tv3) + *Sclerotium rolfsii* |
| T4            | Tv4       | *Trichoderma asperellum* (Tv4) + *Sclerotium rolfsii* |
| T5            | Tv5       | *Trichoderma asperellum* (Tv5) + *Sclerotium rolfsii* |
| T6            | Tv6       | *Trichoderma asperellum* (Tv6) + *Sclerotium rolfsii* |
| T7            | Control   | Sclerotium rolfsii culture |

### Table 6: Antagonistic efficacy of *Trichoderma asperellum* isolates against *Rhizoctonia bataticola* in dual culture technique

| Treatment No. | Code name | Description |
|---------------|-----------|-------------|
| T1            | Tv1       | *Trichoderma asperellum* (Tv1) + *Rhizoctonia bataticola* |
| T2            | Tv2       | *Trichoderma asperellum* (Tv2) + *Rhizoctonia bataticola* |
| T3            | Tv3       | *Trichoderma asperellum* (Tv3) + *Rhizoctonia bataticola* |
| T4            | Tv4       | *Trichoderma asperellum* (Tv4) + *Rhizoctonia bataticola* |
| T5            | Tv5       | *Trichoderma asperellum* (Tv5) + *Rhizoctonia bataticola* |
| T6            | Tv6       | *Trichoderma asperellum* (Tv6) + *Rhizoctonia bataticola* |
| T7            | Control   | *Rhizoctonia bataticola* culture |

### Results and Discussion

**Morphological Characters of *Trichoderma asperellum***

Morphological characters of *Trichoderma asperellum* with respect to radial growth and colony characters were studied on PDA.

The radial growth (mm) of all isolates was measured at 7th days after inoculation. The maximum radial growth (mm) was recorded in Tv1 and Tv4 i.e. 90.00 mm collected from Akola and Amravati Districts on cotton and soybean crop respectively whereas minimum was (87.50 mm) observed inTv3 of Solapur District from Jowar crop. Among six isolates of *Trichoderma asperellum* Tv1, Tv5 and Tv6 are milky white to dark green in colour with white yellow and amber colour pigmentation, respectively having subaerial and disperse mycelial growth. The isolate Tv2 produce greenish yellow colony colour with yellow pigmentation having flat and disperse mycelial growth. The isolate Tv3 had subaerial mycelial growth, milky white to grayish green colony colour with yellow pigmentation. Inisolate Tv4, flat and subaerial mycelial growth was observed with light grey to greenish yellow colony colour and white pigmentation on PDA.

The present results are in agreement with Soesanto et al. (2011) [16] and Khang et al. (2013) [8], who were isolated *Trichoderma* spp. and studied the colony colour as velvetyinous with white and dark green floccose surface along with scattered green patches and yellow to green pigmentation on PDA medium.

### Table 7: Morphological characteristics of *Trichoderma asperellum* collected from different Districts of Maharashtra

| Sr. No. | Isolates | Radial growth (mm) at 7 DAI | Colony growth type | Colony colour | Pigmentation |
|---------|----------|-----------------------------|-------------------|--------------|-------------|
| 1.      | Tv1      | 90.00                       | Sub aerial and disperse | Milky white to dark green | White colour |
| 2.      | Tv2      | 89.67                       | flat and disperse   | Greenish yellow    | Yellow colour |
| 3.      | Tv3      | 87.50                       | Sub aerial and disperse | Milky white to grayish green | Yellow colour |
| 4.      | Tv4      | 90.00                       | Flat and Superficial | Light grey to greenish yellow | White colour |
| 5.      | Tv5      | 87.90                       | Disperse and superficial | Milky white to dark green | Yellow colour |
| 6.      | Tv6      | 89.33                       | Sub aerial and disperse | Milky white to dark green | Amber colour |
Antagonistic efficacy of *Trichoderma asperellum* isolates against *Fusarium solani*, *Scleritium rolfsii* and *Rhizoctonia bataticola* (per cent growth inhibition) at 7 DAI

The per cent growth inhibition of *Rhizoctonia bataticola* by *Trichoderma asperellum* isolates was shown in Table 8. Antagonism between *Trichoderma asperellum* and test pathogen *Rhizoctonia bataticola* indicated that the test pathogen stops growing upon contact with the antagonist *Trichoderma asperellum*. The maximum growth inhibition (80.33\%) of *Rhizoctonia bataticola* was exerted by *Tv3* isolate with minimum radial growth (17.70 mm) which was at par with *Tv5* isolate (79.41\%).

This study is further supported by Shalini and Kotasthane (2006)\[15\], Mayo et al. (2015)\[10]\, Naimi et al. (2010)\[12\] who studied the *in vitro* antifungal ability of the different *Trichoderma* isolates was based on the ability to produce metabolites that may inhibit the growth of *R. solani*.

| Sr. No. | Isolates | Radial growth (mm) at 7th DAI | Per cent inhibition over control |
|---------|----------|------------------------------|---------------------------------|
|         |          | *F. solani* | *S. rolfsii* | *R. bataticola* | *F. solani* | *S. Rolfsii* | *R. bataticola* |
| 1.      | *Tv1*    | 20.32       | 19.30        | 26.57           | 77.43      | 78.55        | 70.47          |
|         |          |              |              | *(59.94)*       | *(62.43)*  | *(57.08)*    |                |
| 2.      | *Tv2*    | 19.17       | 18.47        | 22.00           | 78.70      | 79.47        | 75.55          |
|         |          |              |              | *(62.51)*       | *(63.07)*  | *(60.36)*    |                |
| 3.      | *Tv3*    | 17.53       | 17.00        | 17.70           | 80.52      | 81.11        | 80.33          |
|         |          |              |              | *(63.82)*       | *(64.23)*  | *(63.67)*    |                |
| 4.      | *Tv4*    | 18.20       | 17.63        | 24.93           | 79.78      | 80.41        | 72.3           |
|         |          |              |              | *(63.27)*       | *(63.72)*  | *(58.24)*    |                |
| 5.      | *Tv5*    | 15.80       | 17.90        | 18.53           | 82.44      | 80.11        | 79.41          |
|         |          |              |              | *(65.22)*       | *(61.68)*  | *(60.00)*    |                |
| 6.      | *Tv6*    | 23.60       | 19.70        | 24.87           | 73.78      | 78.11        | 72.36          |
|         |          |              |              | *(59.19)*       | *(60.41)*  | *(58.28)*    |                |
| 7.      | control  | 90          | 90.00        | 90.00           | 00.00      | 00.00        | 00.00          |
|         |          | F test      | Sig          | Sig             | Sig        | Sig          | Sig            |
|         |          | S.E (M)±    | 0.50         | 0.58            | 0.50       | 0.74         | 1.03           |
|         |          | C.D. at (p=0.01) | 2.13    | 2.42            | 2.12       | 3.10         | 4.34           |

In case of *Sclerotium rolfsii*, statistically significant differences were obtained among the different *Trichoderma asperellum* isolates against test pathogen over control. The minimum radial growth (17mm) with maximum per cent inhibition (81.11\%) was recorded in isolate *Tv3* collected from Solapur district which was at par with *Tv4* (80.41\%) and *Tv5* (80.11\%). However, the isolate *Tv6* (78.11\%) showed minimum per cent inhibition over control.

The present findings are in conformity with Bagwan N. B. (2011)\[3\], who tested *Trichoderma* spp. Isolates against *Sclerotium rolfsii* in which, isolate T043 showed maximum (98.70\%) growth inhibition. Rao and Kulkarni (2003)\[13\] also reported the maximum (58.50\%) growth inhibition of *Sclerotium rolfsii* by *Trichoderma asperellum*. Shrinivasulu (2005)\[18\] also reported that *Trichoderma asperellum* was very effective in reducing the radial growth of *Sclerotium rolfsii*.

References

1. Arumugam K, Ramalingam P, Appu M. Isolation of *Trichoderma Asperellum* and *Pseudomonas fluorescens* organism from soil and their treatment against rice pathogens, J Microbiol. Biotech. Res 2013;3(6):77-81.
2. Asad SA, Ali N, Hameed A, Khan SA, Ahmad R, Bilal M et al. Biocontrol efficacy of different isolates of *Trichoderma* against soil borne pathogen *Rhizoctonia solani*. Polish J. Microbio 2014;63(1):95-103.
3. Bagwan NB. Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii*, *Aspergillus niger* and *Aspergillus flavus*. Int. J Pl. Protection 2011;4(1):107-111.
4. Belete E, Ayalew A, Ahmed S. Evaluation of local isolates of *Trichoderma* spp. against Black Root Rot (*Fusarium solani*) on Faba Bean, J Pl. Pathol Microb 2015;6:6.
5. Chet I, Inbar J, Hadar I. Fungal antagonists and mycoparasites, In: The mycota IV: Environmental and microbial relationships. Eds: Wicklow, D.T and B. So
6. Elad Y, Chet L, Henis Y. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 1981;9(1):59-67.
7. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews 2004;2:43-56.
8. Khang VT, Nguyen TMA, Pham MT, Tham NTH. Isolation and selection of *Trichoderma* spp. exhibiting high antifungal activities against major pathogens in Mekong delta Omonrice 2013;19:159-171.
9. Mandal S, Srivastava KD, Agrawal R, Singh DV. Mycoparasitic action of some fungi on blotch pathogen (*Oreochrysis sorokiniana*) of wheat. Indian Phytopath 1999;52(5):39-43.
10. Mayo S, Gutierrez S, Malmierca MG, Lorenzana A, Campelo MP, Hermosa R et al. Influence of *Rhizoctonia*...
solani and Trichoderma spp. In growth of bean (Phaseolus vulgaris L.) and in the induction of plant defense-related genes. Original Research 2015. Doi: 10.3389/fpls.2015.00685

11. Mukherjee PK. Biological control of Chick-pea wilt complex, Ph. D. Thesis, G. B. Pant Univ. Agri. Tech., Pantnagar, India, 1991, pp188.

12. Naeimi S, Okhovvat SM, Nikkhah MJ, Vágvölgyi C, Khosravi V, Kredics L. Biological control of Rhizoctonia solani AG1-1A, the causal agent of rice sheath blight with Trichoderma strains. Phytopathol. Mediterr 2010;49:287-300.

13. Rao SN, Kulkarni S. Effect of Trichoderma spp. on the growth of Sclerotium rolfsii Sacco J BioI. Control 2003;17(2):181-184.

14. Samuels GJ. Trichoderma: a review of biology and systematics of the genus. Mycological Research 1996;100:923-935.

15. Shalini N, Lata KP, Kotasthane AS. Genetic relatedness among Trichoderma isolates inhibiting a pathogenic fungi Rhizoctonia solani, African J Biotech 2006;5(8):580-584.

16. Soesanto L, Utami DS, Rahayuniati RF. Morphological characteristics of four Trichoderma isolates and two endophytic Fusarium isolates. Can. J sci. and Industrial Res 2011, 2(8).

17. Sonawane A, Mahajan M, Renake S. Antifungal activity of a fungal isolates against Pomegranate wilt pathogen Fusarium Int. J Curr. Microbiol. App. Sci Special 2015;2:48-57.

18. Srinivasulu B, Krishnakumar KV, Aruna K, Krishnaprasadji J, Rao DVR. In vitro antagonism of three Trichoderma spp. against Sclerotium rolfsii Sacc., a collar-rot pathogen in elephant foot yam. J BioI. Control 2005;19(2):167-171.

19. Vincent JM. Distortion of fungal hyphae in the presence of certain Inhibitors Nature 1927;159:350.