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# Isolation of *Trichoderma spp*. from arid and semi-arid condition and screening for its *in vitro* bio-efficacy and abiotic stress tolerance

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This study was proposed to isolate *Trichoderma* spp., (VT-1 to VT-18) from healthy rhizosphere (20 cm deep) of different fields and from diverse agro-ecological sub regions of India. The 18 isolates were screened for their antagonist efficacy, drought, temperature, salinity tolerance, volatile, non-volatile and chitinase production. The five fungal phytopathogens were tested for antagonistic potential against eight *Trichoderma* spp., isolates viz. VT7, VT8, VT13, VT14, VT15, VT16, VT17 and VT18 that gave aconsiderable antagonistic effect on mycelial growth of the pathogens in dual cultures compared to the controls. Maximum inhibition occurred in *Magnaporthe oryzae* 90*%, Rhizoctonia solani* 84.70%, *Fusarium ricini* 70%, *Macrophomina phaseolina* 70.50% and *Athelia rolfsii* 64.50% using dual culture assay and through production of non-volatile inhibitors. *Trichoderma* spp. was treated in various concentration of poly ethylene glycol (PEG) 6000 DA, 10-40 Mpa isolates VT2 and VT7 shown highest drought tolerance, salinity tolerance studied by various NaCl concentrations. 0.1-3 M NaCl concentration was adopted on four isolates VT7, VT8, VT9 and VT14 that showed highest saline tolerance at 1.5 M concentration, temperature tolerance growth study was done at a range from 35-44°C to be mesophilic and optimum growth of 44°C was maintained for two isolates VT2 and VT7 that showed highest temperature tolerance in all the experiments compared to the commercial control strains such as *Trichoderma harzianum* (TNAU), *Trichoderma viride* (IIHR); *Trichoderma asperellum* (TRA) was used as control for comparative study.

**Keywords:** Antagonistic activity; drought; mesophilic, *Trichoderma*; volatile

## INTRODUCTION

Increasing the farm productivity in a lesser area by conditioning the crops/plants to the changing environment are the key factor to be focussed in the coming years of the 21st century. The role of rhizospheric microbiome known as the second genome of plants, collectively containing actinomycetes, bacteria and fungi, oomycetes are closely related to plant growth health in protection the changing environments (Li *et al.* 2021).

The role of microbial biocontrol agent on plant protection is well demonstrated. *Trichoderma* is an important biocontrol agents reported to act against major plant pathogens (Prabavathy *et.al*. 2006), as biocontrol agent against root rot diseases (Chenniappan *et al.* 2019), damping off (Jung *et al*., 2003), wilt (Pegg *et al*. 2019), fruit rot (Sadfi-Zouaoui *et al.* 2008) by suppressing the growth of fugal phytopathogens and regulate the rate of plant growth and other plant diseases (Kumar and Khurana, 2021). The secondary metabolites epipolythiodioxopiperazines secreted by *Trichoderma* spp. have proven its role in suppressing the growth of pathogenic microorganisms and stimulating the plant growth (Khan *et al.* 2020).

Besides, the interaction between plant and *Trichoderma* spp. reported to successfully regulate root architecture, increase the length of lateral and primary root that result in the effectiveness of nutrient uptake by the plant (Nieves-Cordones *et*

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*al*., 2020). The diversity of *Trichoderma* spp. as natural decomposition agents of biological agent of bioremediation reported by several studies (Zin and Badaluddin, 2020). Since *Trichoderma* is known to regulate the root growth function as a good biocontrol agent through various mode of actions it is important to identify a few efficient isolates that could with stand the adverse climatic conditions while acting as a biocontrol agent.

## MATERIALS AND METHODS

### *Chemicals and experimental statistics*

All chemicals used were of analytical grade and media components of high purity grade. The microbiological media used were dehydrated media (Hi-Media, Mumbai). Production studies were carried out as batch cultures in 250 ml Erlenmeyer flasks, containing 100 ml of culture media. All the experiments were carried out independently in triplicates and repeated twice. The standard deviation in results was within experimental limits.

### *Isolation of Trichoderma from isolated soil sample*

Soil samples were collected from healthy rhizosphere (20 cm deep) of different fields and from diverse agro-ecological sub regions of India (Fig. 1). *Trichoderma* was isolated from the collected soil samples by the technique as described by Tozlu *et al*. (2018). Twenty grams of each sample were thoroughly mixed with 500 ml distilled water containing 0.02% citric acid. Five ml of prepared solutions were then added to Petri plates containing 15 ml water agar at 50°C and shaking gently to mix it properly. After cooling, 5 cm solidified water agar discs were then transferred to plates containing Davet selective media and were incubated at 25°C. After that, isolates were purified and identified after proper growth based on the morphological characters discribed for *Trichoderma* by microscopic observations (Fig.1). India map showing soil sample collecting locations used for the isolation of *Trichoderma* isolates.

### *In vitro dual culture assay*

*Trichoderma* isolates were evaluated against five phytopathogens *Fusarium oxysporum* f. sp. *ricini, Macrophomina phaseolina, Rhizoctonia solani, Athelia rolfsii* and *Magnaporthe oryzae* by the dual plate culture technique. The mycelia disc of each pathogen measuring 9 mm was placed in the opposite ends of a PDA plate. The plates were incubated for 2 to 5 days at room temperature (28 ± 2 °C), after which, the antagonistic activity of *Trichoderma* spp. against phytopathogens was observed as zone of inhibition (ZOI). The promising antagonistic activity against tested fungal pathogens was measured and the percent inhibition of average radial growth was calculated relative to the control as follows:

L = [(C – T)/C] x 100, Where L is the percentage inhibition of radial mycelial growth, C is radial growth of the pathogen in the control; T is radial growth of the pathogen in the presence of *Trichoderma* (Edington, 1975).

### *Effect of drought tolerance*

The efficiency of drought tolerance by *Trichoderma* spp. was studied using poly ethylene glycol (PEG) 6000 DA. Concentrations of 10, 20, 30 and 40% of PEG were amended with PDA and sterilized. A 5 mm bore was made from each *Trichoderma* spp. actively growing plate and placed on the PEG amended PDA medium. The plates were incubated at 28°C for 7 days and results were recorded in terms of mycelial growth over the control. The sporulation on the plates were recorded based on the dense appearance and were graded as (+++) good, (++) moderate (+) weak and (-) no sporulation. Three commercial *Trichoderma* spp viz., *Trichoderma harzianum* (TNAU), *Trichoderma viride* (IIHR) and *Trichoderma asperellum* (TRA) were used as control for comparative study.

### *Effect of salinity tolerance*

Salinity tolerance by *Trichoderma* spp was studied by using different molars of sodium chloride (NaCl) various concentration 0.1-3 M was prepared in PDA media and sterilized at 121°C for 20 min. Actively growing cultures of *Trichoderma* spp., of 5 mm size were aseptically transferred on to the agar plates and kept in the incubator at 27°C for 7 days. The results were noted based on the mycelial growth and sporulation pattern compared to the control.

### *Effect of temperature tolerance*

Ten mL of PDB was dispensed into 30 mL screw cap tubes and autoclaved. Inoculated with 100µL

of spore suspension (1x10$^6$ spores/mL) of *Trichoderma* isolates and incubated at 36°C, 38°C, 40°C, 42°C and 44°C for a period of 7 days. After the completion of incubation period, mycelial biomass was separated by filtering through sterile Whatman No.1 filter papers and the mycelium was dried in sterile pre-weighed glass Petriplates to constant weight in an oven at 60$^0$C. The final weight of the Petriplates was recorded and the difference in the weight was taken as biomass of the test fungus.

***Evaluation of volatile metabolites***

The effect of the volatile metabolites released by *Trichoderma* on the mycelial growth of the pathogens was evaluated by the "Inverted Plate Technique". The 5 mm mycelial discs of *Trichoderma* obtained from the margin of young cultures were placed centrally on the PDA glass dish (Borocil, 100 × 20 mm) and incubated in 25±1ºC for 0, 24, 48 and 72 h. In the control plates, sterile PDA media discs 5 mm in diameter were placed on the plates as mentioned above. At the end of the incubation period, the top of each Petri dish was replaced with the bottom of the Petri dish inoculated with pathogen and sealed together with adhesive tape. Sealing Petri dishes avoided the escape of volatile compounds of the antagonist and ensured their inhibitory effect on the pathogen. A completely randomized experimental design was used with three replicates. Radial growth of the pathogens was recorded on the 5th day of incubation and growth was calculated, as described above.

***Evaluation of non-volatile metabolites***

The food poisoning technique was used to evaluate the effect of non-volatile metabolites produced by *Trichoderma* on the growth of pathogens. 5 mm discs of young mycelial agar plugs were taken from the edge of growth cultures and were inoculated in 250-ml conical flasks having 100-ml sterilized potato dextrose broth (PDB) and incubated at 25±1ºC. After incubating for 7 days, the culture filtrates were collected and centrifuged at 3000 rpm for 20 min; the supernatants were filtered through 45 ìm Millipore filters using a vacuum pump assembly under aseptic conditions. Just before pouring, culture filtrate was mixed with molten PDA to obtain a final concentration of 5, 10, 15 and 20%. 5-mm discs of the pathogens were inoculated to the Petri dishes amended with culture filtrate and incubated at 25±1ºC. The colony diameter of the pathogens was measured after 5 days and compared with the growth of the pathogen in the control. In control, the PDA was amended with the same amount of sterilized culture filtrate.

***Screening for chitinase production***

Chitinase activity was determined using colloidal chitin at 0.5% (w/v) dissolved in acetate buffer (50mM and pH 5.5) as a substrate. Colloidal chitin was prepared following the method of Mathivanan *et al*. (1998). All the *Trichoderma* isolates were grown in basal medium amended with colloidal chitin. Each 500 mL flask containing 250 mL medium was inoculated with a culture disk (5 mm diameter) taken from the growing margins of fungi on a PDA plate. Three replications were maintained for each isolate. The inoculated flasks were then placed on a rotary shaker at 180 rpm and 28°C. The culture filtrate (250 mL) was used as crude protein extract after incubation for 5 days. The culture broth was filtered through 0.22 ìM bacterial proof filter (Millipore Corporation; Bedford, MA, USA). Culture filtrates were used as sources of crude chitinase and kept throughout the experiment at 4°C.

***Assay of chitinase***

The chitinase assay mixture contained 150 ìl of colloidal chitin and 50 ìl of crude enzyme extract prepared as described above and incubated at 35°C for 120 min. The reaction was stopped by addition of one ml of 3, 5-dinitrosalicylic acid (DNS) and further incubated at 100°C for 5 min. The amount of reducing sugar was determined by measuring absorbance at 540 nm in a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme necessary to release 1µmol reducing sugar per minute.

**RESULTS AND DISCUSSION**

***Isolation of Trichoderma***

A total of 18 *Trichoderma* spp. were isolated from eighteen different plants healthy *rhizospheres*. The *rhizosphere* soil samples were collected from 16 arid and semi arid conditions of diverse agro-ecological sub regions of India. The *Trichoderma spp*. (VT-1 to VT-18) was isolated from

**Table1:** Isolation of *Trichoderma* from healthy rhizospheric soil samples collected from different states

| States | Locations | Crops | Isolate codes |
|---|---|---|---|
| Telangana | Khammam | Chilli | VT-1 |
| Telangana | Nalgonda | Citrus | VT-2 |
| Tamil Nadu | Madurai | Banana | VT-3 |
| Karnataka | Chitradurga | Cotton | VT-4 |
| Maharashtra | Pune | Grapes | VT-5 |
| Andhra Pradesh | Visakhapatnam | Turmeric | VT-6 |
| Andhra Pradesh | Chittoor | Tomato | VT-7 |
| Rajasthan | Phalodi | Bajra | VT-8 |
| Gujarat | Patan | Maize | VT-9 |
| Punjab | Jalandhar | Wheat | VT-10 |
| Assam | Nalbari | Mustard | VT-11 |
| Kerala | Kottayam | Tapioca | VT-12 |
| Madhya Pradesh | Jabalpur | Soyabean | VT-13 |
| Utter Pradesh | Shahjahanpur | Groundnut | VT-14 |
| Sikkim | Gangtok | Maize | VT-15 |
| Uttarakhand | Nainital | Potato | VT-16 |
| Arunachal Pradesh | Changlang | Paddy | VT-17 |
| Bihar | Jamui | Onion | VT-18 |

**Table. 2:** Antagonistic activity of *Trichoderma* isolates against fungal pathogens

| Isolate codes | *Fusarium ricini* (%) | *Macrophomina phaseolina* (%) | *Rhizoctonia solani* (%) |
|---|---|---|---|
| VT-1 | 17.0 | 33.0 | 47.5 |
| VT-2 | 30.8 | 39.9 | 45.0 |
| VT-3 | 10.5 | 26.3 | 36.0 |
| VT-4 | 24.5 | 46.4 | 45.0 |
| VT-5 | 48.0 | 24.0 | 43.9 |
| VT-6 | 29.5 | 33.5 | 24.8 |
| VT-7 | 63.0 | 44.4 | 56.2 |
| VT-8 | 63.0 | 46.0 | 58.5 |
| VT-9 | 33.0 | 33.8 | 38.5 |
| VT-10 | 39.0 | 37.3 | 44.4 |
| VT-11 | 35.0 | 30.3 | 40.9 |
| VT-12 | 33.0 | 26.7 | 38.5 |
| VT-13 | 62.0 | 64.7 | 52.0 |
| VT-14 | 60.0 | 60.0 | 69.5 |
| VT-15 | 60.0 | 61.5 | 52.5 |
| VT-16 | 60.0. | 54.5 | 64.7 |
| VT-17 | 70.0 | 70.5 | 84.7 |
| VT-18 | 60.0 | 60.0 | 82.5 |
| *Trichoderma harzianum* (IIHR) | 72.0 | 55.0 | 81.0 |
| *Trichoderma viride* (TNAU) | 68.0 | 74.0 | 88.0 |
| *Trichoderma asperellum* | 70.0 | 58.0 | 78.0 |

**Table. 3:** *In-vitro Trichoderma* growth under drought condition

| Isolates No. | PEG 6000 Da (%) | | | |
|---|---|---|---|---|
| | 10 (-0.15 Mpa*) | 20 (-0.49 Mpa) | 30 (-1.03 Mpa) | 40 (-1.76 Mpa) |
| VT-1 | 100 (+++) | 100 (+++) | 63.2 (+) | 0.0 |
| VT-2 | 100 (+++) | 100 (+++) | 79.1 (+) | 0.0 |
| VT-3 | 100 (+++) | 75.5 (++) | 22.6 (-) | 0.0 |
| VT-4 | 100 (+++) | 43 (++) | 0.0 | 0.0 |
| VT-5 | 100 (+++) | 73.0 (++) | 28.2 (+) | 0.0 |
| VT-6 | 100 (+++) | 95.5 (+++_ | 65.7 (+) | 0.0 |
| VT-7 | 100 (+++) | 100 (+++) | 76.5 (++) | 0.0 |
| VT-8 | 100 (+++) | 100 (+++) | 71.8 (++) | 0.0 |
| VT-9 | 100 (+++) | 100 (+++) | 65.4 (+) | 0.0 |
| VT-10 | 100 (+++) | 61.5 (++) | 10.5 (-) | 0.0 |
| VT-11 | 100 (+++) | 28.5 (+) | 0.0 | 0.0 |
| VT-12 | 100 (+++) | 100 (+++) | 69.5 (++) | 0.0 |
| VT-13 | 100 (+++) | 92 (+++) | 44.0 (++) | 0.0 |
| VT-14 | 100 (+++) | 75 (+++) | 0.0 | 0.0 |
| VT-15 | 100 (+++) | 100 (+++) | 55.2 (++) | 0.0 |
| VT-16 | 100 (+++) | 100 (+++) | 43.5 (++) | 0.0 |
| VT-17 | 100 (+++) | 85.0 (++) | 0.0 | 0.0 |
| VT-18 | 100 (+++) | 100 (+++) | 55.5 (+) | 0.0 |
| *Trichoderma harzianum* (IIHR) | 100 (+++) | 100 (+++) | 72.7 (++) | 0.0 |
| *Trichoderma viride* (TNAU) | 100 (+++) | 100 (+++) | 74.4 (++) | 0.0 |
| *Trichoderma asperellum* (TRA) | 100 (+++) | 100 (+++) | 71.0 (++) | 0.0 |

(+++): Good, (++) moderate and (+) weak sporulation. (-) No sporulation

rhizospheres soils in potato dextrose agar medium (Table 1). Similar study have been performed by Rani *et al.* (2019) using 10 fungal isolate from the garden soil, kitchen waste and farm soil. However it was represented that only three types of isolates were cultured using Potato dextrose agar and Czapek-Dox Agar media.

### *In vitro antagonistic activity of dual culture plate assay*

All the 18 isolates were screened for their antagonist activity against five fungal phytopathogens. The antagonistic potential of

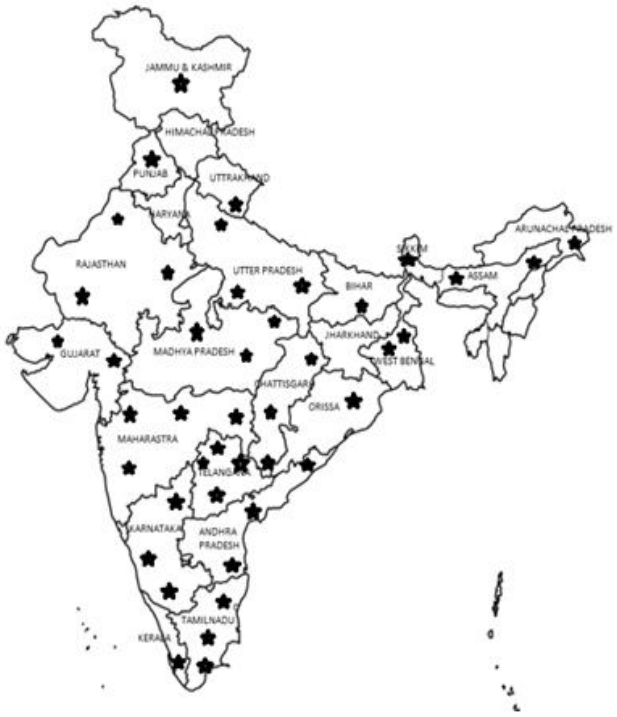


**Fig.1:** Locations depicted in India Map showing soil sample collection sites for isolation of *Trichoderma*

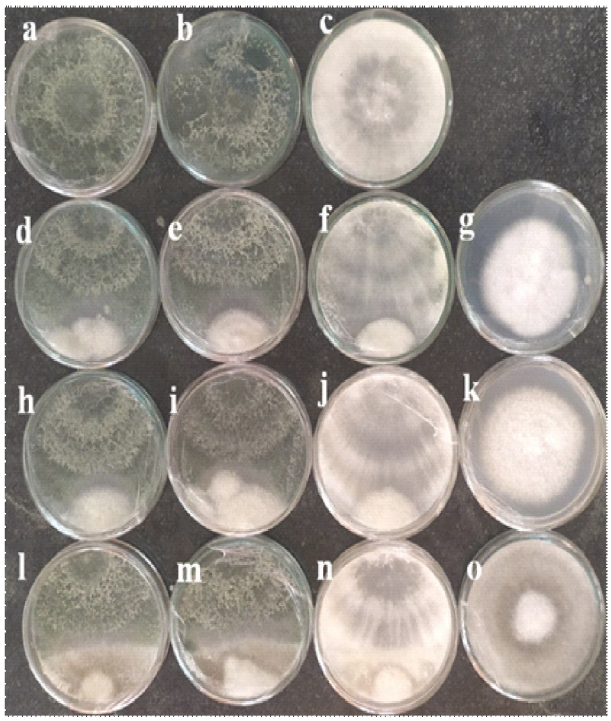


**Fig. 2**. (a)*Trichoderma viride* (b) *Trichoderma asperellum*(c) *Trichoderma harzianum* (d)*T. viride + Fusarium ricini* (e)*T. asperellum + F. ricini* (f) *T. harzianum + F. ricini;* (g) *F. ricini* (h) *T. viride + Rhizactonia solani* (i) *T.asperellum + R. solani* (j) *T. harzianum + R. solani* (k) *R. solani* (l) *T. viride + Macrophomina phaseolina* (m) *T. asperellum + M. phaseolina* (n) *T. harzianum + M. phaseolina* (o) *M. phaseolina*

**Table. 4** : Effect of salinity on growth of *Trichoderma* isolates

| Isolates | Percent growth of *Trichoderma* spp in molar concentrations of Nacl | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.1 | 0.5 | 0.75 | 1.25 | 1.5 | 2.0 | 2.5 |
| VT-1 | 91.0 (++) | 75.8 (+) | 58.4 (-) | 22.0 (-) | 9.2 (-) | 0.0 | 0.0 |
| VT-2 | 93.4 (++) | 71.2 (+) | 40.0 (-) | 18.8 (-) | 6.0 (-) | 0.0 | 0.0 |
| VT-3 | 81.2 (++) | 58.8 (+) | 31.0 (-) | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-4 | 78.2 (+) | 33.0 (-) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-5 | 85.1 (++) | 64.8 (+) | 38.3 (-) | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-6 | 94.8 (++) | 78.3 (+) | 61.5 (-) | 23.2 (-) | 14.5 (-) | 0.0 | 0.0 |
| VT-7 | 96.5 (++) | 82.4 (+) | 65.8 (-) | 30.7 (-) | 19.1 (-) | 0.0 | 0.0 |
| VT-8 | 95.0 (++) | 86.5 (+) | 67.2 (-) | 29.4 (-) | 21.5 (-) | 0.0 | 0.0 |
| VT-9 | 96.2 (++) | 85.8 (+) | 70.1 (-) | 33.6 (-) | 21.8 (-) | 0.0 | 0.0 |
| VT-10 | 71.0 (+) | 21.5 (-) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-11 | 75.8 (+) | 18.5 (-) | 6.5 (-) | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-12 | 65.2 (+) | 21.8 (-) | 9.0 (-) | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-13 | 70.5 (++) | 36.3 (+) | 11.2 (-) | 3.5 (-) | 0.0 | 0.0 | 0.0 |
| VT-14 | 98.9 (++) | 86.8 (-) | 78.2 (-) | 39.4 (-) | 24.2 (-) | 0.0 | 0.0 |
| VT-15 | 85.8 (++) | 69.2 (+) | 32.5 (-) | 8.8 (-) | 0.0 | 0.0 | 0.0 |
| VT-16 | 76.2 (++) | 51.4 (+) | 11.8 (-) | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-17 | 87.5 (++) | 53.2 (+) | 39.0 (-) | 12.1 (-) | 2.1 (-) | 0.0 | 0.0 |
| VT-18 | 92.8 (++) | 79.4 (+) | 63.5 (-) | 35. 4 (-) | 9.6 (-) | 0.0 | 0.0 |
| *Trichoderma harzianum* (IIHR) | 93.0 (++) | 80.5 (+) | 60.5 (-) | 22.3 (-) | 15.5 (-) | 0.0 | 0.0 |
| *Trichoderma viride* (TNAU) | 98.5 (++) | 85.6 (+) | 62.11 (-) | 28.0 (-) | 16.4 (-) | 0.0 | 0.0 |
| *Trichoderma asperellum* (TRA) | 96.5 (++) | 81.0 (+) | 76.3 (-) | 35.5 (-) | 22.0 (-) | 0.0 | 0.0 |

eight *Trichoderma* isolates viz. VT-7, VT-8, VT-13, VT-14, VT-15, VT-16, VT-17 and VT-18 had considerable antagonistic effect on mycelial growth of the pathogens in dual cultures compared to the control. Among them three isolates namely VT-18, VT-16 and VT-13 have exihibited excellent antagonistic activity (>60%) against *Fusarium ricini Macrophomina phaseolina, Rhizoctonia solani, Athelia rolfsii* and *Magnaporthe oryzae*. In particular VT-17 revealed 70% antagonist agent *F. ricini* that was almost equivalent to *T. harzianum* (72%) *T. viride* (68%) and *T. asperellum* (70%). The same isolate VT-17 also provided 70.5% antagonist against *M. phaseolina* (Saravanakumar *et al.,* 2017). The maximum antagonistic activity was shown by *T. viride* (74%) against *M. phaseolina. T. harzianum, T. viride* and *T. asperellum*, isolates VT-17- VT18 have exihibited antagonism above 80% against *R. solani* (Inayati *et al*. 2019). *Trichoderm*a isolate VT-16 revealed 64.5% antagonist against *Athelia rolfsii* in comparison with *T. harzianum* (64%) *T. viride* (65%) and *T. asperellum* (68%) as shown in Fig. 1 and Table 2.

### *Effect of Drought*

Growth of *Trichoderma* isolates (VT-1-VT-18) on PDB medium supplemented with PEG 6000 at the

**Table 5 :** Effect of temperature on Biomass production of *Trichoderma* isolates.

| *Trichoderma* isolates | Biomass (g) | | | | |
|---|---|---|---|---|---|
| | 36°C | 38°C | 40°C | 42°C | 44°C |
| VT-1 | 0.45 ± 0.03 | 0.043 ± 0.008 | 0.041 ± 0.001 | 0.036 ± 0.001 | 0.035 ± 0.003 |
| VT-2 | 0.58 ± 0.03 | 0.056± 0.004 | 0.046 ± 0.001 | 0.05 ± 0.001 | 0.038 ± 0.003 |
| VT-3 | 0.40 ± 0.02 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-4 | 0.63 ± 0.03 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-5 | 0.46 ± 0.03 | 0.049 ± 0.004 | 0.0432 ± 0.0 | 0.0 | 0.0 |
| VT-6 | 0.82 ± 0.02 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-7 | 0.86 ± 0.01 | 0.05 ± 0.003 | 0.036± 0.004 | 0.0 | 0.0 |
| VT-8 | 0.75 ± 0.04 | 0.054 ± 0.003 | 0.042 ± 0.001 | 0.04 ± 0.001 | 0.038 ± 0.003 |
| VT-9 | 0.78 ± 0.01 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-10 | 0.62 ± 0.02 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-11 | 0.46 ± 0.01 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-12 | 0.48 ± 0.01 | 0.042 ± 0.002 | 0.039 ± 0.001 | 0.04 ± 0.002 | 0.029 ± 0.001 |
| VT-13 | 0.81 ± 0.02 | 0.06 ± 0.003 | 0.038± 0.004 | 0.0 | 0.0 |
| VT-14 | 0.36 ± 0.02 | 0.041 ± 0.003 | 0.0432 ± 0.001 | 0.0 | 0.0 |
| VT-15 | 0.8 ± 0.01 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-16 | 0.85 ± 0.01 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-17 | 0.72 ± 0.02 | 0.0 | 0.0 | 0.0 | 0.0 |
| VT-18 | 0.68 ± 0.01 | 0.038 ± 0.002 | 0.031 ± 0.001 | 0.05 ± 0.002 | 0.0 |
| *Trichoderma harzianum* (IIHR) | 0.65± 0.02 | 0.046± 0.004 | 0.023 ± 0.001 | 0.0 | 0.0 |
| *Trichoderma viride* (TNAU) | 0.83 ± 0.01 | 0.062 ± 0.002 | 0.049 ± 0.001 | 0.028 ± 0.002 | 0.029 ± 0.001 |
| *Trichoderma asperellum* (TRA) | 0.79 ± 0.03 | 0.019 ± 0.007 | 0.0 | 0.0 | 0.0 |

levels of 10 to 40%, showed a marked variation among the isolates (Table 3). Moreover, all tested isolates were able to grow in PEG 6000 concentrations at up to 30%. The growth of the *Trichoderma* isolates was negative when they were incubated in PDA placed was 40% PEG. It was observed that *Trichoderma* isolates were tolerant upto 30% of PEG 6000 with various ranging 10.5% in VT-10 to 79% in VT-7 in comparison with 71-74.4% growth in *T. harzianum, T. viride* and *T. asperellum* (Table 3). Similar results in *T. viride, T. harzianum* and *T. koningii* on PDB medium supplemented with PEG 6000 at the levels of 5 to 25%, showed a marked variation among these strains. All tested strains were able to grow in PEG 6000 concentrations at up to 25%. After incubation time of 7 days on *Trichoderma* strains, viable cell numbers showed a decrease in the growth with increasing PEG concentrations (Nahrawy *et al.* 2020).

### *Effect of salinity*

Effects of salt stress on *Trichoderma* spp. treated with NaCl amended Potato dextrose agar medium was studied on isolates VT-7, VT-8, VT-9 and VT-14 showed low growth of 24.2% in 1.5M high concentrations of NaCl and 98.9% of growth in less than 0.1M concentration of NaCl. The results were

**Table. 6:** Primary screening for chitinolytic activity of *Trichoderma*

| S. No. | Isolate codes | Primary screening (mm) | Secondary of screening estimation of amount of N-acetyl glucosamine (U/mL) |
|---|---|---|---|
| 1 | VT-1 | 10 | 6.5 |
| 2 | VT-2 | 12 | 7.2 |
| 3 | VT-3 | 10 | 6.5 |
| 4 | VT-4 | 15 | 7.6 |
| 5 | VT-5 | 18 | 14.0 |
| 6 | VT-6 | 16 | 10.6 |
| 7 | VT-7 | 20 | 16.0 |
| 8 | VT-8 | 22 | 30.0 |
| 9 | VT-9 | 10 | 6.5 |
| 10 | VT-10 | 16 | 10.8 |
| 11 | VT-11 | 10 | 6.5 |
| 12 | VT-12 | 16 | 10.6 |
| 13 | VT-13 | 18 | 14.0 |
| 14 | VT-14 | 28 | 32.5 |
| 15 | VT-15 | 26 | 33.4 |
| 16 | VT-16 | 20 | 16.0 |
| 17 | VT-17 | 22 | 30.5 |
| 18 | VT-18 | 30 | 35.0 |
| 19 | *Trichoderma harzianum* (IIHR) | 20 | 16.0 |
| 20 | *Trichoderma viride* (TNAU) | 18 | 14.0 |
| 21 | *Trichoderma asperellum* | 16 | 12.0 |

noted based on the mycelial growth and sporulation pattern and compared to the commercial control strains viz., *Trichoderma harzianum* (TNAU), *Trichoderma viride* (IIHR) and *Trichoderma asperellum* (TRA) (Table-4). Similarly *T. atroviride* can grow in 100mM of NaCl concentration and promoted the growth of cucumber under 100 mM and 200 mM NaCl, especially for the root (Zhang *et al*. 2022), effect of different concentrations of NaCl (0, 100, and 200 mM) on *T. harzianum* growth (Ahmad *et al.* 2015).

### *Effect of temperature*

Effects of temperature tolerance were evaluated in shaking condition incubating various temperatures in 7 days. After the incubation period, mycelial biomass was separated by filtering through sterile Whatman 1 filter papers and the mycelium was dried in sterile pre-weighed glass petriplates to constant weight in an oven at 60°C. The final weight of the petriplates was recorded and the difference in the weight was recorded for biomass. The 18 isolates VT-3, VT-4, VT-6, VT-9, VT-10, VT-11, VT-15 VT-16 and VT-17 grown in 60°C showed low biomass of 0.40 g. Whereas in high temperature of 44°C grown isolates VT-1, VT-2, VT-8 and VT-12 gave a maximum biomass compared to the commercial control strains *Trichoderma harzianum* (TNAU), *Trichoderma viride (*IIHR)and *Trichoderma asperellum*(TRA) (Table-5). Earlier research work on influence of temperature on the growth, sporulation, colonization and survival of *Trichoderma* spp. strains T065, T071, T154, and T214 25°C was optimum growth was observed (Guzmán Carro-Huerga, *et al.* 2021).

### *Primary screening for chitinolytic activity*

All the 18 *Trichoderma spp.* isolates showed a qualitative assay of chitinolytic activity with a zone of clearance over 10 to 30mm. *Trichoderma spp*., obtained from healthy rhizospheres soil sample VT-8 (Bajra), VT-14 (Ground Nut), VT-15 (Maize), VT-

17 (paddy rice field) and VT-18 (Onion) remarkably hydrolyzed the colloidal chitin and produced a prominent and maximum clear zone in CCA plate. Among the five chitinolytic fungi tested in the secondary screening, the culture filtrate of the healthy rhizospheres isolates VT-8, VT-14, VT-15, VT-17 and VT-18 produced maximum clear zone of 30mm in CCA (Table 6). Trichoderma spp. produces various hydrolytic enzymes such as chitinase, N-acetylglucosaminidase, â-1,3-glucanase, cellulase, endoglucanase, â-glucosidase, protease and amylase (De Marco *et al*. 2003). Screening on qualitative assay of chitinolytic activity of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India using different concentration of bromocresol purple dye (Agrawal and Kotasthane, 2012).

Among the five *Trichoderma spp*. isolates quantitative analysis of chitinase enzymes was tested in PDB amended colloidal chitin substrate (0.5%). High chitinase production five isolates such as; VT-18 (35 U /ml), VT-17 (30.5 U/ml), VT-15 (33.4 U/ml), VT-14 (32.6 U/ml) and VT-8 (30 U/ml) were compared to the positive commercial strains *Trichoderma harzianum* (TNAU), *Trichoderma viride* (IIHR) and *Trichoderma asperellum* (TRA). Similar result to the 22 U/mL after 96 h of *T. asperellum* PQ34 (Loc et al., 2020*). Trichoderma koningiopsis* UFSMQ40 grown using solid state fermentation to yield chitinase production of 10.76 U/gds-1 (Baldoni *et al*. 2020).

## CONCLUSION

The results clearly recorded that 18 *Trichoderma* isolates exhibited anti-fungal activity, drought, salinity, temperature, as well as chitinase production. Five isolates namrely VT-8, VT-14, VT-15, VT-17 and VT-18 showed considerable biocontrol activity and growth when compared to commercial positive isolates viz., *Trichoderma harzianum* (TNAU), *Trichoderma viride* (IIHR) and *Trichoderma asperellum* (TRA). These 5 isolates were further used for field studies as well as bio-control formulation

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