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# *In-vitro* screening of *Trichoderma* isolates for their antagonistic potential against *Rhizoctonia solani* causing aerial blight of Soybean

ARUNKUMAR, BHUPESH CHANDRA KABDWAL and ROOPALI SHARMA*

*Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar-263145 (U. S. Nagar Uttarakhand)*
**Corresponding author's email id: roopalibiocontrol@gmail.com*

**ABSTRACT:** *Glycine max* (L.) Merrill, belonging to the family Fabaceae, is one of the oldest oilseed crops grown throughout the world. Being an important source of soy milk and cheese, the crop is also known as the "Cow of the field" and "Gold from Soil" in China. The present investigation was carried out to evaluate *Trichoderma* isolates against *Rhizoctonia solani* for their antagonistic potential through dual culture, volatile, and non-volatile assays. *Trichoderma* species were isolated from rhizospheric soils from different geographical regions of Uttarakhand (Plains, Kumaon, and Garhwal divisions). Fifty-two *Trichoderma* spp. isolates were evaluated for their ability to parasitize and inhibit *R. solani*. Two isolates, *Trichoderma asperellum* PATB-7 and PATB-41, achieved 100% parasitization of *R. solani* after 12 days after contact (DAC) through dual culture technique. In volatile compound assays, *T. asperellum* PATB-15 (62.92%) and PATB-41 (62.08%) showed inhibition of *R. solani* mycelial growth. Non-volatile metabolite studies using 10 selected isolates revealed *T. asperellum* PATB-41 as the most effective, inhibiting *R. solani* mycelial growth by 30.83% at 25% concentration and 61.62% at 50% concentration, followed by T1- *Trichoderma asperellum* PATB-7 (54.58%). These isolates could be further used for glasshouse and field conditions for eco-friendly management of aerial blight of soybean.

**Key words:** Dual culture, *in-vitro,* non-volatile, *Rhizoctonia solani, Trichoderma* spp, volatile

Soybean is one of the oldest oilseed crops grown throughout the world. Being an important source of soy milk and cheese with its protein content of 40% and oil content of 20%, the plant is considered more of an oilseed crop than a pulse and provides 25% of the world's edible oil (Agarwal, 2013). Major biotic constraints in the production of the crop include all the diseases and insects which damage the plant parts and render it unproductive. The abiotic stresses predispose the plant to biotic factors which further weaken the crop and reduce the yields (Strange and Scott, 2005). So far in India, only twenty-three diseases have been reported from different soybean-producing areas. *Rhizoctonia solani* is primarily soil-borne, and during heavy rains, soil splashes onto the leaves serve as the initial inoculum. Aerial blight symptoms often occur on soybean plants that are 45 to 60 days old (Prasad, 2005). The fungus can spread quickly and over a larger area in the soil, where it can persist as mycelia or sclerotia. Intensified use of chemicals has led to accumulation of toxic compounds in humans and the environment. Therefore, management of the disease by biocontrol agents is an eco-friendly and cost-effective approach. Among various biocontrol agents, *Trichoderma* is most widely used against various root, aerial, and soil-borne pathogens. The present study aims to evaluate the antagonistic potential of different *Trichoderma* isolates against *R. solani* using *in-vitro* methods to identify effective biocontrol agents for managing soybean aerial blight disease. These biocontrol agents include fungi like *Trichoderma harzianum* and *Trichoderma asperellum*, as well as bacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* (AICRP, 2023).

## MATERIALS AND METHODS

### *Soil Sample Collection*

Extensive collections of rhizospheric soil samples were undertaken from various farming situations across agro-ecological locations in the districts of Nainital, Udham Singh Nagar, Almora, Bageshwar, Champawat, Pithoragarh, Pauri Garhwal, and Dehradun in Uttarakhand. Generally, healthy plants were selected from the standing crops at each location, and rhizospheric soil was collected. The plants were gently and carefully uprooted, and the

soil tightly adhering to the roots was gathered. Five such samples were randomly collected from each crop field, mixed, and one-fourth of the combined sample was used as the composite rhizospheric soil sample for the region. Detailed locations and geographical coordinates are listed in Table 1.

***Isolation of Trichoderma***

Soil samples were air-dried for four hours. Isolation was performed using the serial dilution technique (Krassilnikov, 1950; Throndsen, 1978). Trichoderma selective medium (TSM) was used for isolating *Trichoderma* isolates (Elad *et al.,* 1981). Trichoderma isolates were purified using a single spore culture technique. The purified *Trichoderma* isolates were sub-cultured on Potato dextrose agar (PDA) plates and incubated at 26°C for 5 days. These pure cultures were then maintained in refrigeration for maintained of their genetic purity for further evaluations.

***Assay of mycoparasitic effects***

**Mycelial parasitization/ Dual culture technique**

To determine the effect of *Trichoderma* isolates on mycelial growth of *R. solani*, a dual culture method was used (Morton and Stroube, 1955). *Trichoderma* isolates and *R. solani* were studied on PDA. Twenty ml of PDA was poured into plates (90 mm) and allowed to solidify. Mycelial discs (5 mm) taken from the actively growing colonies of *R. solani* and *Trichoderma* were placed simultaneously on opposite sides of the PDA plates, 1 cm apart from the periphery. The Petri plates inoculated with each pathogen along with *Trichoderma* isolates were incubated at 26±2°C. Each treatment was replicated thrice. First observations were taken just after contact, and the radial growth of *R. solani* was measured and considered as a check. After contact, observations were taken regularly at 3-day intervals until the antagonist completely parasitized or overgrew the *R. solani*, or the antagonist stopped growing over the *R. solani*. Percent parasitization of the test pathogen was calculated by comparing the growth of the *R. solani* (after parasitization) with its growth just after contact (check). Percent mycelial parasitization was calculated using the following formula (Vincent, 1974):

$$\text{Percent mycelial parasitization} = \frac{C-T}{C} \times 100$$

Where, C = Growth of test pathogen just after contact with antagonist; T = Growth of test pathogen after parasitization by antagonist

***Assay for volatile effects***

For antifungal activity of volatiles, the first set of Petri plates containing PDA was inoculated with a 5 mm disc of *Trichoderma* isolate growing on PDA, while the second set of Petri plates containing PDA was inoculated with a 5 mm plug of *R. solani* in the center of the plate. The plates inoculated with the *R. solani* were placed in an inverted position over the *Trichoderma* isolate culture plates after removing the lids. Both plates were sealed together with parafilm and incubated at 26±2°C for 4-7 days. This ensured that both organisms were growing in the same atmosphere. Petri plates inoculated with a 5 mm disc of *R. solani* and kept separately served as a control (Dennis and Webster, 1971). Three replications were maintained for each *R. solani* treatment. The radial growth of *R. solani* colonies was recorded and compared with the control. Per cent growth inhibition of *R. solani* was calculated using the following formula (Vincent, 1974):

$$\text{Per cent mycelial inhibition (I)} = \frac{C-T}{C} \times 100$$

Where, C = Growth of test pathogen in control; T = Growth of test pathogen in treatment

***Assay of non-volatile effect***

*Trichoderma* isolates were cultured in Potato dextrose broth (PDB) and incubated at 26±2 °C for a week. The supernatant was extracted and filtered using a bacterial-proof filter. Cell-free cultures of *Trichoderma* isolates were obtained by passing through Whatman filter paper (642 equivalent to Grade 2 - HiMedia) followed by a syringe filter with 0.22 µm pore size. The filtrates thus obtained were tested at two concentrations (25% and 50%) against *R. solani* using the poison food technique (Dennis and Webster, 1971). The radial growth (mm) of *R. solani* in treated plates was measured when *R. solani* in control plates reached full growth. Percent mycelial inhibition was calculated using the following formula (Vincent, 1974):

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

Where, C = growth of test pathogen in control; T = growth of test pathogen in treatment

***Statistical Analysis***

The data were analyzed statistically using a Completely Randomized Design (CRD) under laboratory conditions. Data recorded were first transformed to make them homogeneous before analysis. The treatments were compared by means of critical differences using OPSTAT software and WASP - Web Agri Stat Package 2.0.

## RESULTS AND DISCUSSION

In the dual culture technique, 52 *Trichoderma* isolates [*Trichoderma asperellum* (24); *T. harzianum* (12); *T. viride* (05); *T. asperelloides* (03); *T. atroviride* (02); *T. yunnanense* (02); *T. cerebriforme* (01); *T. rugulosum* (01); *T. afroharzianum* (01) & *T. lixii* (01)] were evaluated for their ability to parasitize *R. solani*. The percentage of parasitization was calculated at 3, 6, 9, and 12 days after contact (DAC). Among these isolates, those exhibiting complete and rapid parasitization were selected. Notably, isolates *Trichoderma asperellum* PATB-7 and *Trichoderma asperellum* PATB-41 showed 100% parasitization of *R. solani* at 12 DAC, which were at par with isolates PATB-2, PATB-5, PATB-8, PATB-9, PATB-11, PATB-12, PATB-15, PATB-18, PATB-19, PATB-20, PATB-21, PATB-23, PATB-25, PATB-44, PATB-45, and PATB-50. Other isolates were also found effective in mycelial parasitization of *R. solani*, with inhibition ranging from 81.09% to 100% (Table 2; Fig. 1a; Fig. 1b & Fig. 1c). The above-mentioned isolates were found more effective against *R. solani* than other isolates comparatively. The percent mycelial parasitization of remaining isolates at different days after contact with the pathogen is mentioned in Table 2. Nurhayati *et al.* (2021) conducted a dual culture experiment in which *T. asperellum* showed 64.23% mycelial inhibition of *R. solani*. Similarly, Seema and Devaki (2012) conducted tests using *T. harzianum* and *T. viride*, which showed mycelial inhibition of 67% and 70%, respectively. Manandhar *et al.* (2019) evaluated *Trichoderma* spp. Ten isolates of *Trichoderma* were tested in dual culture with the soil-borne pathogen *R. solani*; six of the tested *Trichoderma* isolates showed more than 80% inhibition of its radial colony growth. Chao and Zhuang (2019) investigated the biocontrol potential of 8 *Trichoderma* species against the phytopathogenic fungus *R. solani*. *Trichoderma* isolates were first evaluated *in-vitro* by dual culture tests for their antagonism, mycoparasitic ability, and antifungal activity against *R. solani*. The highest inhibition percentage reached 82% as expressed by the *Trichoderma simmonsii* isolate 8702. According to Wu *et al.* (2017), *T. asperellum* had a mycoparasitic mechanism in the form of hyphae coiling involving cell wall-degrading enzymes (CWDEs) comprising of chitinase, glucanase, and protease which degraded pathogenic cell walls. *Trichoderma* hyphae coiled pathogenic hyphae and caused hyphal abnormalities and pathogenic cell lysis (Romero-Cortes *et al.,* 2019; Zhang & Zhuang, 2020). The results obtained in this study accordance with the above mention reports under *in-vitro* conditions.

In the volatile assay, compounds produced by *Trichoderma* isolates showing more than 50% inhibition of *R. solani* were selected. Results revealed (Table 3) that volatile compounds produced by *Trichoderma* isolates were effective in inhibiting the radial growth of *R. solani* tested *in-vitro*. Maximum growth inhibition was observed in isolate *Trichoderma asperellum* PATB-15, which was found effective against *R. solani*, showing 62.92% mycelial inhibition. This was on par with isolate *Trichoderma asperellum* PATB-41 (62.08%). Other isolates were also found effective in mycelial inhibition of *R. solani*, with inhibition ranging from 5.00% to 62.92% (Table 3; Fig. 2a & Fig. 2b). Isolates PATB-7, PATB-11, PATB-20, PATB-21, PATB-23, PATB-25, PATB-44, and PATB-50 showed 55.42%, 56.88%, 54.79%, 54.58%, 59.17%, 60.00%, 58.75%, and 60.00% inhibition, respectively. The above-mentioned isolates were found to be more effective against *R. solani* compared to other isolates. Halifu *et al.* (2020) studied the volatile metabolites of *T. virens* ZT05 and showed that it has an inhibitory effect on *R. solani* in the up-and-down culture

**Table 1: List of *Trichoderma* isolates obtained through isolation from rhizospheric soil samples**

| Isolate code | Biocontrol agent | Village name | Block | District | Latitude | Longitude |
|---|---|---|---|---|---|---|
| PATB-1 | *Trichoderma asperellum* | Akhoriya | Chaukhutiya | Almora | 29°50'20.782''N | 79°23'11.41''E |
| PATB-2 | *Trichoderma cerebriforme* | Someshwer | Someshwer | Almora | 29°46'51.824''N | 79°36'5.728''E |
| PATB-3 | *Trichoderma yunnanense* | Chitreshwar | Chaukhutiya | Almora | 29°48'57.355''N | 79°24'53.602''E |
| PATB-4 | *Trichoderma rugulosum* | Nanakmatta | Sitarganj | U S Nagar | 28°56'39.746''N | 79°48'33.404''E |
| PATB-5 | *Trichoderma asperellum* | Patal Bhuvaneshwar | Gangolihat | Pithoragarh | 29°41'17.2"N | 80°05'32.1"E |
| PATB-6 | *Trichoderma asperellum* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |
| PATB-7 | *Trichoderma asperellum* | Chhana | Hawalbag | Alomora | 29°50'52.082''N | 79°21'58.146''E |
| PATB-8 | *Trichoderma asperellum* | Halduchaur | Haldwani | Nainital | 29°9'3.097''N | 79°31'19.097''E |
| PATB-9 | *Trichoderma viride* | Gangolihat | Gangolihat | Pithoragarh | 29°40'11.1"N | 80°02'06.1"E |
| PATB-10 | *Trichoderma asperellum* | Patotiya | Nainidanda | Pauri Garhwal | 29°48'18.3"N | 78°57'18.4"E |
| PATB-11 | *Trichoderma harzianum* | Vijay Rampura | Bajpur | U S Nagar | 29°09'10.9"N | 79°13'31.3"E |
| PATB-12 | *Trichoderma asperellum* | Nainidanda | Nainidanda | Pauri Garhwal | 29°44'22.1"N | 79°00'46.4"E |
| PATB-13 | *Trichoderma asperellum* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |
| PATB-14 | *Trichoderma harzianum* | Dhumakot | Nainidanda | Pauri Garhwal | 29°45'08.6"N | 79°00'39.5"E |
| PATB-15 | *Trichoderma asperellum* | Gaula Par | Haldwani | Nainital | 29°10'35.3"N | 79°35'25.1"E |
| PATB-16 | *Trichoderma harzianum* | Jogida | Nainidanda | Pauri Garhwal | 29°43'56.3"N | 79°00'26.3"E |
| PATB-17 | *Trichoderma harzianum* | Silagaon | Nainidanda | Pauri Garhwal | 29°43'56.3"N | 79°00'26.3"E |
| PATB-18 | *Trichoderma viride* | Tanakpur | Champawat | Champawat | 29°04'20.6"N | 80°06'18.8"E |
| PATB-19 | *Trichoderma asperellum* | Bajpur | Bajpur | U S Nagar | 29°9'44.405''N | 79°9'33.419''E |
| PATB-20 | *Trichoderma yunnanense* | Tanakpur | Champawat | Champawat | 29°04'20.6"N | 80°06'18.8"E |
| PATB-21 | *Trichoderma viride* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |
| PATB-22 | *Trichoderma harzianum* | Dhaniya kot | Landsdowne | Pauri Garhwal | 29°79'61.14''N | 78°73'59.302''E |
| PATB-23 | *Trichoderma asperellum* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |
| PATB-24 | *Trichoderma asperellum* | Halduchaur | Haldwani | Nainital | 29°9'3.097''N | 79°31'19.097''E |
| PATB-25 | *Trichoderma asperelloides* | Halduchaur | Haldwani | Nainital | 29°9'3.097''N | 79°31'19.097''E |
| PATB-26 | *Trichoderma asperellum* | Satpuli sain | Dwarikal | Pauri Garhwal | 29°55'20.622''N | 78°41'49.945''E |
| PATB-27 | *Trichoderma harzianum* | Dewala Malla | Haldwani | Nainital | 29°12'43.6"N | 79°34'02.2"E |
| PATB-28 | *Trichoderma asperelloides* | Gaula par | Haldwani | Nainital | 29°10'35.3"N | 79°35'25.1"E |
| PATB-29 | *Trichoderma viride* | Gwalakot | Hawalbag | Alomora | 29°48'58''N | 79°18'40''E |
| PATB-30 | *Trichoderma afroharzianum* | Chausali | Hawalbag | Alomora | 29°34'44.554''N | 79°37'49.384''E |
| PATB-31 | *Trichoderma asperellum* | Munsyari | Munsyari | Pithoragarh | 30°04'17.1"N | 80°13'57.4"E |
| PATB-32 | *Trichoderma harzianum* | Sarmoli | Munsyari | Pithoragarh | 30°04'45.8"N | 80°14'09.0"E |
| PATB-33 | *Trichoderma harzianum* | Rampur | *Kaladhungi* | Nainital | 29°14'49.273''N | 79°22'4.752''E |
| PATB-34 | *Trichoderma asperellum* | Bahuli | Bageshwar | Bageshwar | 29°51'12.7"N | 79°44'50.3"E |
| PATB-35 | *Trichoderma harzianum* | Ganeshpur | Bajpur | U S Nagar | 29°3'14.904''N | 79°27'47.088''E |
| PATB-36 | *Trichoderma harzianum* | Bhatoli | Bageshwar | Bageshwar | 29°50'29.0"N | 79°45'06.2"E |
| PATB-37 | *Trichoderma viride* | Chhatpur | Rudrapur | U S Nagar | 29°1'55.33''N | 79°23'30.026''E |
| PATB-38 | *Trichoderma atroviride* | Chandan nagar | Gadarpur | U S Nagar | 29°3'25.614''N | 79°23'33.757''E |
| PATB-39 | *Trichoderma asperellum* | Garur | Garur | Bageshwar | 29°53'51.4"N | 79°36'38.2"E |
| PATB-40 | *Trichoderma harzianum* | Chakarpur | Bajpur | U S Nagar | 29°9'8.29''N | 79°9'3.604''E |
| PATB-41 | *Trichoderma asperellum* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |
| PATB-42 | *Trichoderma asperellum* | Maltha | Ekeshwar | Pauri Garhwal | 29°56'18.388''N | 78°44'49.837''E |
| PATB-43 | *Trichoderma asperellum* | Jawahar Nagar | Rudrapur | U S Nagar | 29°0'17.072''N | 79°31'28.721''E |
| PATB-44 | *Trichoderma asperellum* | Champawat | Champawat | Champawat | 29°20'30.8"N | 80°05'55.0"E |
| PATB-45 | *Trichoderma asperellum* | Bailpadva | Ramnagar | Nainital | 29°28'22.87''N | 79°28'22.87''E |
| PATB-46 | *Trichoderma asperelloides* | Lama chauda | Kutauli | Nainital | 29°13'52.115''N | 79°26'13.333''E |
| PATB-47 | *Trichoderma asperellum* | Chitreshwar | Chaukhutiya | Almora | 29°48'57.355''N | 79°24'53.602''E |
| PATB-48 | *Trichoderma atroviride* | Gadaria bang | Kichha | U S Nagar | 28°58'6.503''N | 79°31'29.629''E |
| PATB-49 | *Trichoderma asperellum* | Bajpur | Bajpur | U S Nagar | 29°9'44.405''N | 79°9'33.419''E |
| PATB-50 | *Trichoderma asperellum* | Sheetpuri | Bajpur | U S Nagar | 29°09'07.1"N | 79°12'37.4"E |
| PATB-51 | *Trichoderma harzianum* | Vikas Nagar | Dehradun | Dehradun | 30°28'18.4"N | 77°45'37.5"E |
| PATB-52 | *Trichoderma lixii* | Pantnagar | Rudrapur | U S Nagar | 29°1'5.444''N | 79°28'58.648''E |

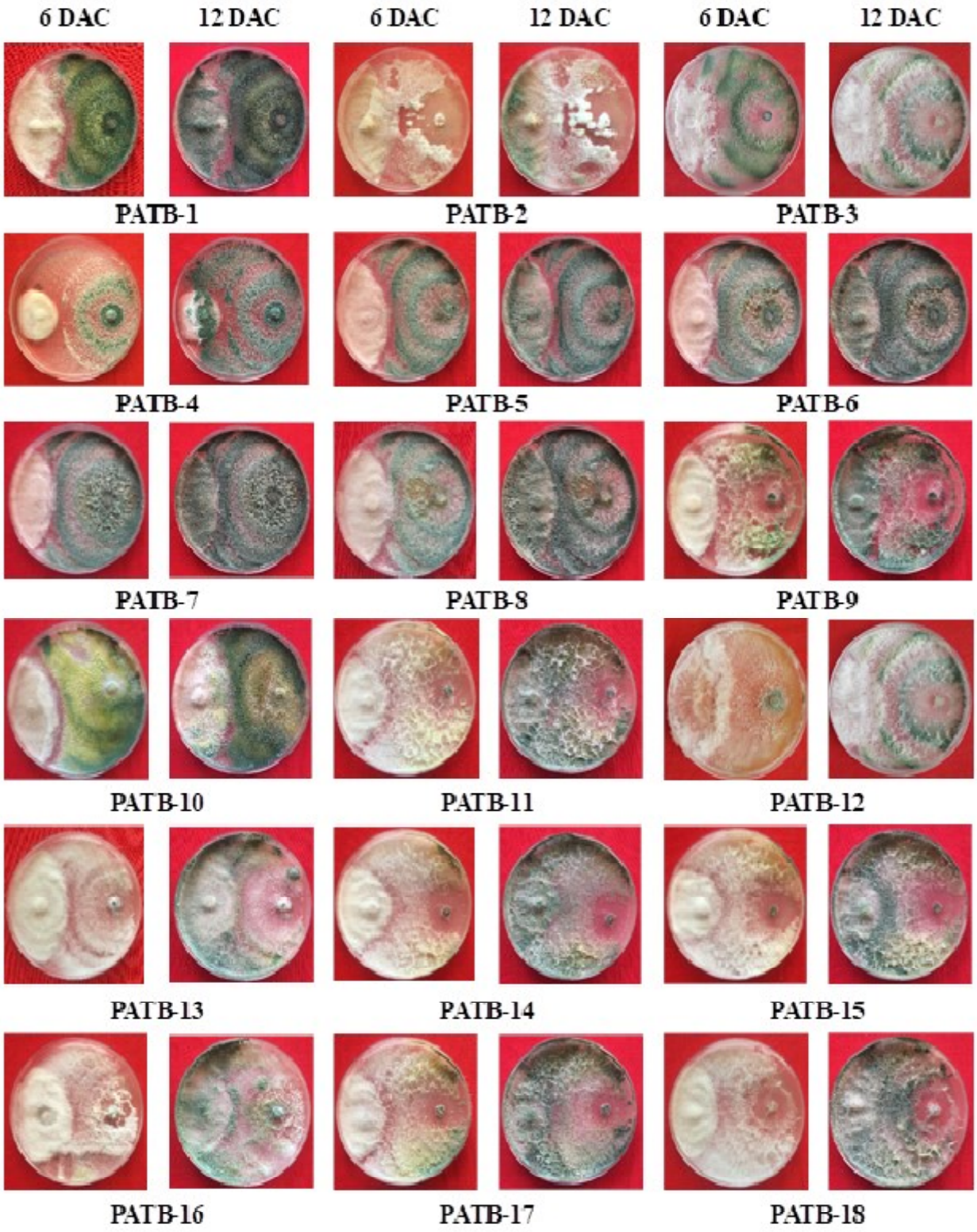



**Fig. 1a: Effect of *Trichoderma* isolates on mycelial parasitization of *R. solani* under *in-vitro* by dual culture technique (Isolates PATB-1 to PATB-18)**

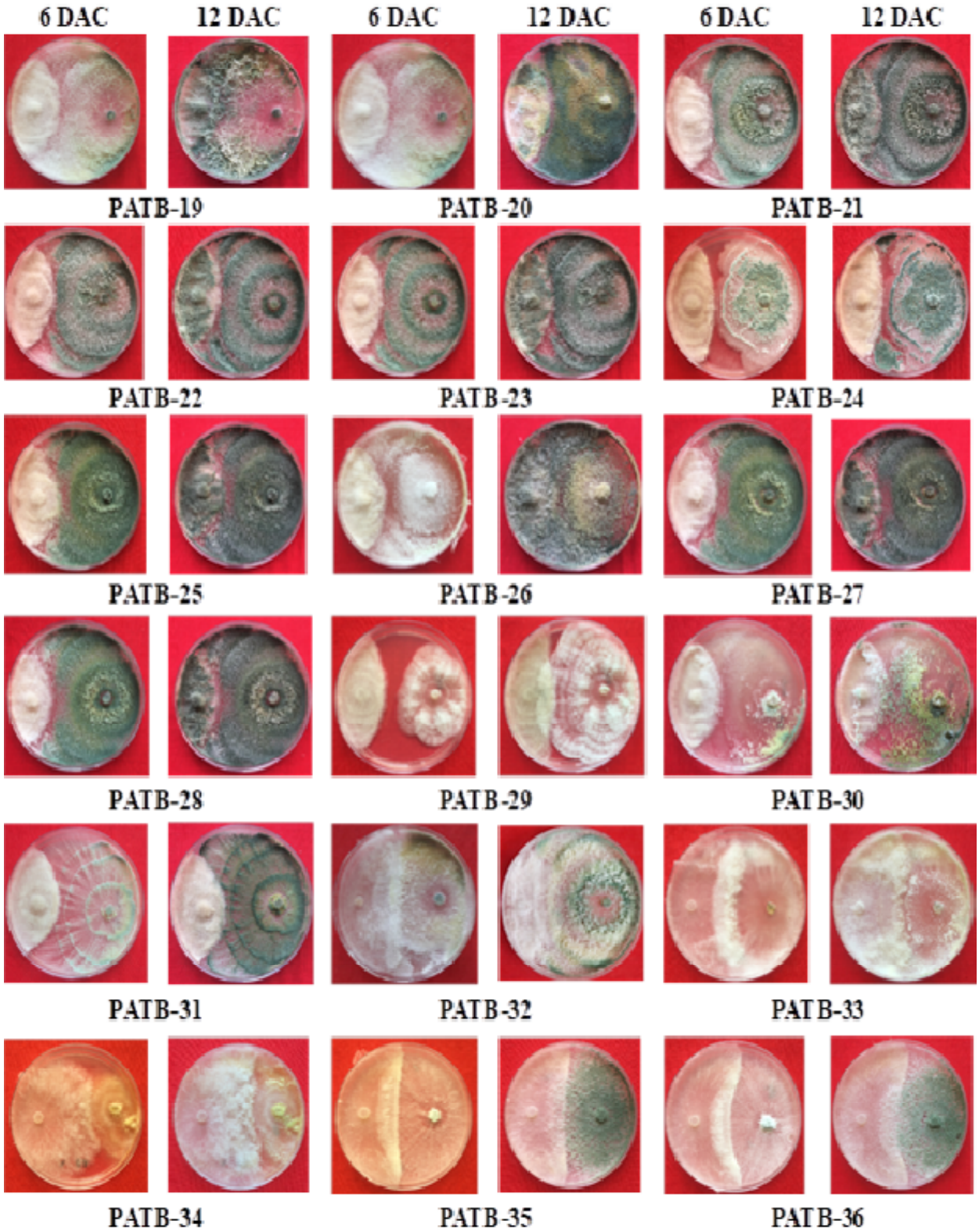



**Fig. 1b: Effect of *Trichoderma* isolates on mycelial parasitization of *R. solani* under *in-vitro* by dual culture technique (Isolates PATB-19 to PATB-36)**

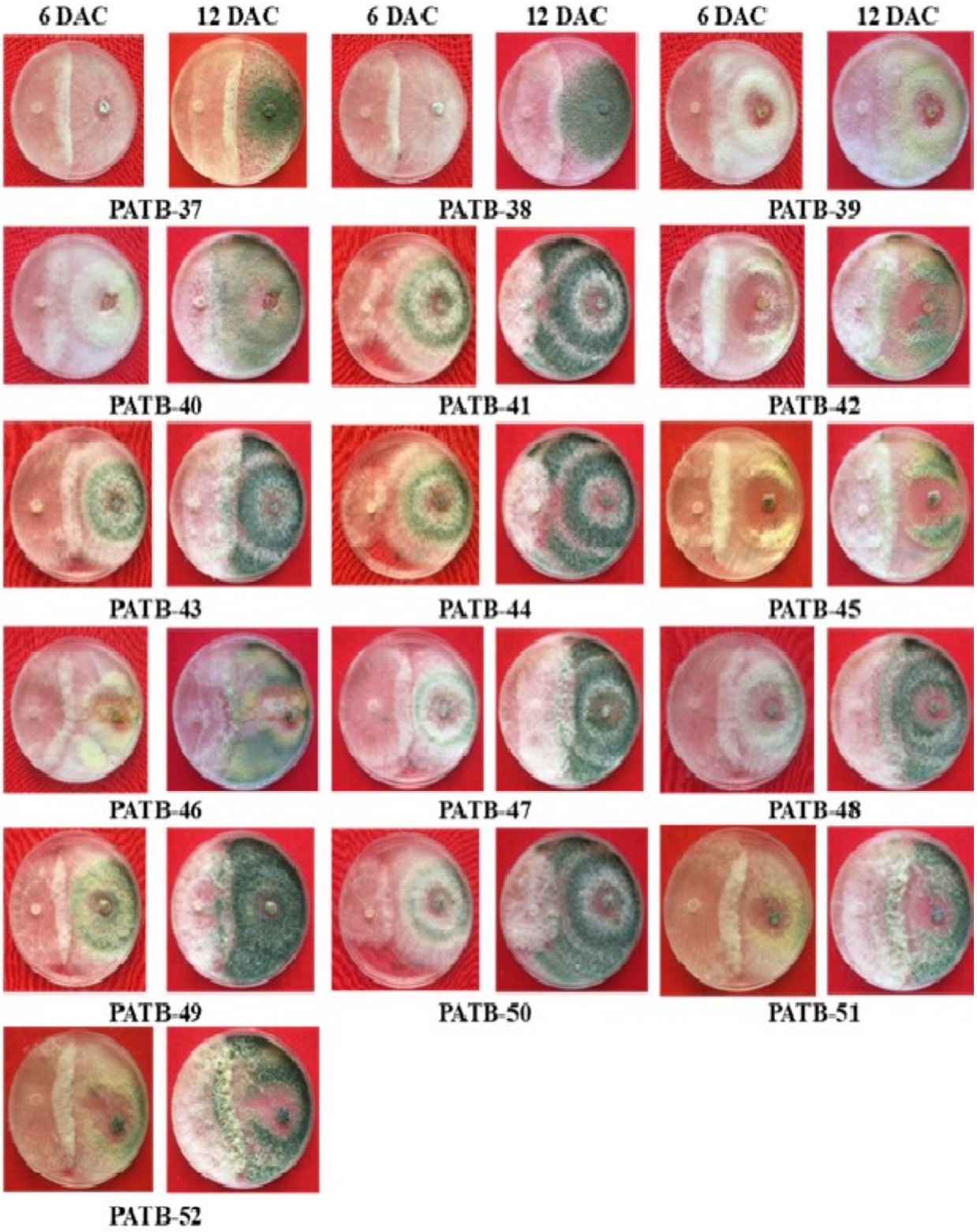



**Fig. 1c: Effect of *Trichoderma* isolates on mycelial parasitization of *R. solani* under *in-vitro* by dual culture technique (Isolates PATB-37 to PATB-52)**

**Table 2. *In-vitro* screening of *Trichoderma* isolates against *R. solani* through dual culture technique**

| Sl. No | *Trichoderma* isolates | Isolate code* | Per cent mycelial parasitization (%)** | | | |
|---|---|---|---|---|---|---|
| | | | Days after contact (DAC) | | | |
| | | | **3** | **6** | **9** | **12** |
| 1 | *Trichoderma asperellum* | PATB-1 | 34.13 | 83.95 | 90.01 | 93.01 |
| 2 | *Trichoderma cerebriforme* | PATB-2 | 46.52 | 81.11 | 91.02 | 94.05 |
| 3 | *Trichoderma yunnanense* | PATB-3 | 40.24 | 77.08 | 85.35 | 89.79 |
| 4 | *Trichoderma rugulosum* | PATB-4 | 43.93 | 83.94 | 87.30 | 89.70 |
| 5 | *Trichoderma asperellum* | PATB-5 | 38.82 | 85.41 | 90.28 | 94.20 |
| 6 | *Trichoderma asperellum* | PATB-6 | 44.58 | 82.57 | 90.21 | 92.43 |
| 7 | *Trichoderma asperellum* | PATB-7 | 36.61 | 85.57 | 96.32 | 100.00 |
| 8 | *Trichoderma asperellum* | PATB-8 | 48.38 | 82.37 | 91.73 | 94.81 |
| 9 | *Trichoderma viride* | PATB-9 | 48.35 | 84.62 | 92.29 | 93.41 |
| 10 | *Trichoderma asperellum* | PATB-10 | 44.94 | 78.66 | 89.89 | 93.26 |
| 11 | *Trichoderma harzianum* | PATB-11 | 62.99 | 85.83 | 95.56 | 97.78 |
| 12 | *Trichoderma asperellum* | PATB-12 | 31.76 | 77.31 | 88.69 | 93.72 |
| 13 | *Trichoderma asperellum* | PATB-13 | 54.08 | 80.15 | 89.14 | 92.79 |
| 14 | *Trichoderma harzianum* | PATB-14 | 36.71 | 72.47 | 83.65 | 90.78 |
| 15 | *Trichoderma asperellum* | PATB-15 | 58.77 | 74.49 | 91.06 | 98.04 |
| 16 | *Trichoderma harzianum* | PATB-16 | 34.13 | 75.92 | 87.54 | 90.66 |
| 17 | *Trichoderma harzianum* | PATB-17 | 43.40 | 81.46 | 89.17 | 91.32 |
| 18 | *Trichoderma viride* | PATB-18 | 61.66 | 74.38 | 89.53 | 96.55 |
| 19 | *Trichoderma asperellum* | PATB-19 | 70.85 | 75.05 | 91.73 | 96.87 |
| 20 | *Trichoderma yunnanense* | PATB-20 | 67.03 | 79.18 | 87.92 | 97.78 |
| 21 | *Trichoderma viride* | PATB-21 | 51.65 | 83.89 | 93.02 | 97.74 |
| 22 | *Trichoderma harzianum* | PATB-22 | 46.67 | 84.44 | 91.11 | 92.22 |
| 23 | *Trichoderma asperellum* | PATB-23 | 48.31 | 87.62 | 95.48 | 97.78 |
| 24 | *Trichoderma asperellum* | PATB-24 | 22.28 | 63.87 | 82.50 | 89.80 |
| 25 | *Trichoderma asperelloides* | PATB-25 | 44.53 | 88.15 | 98.04 | 98.01 |
| 26 | *Trichoderma asperellum* | PATB-26 | 53.25 | 85.16 | 94.21 | 92.06 |
| 27 | *Trichoderma harzianum* | PATB-27 | 46.09 | 85.73 | 92.33 | 92.29 |
| 28 | *Trichoderma asperelloides* | PATB-28 | 50.48 | 84.68 | 89.92 | 88.58 |
| 29 | *Trichoderma viride* | PATB-29 | 28.68 | 40.14 | 75.76 | 84.01 |
| 30 | *Trichoderma afroharzianum* | PATB-30 | 37.97 | 59.47 | 79.95 | 83.44 |
| 31 | *Trichoderma asperellum* | PATB-31 | 37.78 | 55.56 | 75.56 | 82.22 |
| 32 | *Trichoderma harzianum* | PATB-32 | 48.65 | 58.10 | 77.47 | 84.90 |
| 33 | *Trichoderma harzianum* | PATB-33 | 26.84 | 35.27 | 52.25 | 82.60 |
| 34 | *Trichoderma asperellum* | PATB-34 | 9.14 | 29.72 | 44.01 | 81.09 |
| 35 | *Trichoderma harzianum* | PATB-35 | 33.95 | 37.51 | 54.48 | 84.83 |
| 36 | *Trichoderma harzianum* | PATB-36 | 31.78 | 38.19 | 50.00 | 85.49 |
| 37 | *Trichoderma viride* | PATB-37 | 24.47 | 36.98 | 52.86 | 87.51 |
| 38 | *Trichoderma atroviride* | PATB-38 | 21.60 | 32.25 | 46.28 | 90.89 |
| 39 | *Trichoderma asperellum* | PATB-39 | 23.60 | 29.06 | 43.44 | 90.13 |
| 40 | *Trichoderma harzianum* | PATB-40 | 28.00 | 33.06 | 41.01 | 86.10 |
| 41 | *Trichoderma asperellum* | PATB-41 | 46.67 | 73.03 | 85.67 | 100.00 |
| 42 | *Trichoderma asperellum* | PATB-42 | 29.16 | 62.91 | 68.51 | 89.85 |
| 43 | *Trichoderma asperellum* | PATB-43 | 33.06 | 38.38 | 46.00 | 92.24 |
| 44 | *Trichoderma asperellum* | PATB-44 | 10.86 | 30.39 | 74.46 | 98.41 |
| 45 | *Trichoderma asperellum* | PATB-45 | 20.58 | 29.92 | 43.92 | 93.44 |
| 46 | *Trichoderma asperelloides* | PATB-46 | 15.34 | 21.32 | 33.99 | 78.41 |
| 47 | *Trichoderma asperellum* | PATB-47 | 26.01 | 42.19 | 48.68 | 85.50 |
| 48 | *Trichoderma atroviride* | PATB-48 | 24.24 | 37.76 | 45.85 | 92.84 |
| 49 | *Trichoderma asperellum* | PATB-49 | 19.81 | 27.35 | 43.36 | 84.84 |
| 50 | *Trichoderma asperellum* | PATB-50 | 17.76 | 52.47 | 63.06 | 99.12 |
| 51 | *Trichoderma harzianum* | PATB-51 | 29.31 | 35.49 | 49.74 | 92.43 |
| 52 | *Trichoderma lixii* | PATB-52 | 26.38 | 35.87 | 45.32 | 88.88 |
| | C.D (0.05) | | 7.73 | 5.42 | 5.38 | 6.674 |
| | S. Em (±) | | 2.75 | 1.93 | 1.91 | 2.376 |

**Note:** * PATB- **Pa**nt **T**richoderma **B**ioagent: ** Mean of three replications

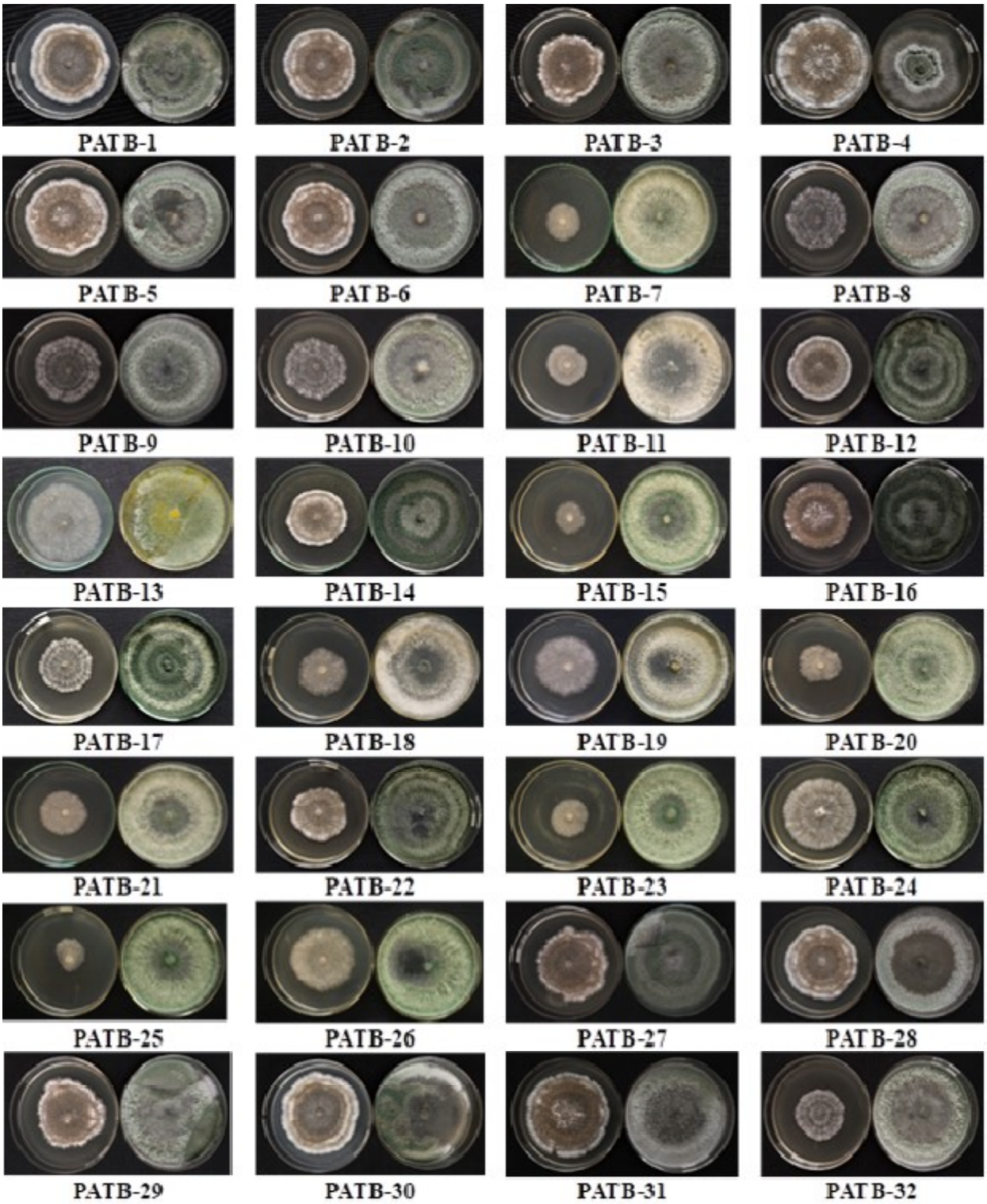



**Fig. 2a: Effect of *Trichoderma* isolates on mycelial inhibition of *R. solani* under *in-vitro* by volatile assay (Isolates PATB-1 to PATB-32)**

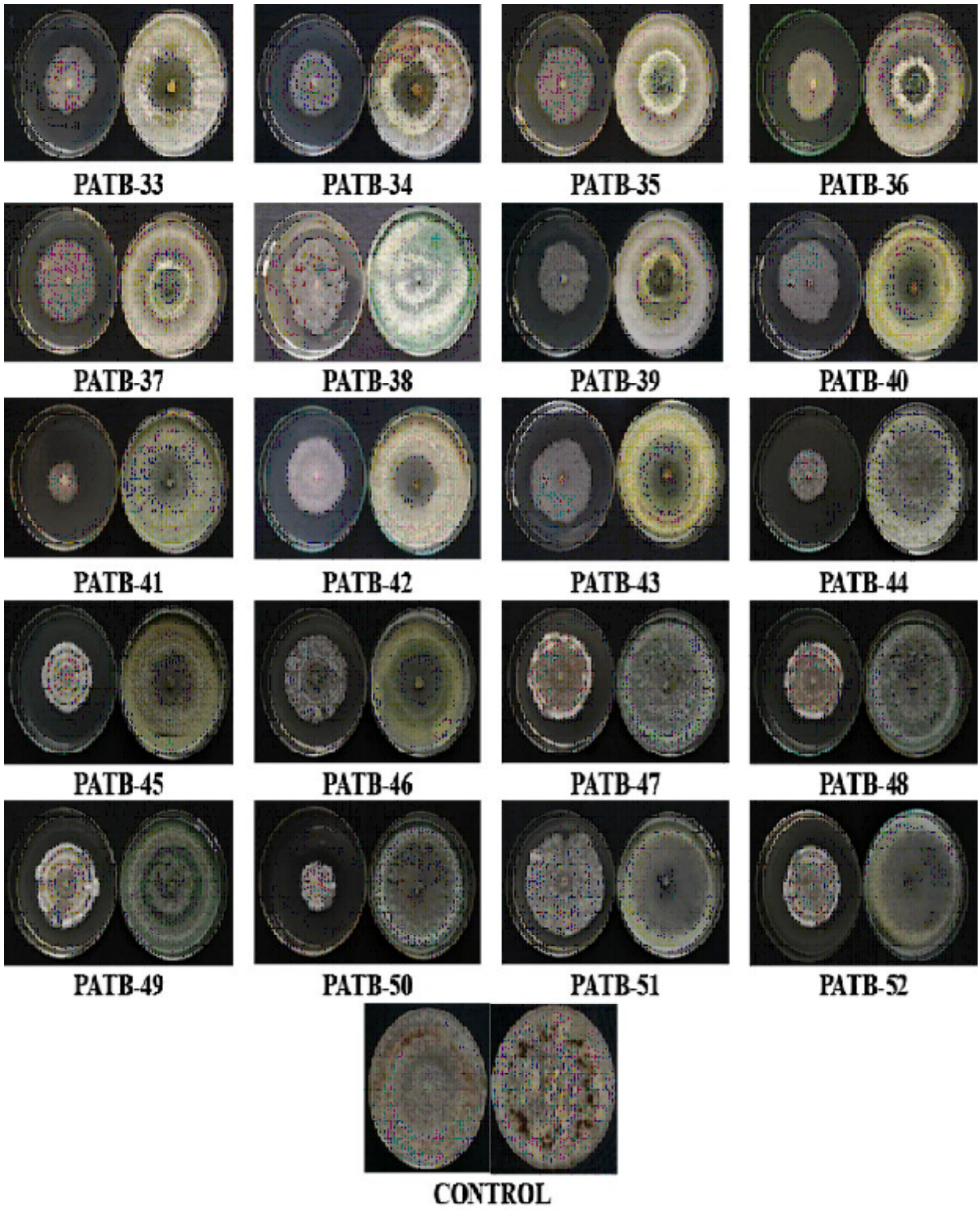



**Fig. 2b: Effect of *Trichoderma* isolates on mycelial inhibition of *R. solani* under *in-vitro* by volatile assay (Isolates PATB-33 to PATB-52)**

**Table 3: *In-vitro* screening of *Trichoderma* isolates against *R. solani* through volatile assay**

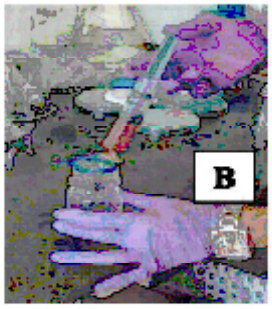
| Sl. No. | *Trichoderma* isolates | Isolate code* | Radial growth of the pathogen (mm)** | Per cent inhibition of mycelia (%)*** |
|---|---|---|---|---|
| 1 | *Trichoderma asperellum* | PATB-1 | 56.33 | 29.58 (32.84) |
| 2 | *Trichoderma cerebriforme* | PATB-2 | 61.00 | 23.75 (29.14) |
| 3 | *Trichoderma yunnanense* | PATB-3 | 62.67 | 21.67 (27.70) |
| 4 | *Trichoderma rugulosum* | PATB-4 | 76.00 | 5.00 (7.59) |
| 5 | *Trichoderma asperellum* | PATB-5 | 69.33 | 13.33 (21.22) |
| 6 | *Trichoderma asperellum* | PATB-6 | 61.67 | 22.92 (28.54) |
| 7 | *Trichoderma asperellum* | PATB-7 | 35.67 | 55.42 (48.09) |
| 8 | *Trichoderma asperellum* | PATB-8 | 53.00 | 33.75 (35.50) |
| 9 | *Trichoderma viride* | PATB-9 | 50.67 | 36.67 (37.24) |
| 10 | *Trichoderma asperellum* | PATB-10 | 52.00 | 35.00 (36.23) |
| 11 | *Trichoderma harzianum* | PATB-11 | 34.50 | 56.88 (48.93) |
| 12 | *Trichoderma asperellum* | PATB-12 | 56.33 | 29.58 (32.90) |
| 13 | *Trichoderma asperellum* | PATB-13 | 52.33 | 34.58 (35.93) |
| 14 | *Trichoderma harzianum* | PATB-14 | 51.67 | 35.42 (36.49) |
| 15 | *Trichoderma asperellum* | PATB-15 | 29.67 | 62.92 (52.47) |
| 16 | *Trichoderma harzianum* | PATB-16 | 47.67 | 40.42 (39.44) |
| 17 | *Trichoderma harzianum* | PATB-17 | 47.67 | 40.42 (39.45) |
| 18 | *Trichoderma viride* | PATB-18 | 44.00 | 45.00 (42.11) |
| 19 | *Trichoderma asperellum* | PATB-19 | 52.67 | 34.17 (35.74) |
| 20 | *Trichoderma yunnanense* | PATB-20 | 36.17 | 54.79 (47.73) |
| 21 | *Trichoderma viride* | PATB-21 | 36.33 | 54.58 (47.61) |
| 22 | *Trichoderma harzianum* | PATB-22 | 51.00 | 36.25 (36.97) |
| 23 | *Trichoderma asperellum* | PATB-23 | 32.67 | 59.17 (50.26) |
| 24 | *Trichoderma asperellum* | PATB-24 | 70.00 | 12.50 (20.63) |
| 25 | *Trichoderma asperelloides* | PATB-25 | 32.00 | 60.00 (50.74) |
| 26 | *Trichoderma asperellum* | PATB-26 | 56.33 | 29.58 (32.90) |
| 27 | *Trichoderma harzianum* | PATB-27 | 56.67 | 29.17 (32.64) |
| 28 | *Trichoderma asperelloides* | PATB-28 | 55.33 | 30.83 (33.69) |
| 29 | *Trichoderma viride* | PATB-29 | 56.00 | 30.00 (33.19) |
| 30 | *Trichoderma afroharzianum* | PATB-30 | 62.00 | 22.50 (28.26) |
| 31 | *Trichoderma asperellum* | PATB-31 | 69.67 | 12.92 (21.02) |
| 32 | *Trichoderma harzianum* | PATB-32 | 48.00 | 40.00 (39.21) |
| 33 | *Trichoderma harzianum* | PATB-33 | 44.00 | 45.00 (42.11) |
| 34 | *Trichoderma asperellum* | PATB-34 | 49.67 | 37.92 (37.98) |
| 35 | *Trichoderma harzianum* | PATB-35 | 52.67 | 34.17 (35.75) |
| 36 | *Trichoderma harzianum* | PATB-36 | 50.00 | 37.50 (37.74) |
| 37 | *Trichoderma viride* | PATB-37 | 51.67 | 35.42 (36.50) |
| 38 | *Trichoderma atroviride* | PATB-38 | 60.00 | 25.00 (29.96) |
| 39 | *Trichoderma asperellum* | PATB-39 | 48.33 | 39.58 (38.96) |
| 40 | *Trichoderma harzianum* | PATB-40 | 55.00 | 31.25 (33.97) |
| 41 | *Trichoderma asperellum* | PATB-41 | 30.33 | 62.08 (51.97) |
| 42 | *Trichoderma asperellum* | PATB-42 | 49.50 | 38.13 (38.11) |
| 43 | *Trichoderma asperellum* | PATB-43 | 45.67 | 42.92 (40.90) |
| 44 | *Trichoderma asperellum* | PATB-44 | 33.00 | 58.75 (50.02) |
| 45 | *Trichoderma asperellum* | PATB-45 | 47.33 | 40.83 (39.70) |
| 46 | *Trichoderma asperelloides* | PATB-46 | 53.67 | 32.92 (34.97) |
| 47 | *Trichoderma asperellum* | PATB-47 | 54.00 | 32.50 (34.74) |
| 48 | *Trichoderma atroviride* | PATB-48 | 51.33 | 35.83 (36.75) |
| 49 | *Trichoderma asperellum* | PATB-49 | 51.67 | 35.42 (36.50) |
| 50 | *Trichoderma asperellum* | PATB-50 | 32.00 | 60.00 (50.75) |
| 51 | *Trichoderma harzianum* | PATB-51 | 64.00 | 20.00 (26.50) |
| 52 | *Trichoderma lixii* | PATB-52 | 52.33 | 34.58 (35.99) |
| 53 | Control | - | 90 | 0.00 (0) |
| | | | C.D. (0.05) | 4.193 |
| | | | S Em± | 1.493 |

**Note:** * PATB- Pant Trichoderma Bioagent; ** Mean of three replications; *** Figures in the parenthesis are arc sine transformed values

**Table 4: *In-vitro* screening of potential *Trichoderma* isolates against *R. solani* through non-volatile metabolites assay**

| Treatments | Isolate code* | Per cent inhibition over control (%)** | | |
|---|---|---|---|---|
| | | 25 % | 50 % | Mean Factor (A) |
| T1- *Trichoderma asperellum* | PATB-7 | 22.50 | 54.58 | 38.54 |
| T2- *Trichoderma harzianum* | PATB-11 | 11.20 | 45.83 | 28.54 |
| T3- *Trichoderma asperellum* | PATB-15 | 8.75 | 32.91 | 20.83 |
| T4- *Trichoderma yunnanense* | PATB-20 | 10.41 | 35.83 | 23.12 |
| T5- *Trichoderma viride* | PATB-21 | 11.25 | 22.08 | 16.66 |
| T6- *Trichoderma asperellum* | PATB-23 | 30.41 | 48.33 | 39.37 |
| T7- *Trichoderma asperelloides* | PATB-25 | 27.91 | 54.16 | 41.04 |
| T8- *Trichoderma asperellum* | PATB-41 | 30.83 | 61.25 | 46.04 |
| T9- *Trichoderma asperellum* | PATB-44 | 10.83 | 50.00 | 30.41 |
| T10- *Trichoderma asperellum* | PATB-50 | 10.00 | 44.58 | 27.29 |
| T-11 Control | - | 0 | 0 | 0 |
| | Mean Factor (B) | 15.833 | 40.871 | Treatments (A×B) |
| | C.D (0.05) | 4.634 | 1.976 | 6.553 |
| | S. Em ± | 1.24 | 1.54 | 2.31 |

**Note:** * PATB- Pant Trichoderma Bioagent; ** Mean of three replications



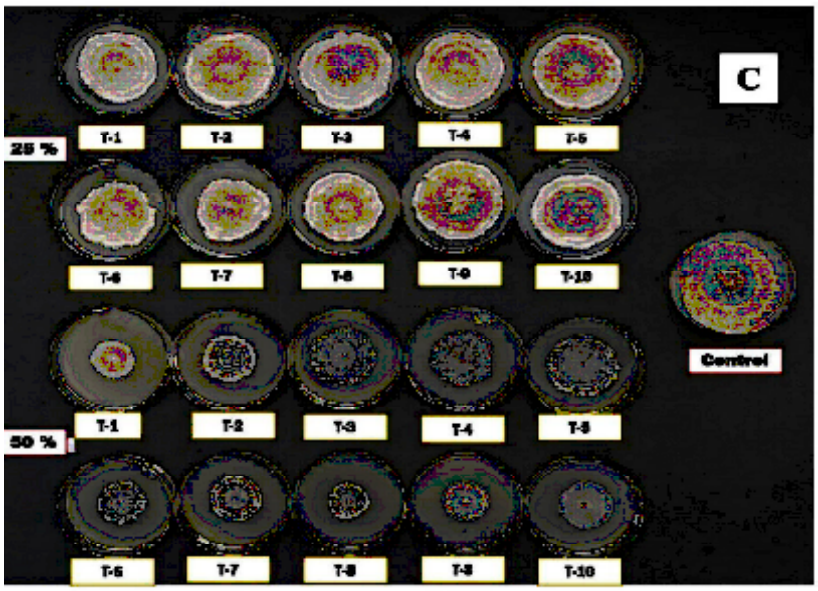




**Fig. 3: Effect of *Trichoderma* isolates on mycelial inhibition of *R. solani* under *in-vitro* by non-volatile metabolites assay, A-Culture filtrate of *Trichoderma* isolates through Whatman filter paper; B-Cell free culture filtered through 0.22 µm bacterial proof syringe; C- Per cent inhibition of mycelial growth of *R. solani***

method. The inhibitory effect on *R. solani* reached up to 80.10%, with slow and sparse growth of *R. solani* observed after five days of up-and-down culture. The results obtained in this study are in accordance with the above-mentioned reports under *in-vitro* conditions.

The effect of non-volatile compounds produced by the ten potential isolates obtained through dual culture and volatile study were further evaluated for their biocontrol potential. These were evaluated at 25 and 50 per cent for its antibiosis effect on radial growth of *R. solani*. Among them at 25% concentration, T8- *Trichoderma asperellum* PATB-41 showed maximum growth inhibition of 30.83 % which is at par with T6-*Trichoderma asperellum* PATB-23 (30.41%) and minimum by T3-*Trichoderma asperellum* PATB-15 (8.75%). At 50 % concentration maximum per cent inhibition of mycelial growth was observed in T8- *Trichoderma asperellum* PATB-41 (61.62%) which is followed by T1- *Trichoderma asperellum* PATB-7 (54.58%) and least by T3- *Trichoderma asperellum* PATB-15 (32.91%) (Table 4; Fig. 3 & Fig. 4). The present findings are in accordance with the observations reported by Weindling (1938) reported that culture filtrate of *T. lignorum* was toxic to *R. solani* and other fungi at higher dilution. Inhibition of mycelial growth of pathogens may be due to the presence of effective secondary metabolites produced by the test antagonist in liquid medium. (Hajieghrari, 2008) in

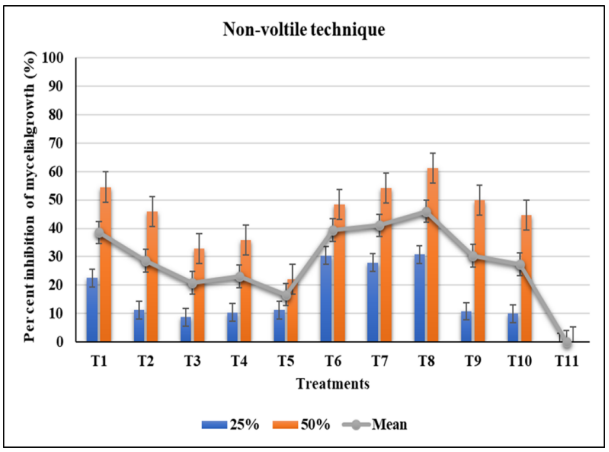



**Fig. 4: Graphical representations of non-volatile assay against *R. solani* by potential *Trichoderma* isolates under *in-vitro* conditions**

*R. solani* by culture filtrates of different *Trichoderma* spp. Abbas *et al.* (2017) stated that the *Trichoderma* spp. are the potential biocontrol agents which inhibit *R. solani* by direct confrontation through mycoparasitic and antibiosis through production of volatile and non-volatile inhibitory compounds. Mayo-Prieto *et al.* (2020) reported that *Trichoderma* isolate was able to inhibit *R. solani* growth. Among antifungal treatment of *T. asperellum* isolate UGM-LHAF, 50% culture filtrate of *T. asperellum* isolate UGM-LHAF showed the strongest radial growth inhibition. The culture filtrate of *T. asperellum* was also known to contain secondary metabolites in the form of trichodermaerin, asperilin, methylcordysinin A, steroid, ergosta, beta-sitosterol, pyrone, viridin, viridiol, harzianolide, ferulic acid, viridiofungin A, cyclonerodiol, massoilactone, and gliovirin which were antifungal (Vinale *et al.,* 2009; Stracquadanio *et al.,* 2020).

## CONCLUSION

The pathogen *Rhizoctonia solani* significantly impacts economically important crops, causing severe losses. This study identified *Trichoderma* isolates with strong antagonistic potential against *R. solani*, particularly *T. asperellum* PATB-41 and *T. asperellum* PATB-7. These isolates exhibit mycoparasitism through hyphal coiling and cell wall-degrading enzymes (Wu *et al.,* 2017). They may also produce volatile and non-volatile metabolites with antifungal properties, including trichodermaerin, asperilin, and gliovirin (Vinale *et al.,* 2009; Stracquadanio *et al.,* 2020). These findings suggest that *T. asperellum* isolates could be effective biocontrol agents against *R. solani* causing aerial blight of soybean.

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