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Research Article

# Efficacy of indigenous *Trichoderma* isolates against *Pythium deliense* Meurs, associated with soft rot of ginger

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**Abstract:** Yellowing and rotting of ginger rhizomes are a severe concern in many ginger-growing tracts of Kerala, where *Pythium deliense* Meurs has recently emerged as a pathogen from the rhizosphere of infected rotten rhizomes, which is proven to be pathogenic by Koch`s postulates. The present study evaluates the antagonistic potential of ten isolates of *Trichoderma* spp., isolated from the roots of healthy ginger, against *P. deliense*, causing soft rot of ginger by dual plate method. The efficacy of volatile and nonvolatile metabolite produced by the antagonistic *Trichoderma* spp. under *in vitro* conditions was evaluated against the pathogen. Among the ten isolates, eight isolates such as, ZGC T16, ZGC T17, ZGC T20, ZGC T23, ZGC T28, ZGC T30, ZGC T35, and ZGC T36 showed inhibition on mycelial growth, above 70% in a dual plate assay. The highest inhibition percentage was showed by ZGC T20 (99.19%) followed by ZGC T17 (87.00%), identified as *Trichoderma ressei* and *Trichoderma virens* respectively, under dual culture method and moderate inhibition due to volatile metabolites (3.5-39.55%). Nonvolatile metabolites produced by ZGC T20 (65.55%), ZGC T17 (53.95%), and ZGC T36 (53.82%) showed comparatively less efficiency. The potential ones (ZGC T20 & ZGC T17) were further evaluated under the pot culture study and in vivo greenhouse conditions. The study revealed that *T. ressei* and *T. virens* have high efficiency in preventing the soft rot of ginger caused by *P. deliense*.

**Keywords:** Biocontrol, Ginger, *Pythium deliense*, soft rot, *Trichoderma*

## Introduction

Ginger is one of the most widely consumed spices in the world. Ginger`s name comes from the Sanskrit word srngaveram, meaning "horn root" based on its appearance (Ansari *et al*. 2021). Ginger is an aromatic and spicy perennial herbaceous plant with branched rhizomes. India is the prominent producer of ginger in the world. Ginger has many pharmacological properties such as an antioxidant, anti-inflammatory, antinausea compound and anticancer agent (Ahmad *et al*. 2001; Minghetti *et al*. 2007; Aggarwal *et al*. 2008; Wu *et al*. 2008). Considering the vast importance and demands, the total area under its cultivation has increased from time to time with enhanced production. Still, unfortunately, the production has declined over time due to destructive rhizome rot or soft rot disease (Rai *et al*. 2018). The important pathogens that cause rhizome rot in ginger are

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*Pythium spp*., like *P. aphanidermatum, P. myriotylum, P. deliense, P. splendens, P. gracile, etc*, and reported to destroy 80-90% of the crop annually. Among the 11 soft rot-causing species of *Pythium* recovered by Dohroo (2005), *P. myriotylum*, and *P. aphanidermatum* were the more prevalent pathogens. Applying chemical fungicides continues to be an important and effective method for plant disease control, including soft rot of ginger. The extensive use of fungicides has become a serious environmental and human health risk since ginger rhizomes are consumed directly. From among numerous fungicides, metalaxyl is most exploited alone and in combination with other fungicides such as Apron 35 WS and Dithane M 45 for excellent control of soft rot pathogens (Chase *et al*. 1986; Ramachandran *et al*. 1989; Dake 1995; Hwang *et al*. 2001; Luong *et al*. 2010). To overcome the complications related to the use of fungicides, a biological control method for the management of *Pythium* would be environment-friendly and cost-effective.

As a potential biocontrol agent to combat the soft rot pathogens, *Trichoderma* spp. have been extensively studied and reported as an excellent candidate with their efficacy to enhance root growth, crop productivity, and uptake of nutrients. Trichoderma mainly controls plant disease-causing pathogens in three ways: antibiosis, mycoparasitism, and competition for nutrients. *Pythium myriotylum*-infected ginger was recovered by volatile and nonvolatile secondary metabolites produced by *Trichoderma* spp. (Rathore *et al*. 1992). Ram *et al*. (2000) reported ginger seeds coated with *Trichoderma* spp. showed significant control of *Pythium* soft rot compared to untreated control. The significant inhibitory ability of *Trichoderma harzianum* and *T. saturnisporum* against *P. splendens* was reported by Shanmugam *et al*. (2013).

*Pythium deliense* Meurs was isolated and identified from the soft rot-infected ginger in 2019 (Vafa *et al*. 2021). *P. deliense* infection on the rhizome, results in the yellowing of leaf margins, decay of pseudostem, and complete rotting of rhizomes, leading to severe crop loss. Since the native isolates are more adapted to the rhizosphere soil, an attempt was made to evaluate the antagonistic potential of native *Trichoderma* isolates. The isolates were isolated from ginger roots collected from major ginger-growing areas Balussery, Kallanode, Koorachundu, Kootalida, Pullurampara, Adhavanad, Vattavada, Adimali and kallarkuty in Kerala. They were evaluated for their potentiality as biocontrol agents against *P. deliense*, the predominant species causing rhizome rot in ginger, under *in vitro* and *in vivo* conditions.

## Materials and Methods

### Isolation of *Trichoderma*

Healthy ginger root samples were collected from major ginger growing tracts of Kerala and used to isolate *Trichoderma* spp. by direct plate technique, using *Trichoderma* Selective Medium (TSM) (Elad and Chet 1983). Samples were washed thoroughly with running tap water, passed through sterile distilled water three times, and dried using sterile filter paper. The dried ginger roots were plated on a solidified Rose Bengal Agar medium. The plates were incubated at 28 ± 1 $^{o}$C to emerge the *Trichoderma* from the plated roots samples. The isolates of *Trichoderma* obtained were purified and maintained on PDA slants at 4$^{0}$C for further studies.

### Identification of *Trichoderma* isolates

Following the keys provided by Rifai (1969) and Samuels *et al*. (2004), morphological characteristics such as colony features, including the shapes and sizes of conidia, the branching patterns of conidiophores, the shapes and sizes of phialides, and the production of chlamydospores were used to identify *Trichoderma* isolates up to species level. Slide cultures were set up for microscopic analysis, which was done by mounting the cultures in Lactophenol cotton blue.

A 3mm × 3mm square section of PDA was cut and placed in the center of the microscopic slide to prepare the slide cultures. A microscope

cover slip was placed over the four sides of the agar square after the four sides had been introduced with fungus mycelia. To keep the environment in the Petri dish moist for fungal growth, the coverslips were put in the plates with a cotton swab that had been soaked in water and filter paper. The mycelia were allowed to develop in the Petri dish for two days while it was covered and incubated at room temperature.

Using sequences from the ribosomal DNA's internal transcribed spacer region (ITS rDNA), as White *et al*. (1990) described, morphological identity was verified. Liquid nitrogen was used to homogenize about 100 mg of the tissue or mycelium, and the powdered tissue was then put into a microcentrifuge tube. DNA was extracted using a kit (the NucleoSpin® Plant II Kit). Using the universal primers ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30), the ITS rDNA region of the isolates was amplified. ExoSAP-IT treatment was used to purify the PCR product. The BigDye Terminator v3.1 technique was used for sequencing. The sequences were then aligned and put through a BLAST search to compare them to comparable *Trichoderma* sequences stored at the National Centre for Biotechnology Information (NCBI).

**Pathogen**

Pathogenic strain of *Pythium*, *viz., P. deliense* strain ZGC P15 (Acc. No. OQ356351), used for the experiment, which was isolated from the soft rot infected ginger (Kozhikode, Kerala—11.5756° N, 75.8165° E). *P. deliense* was characterized by floral cottony mycelium with filamentous inflated/torulated sporangia (8-12 µm in diameter), and oospores were highly aplerotic. This strain was a fast-growing oomycete with smooth oogonia and bending of oogonial stalks towards the antheridia, a unique characteristic of *P. deliense* (Vafa *et al*. 2021).

***In vitro* assay – dual culture**

The antagonistic ability of *Trichoderma* spp. were evaluated under *in vitro* conditions using a dual culture technique (Morton and Stroube 1955). A 5mm diameter culture disc cut from the actively growing margins of 48h old cultures of both pathogen and antagonists were placed equidistant from the edge of the plate (90 mm base) at the opposite side to each other in solidified PDA medium, in sterile petri plates, and incubated under laboratory conditions at 28 ˚C for six days. The petri plates with PDA medium and inoculated with the pathogen alone were the control. Each treatment was maintained as three replications. The plates were observed regularly for the radial growth of the pathogen. The antagonistic activity of each *Trichoderma* isolate was scored using Bell's Scale (1982). The percentage inhibition was calculated by the formula

$$PI = \frac{C - T}{C} \times 100$$

*PI* = Percentage inhibition, *C* = Radial growth of the pathogen in control plate (mm)
*T* = Radial growth of the pathogen in Dual culture (mm)

**Effect of nonvolatile metabolites**

The activity of nonvolatile metabolites of *Trichoderma* isolates was studied using the culture filtrates of the antagonist (Dennis and Webster 1971). Mycelial discs of 5 mm size from a 48 h-old culture of *Trichoderma* isolates were grown in 250 ml conical flasks containing 150 ml Potato Dextrose Broth (PDB) and incubated at 28 ˚C for 14 days. After 14 days of growth of the *Trichoderma* isolates in liquid broth, the medium was filtered using Whatman No.1 filter paper to remove the mycelial mats. Then, the supernatant was filtered and sterilized using Millipore membrane filter paper (0.22 µm). The 10%, 25%, and 50% filtrates were incorporated into Potato Dextrose Agar (PDA) before pouring into petri dishes. The mycelial disc (5 mm) of the pathogen was inoculated at the center of the Petri plates on a solidified PDA medium incorporated with biocontrol culture filtrate. The plates were then incubated at 28 ˚C for 2-3 days. The pathogen on plain

PDA was maintained as control. Each treatment was replicated thrice. The radial growth of the pathogen was recorded, and the percentage of inhibition was calculated as described above.

**Effect of volatile metabolites of biocontrols on pathogen**

The effect of volatile metabolites was studied using the method followed by Dennis and Webster (1971). The antagonists were grown by inoculating centrally on 90 mm Petri dishes (base) containing 20 ml PDA medium and incubated for 48 h at room temperature. The lid of each Petri plate was removed, and the lower lid was sealed with the lower lid of another petri plate containing PDA medium, inoculated with the mycelial disc (5 mm) of the pathogen. Petri plates with pathogen inverted over plates containing only PDA served as control. The plates were sealed with adhesive tape and incubated at 28 $^0$C for 2-3 days. The radial growth of the pathogen in each plate was recorded, and the percentage of inhibition was calculated, as mentioned earlier.

**Greenhouse studies**

The greenhouse studies were conducted at the Botanical Garden of the Department of Botany, the Zamorin's Guruvayurappan College Kozhikode. Pot culture experiments were carried out to analyze the effect of shortlisted *Trichoderma* spp. against *Pythium* soft rot incidence under challenge-inoculated conditions. For the study, ginger variety IISR Varada was used under greenhouse conditions (24–28 °C). The experiment consisted of two treatments, and three replications were maintained for each treatment. Polythene bags filled with the pre-sterilized potting mixture (1 kg mixture/pot) comprised of soil, sand, and farmyard manure in a 1:1:1 proportion. The treatments consisted of one absolute control without any amendments and one positive control with the pathogen for comparison. The *Trichoderma* spp. ZGC T20 & ZGC T17 were used for studies, which were shortlisted from the high inhibitory value obtained from *in vitro* studies. Approximately 25 g ginger rhizome was dipped in a conidial suspension of selected *Trichoderma* isolates, as per the experimental design, planted in a pot, and watered twice a week to maintain average moisture. For challenge inoculation, *P. deliense* was multiplied in PD broth for 10 days. Each culture was macerated using a mixer grinder and incorporated as 100 ml per pot (equivalent to 1 g mycelium in 100 ml) (Tripathi and Grover 1975). The pathogen inoculation was done one month after planting ginger seeds in pots. In planta analysis was mainly concentrated on disease incidence and was calculated using the formula (Bhai *et al*. 2019):

$$\text{Disease incidence } \% = \frac{\text{No . of pseudostems infected}}{\text{Total no. pseudostems produced } (\text{in each pot})} \times 100$$

**Statistical analysis**

Statistical analysis was performed following a completely randomized design (CRBD) with three replications in each treatment. The data were subjected to analysis of variance (ANOVA) using statistical R software. Significance of various treatments was evaluated by post-hoc analysis using Least Significance Data (LSD) (0. 05) at 5% level of significance.

**Results**

**Isolation & identification of *Trichoderma* isolates**

From the healthy root surface of ginger, 10 isolates were isolated, including six from Kozhikode, three from Idukki, and one from Malappuram (Table 1). These isolates were identified as *Trichoderma* spp. based on morphological characteristics, mycelial growth, colony color and texture, and characters of conidia, conidiophores, phialides, and chlamydospore (Fig. 1). From this, five isolates were identified up to species level and confirmed by the Rajiv Gandhi Centre for Biotechnology with accession no (Table 1).

**Table 1** Details of *Trichoderma* isolates collected – Ginger fields.

| Isolate No | Location | Longitude and Latitude | *Trichoderma* spp. | District | Accession No. |
|---|---|---|---|---|---|
| ZGC T10 | Balussery | 11.4413° N, 75.8202° E | *Trichoderma* spp. | Kozhikode | - |
| ZGC T16 | Kallanode | $11.534369^0$ N $75.8762612^0$ E. | *Trichoderma* spp. | Kozhikode | - |
| ZGC T17 | Kallanode | $11.5343697^0$ N $75.8762612^0$ E | *T. virens* | Kozhikode | - |
| ZGC T20 | Adhavanad | 10.8701° N, 76.0363° E | *T. ressei* | Malappuram | OQ845837 |
| ZGC T23 | Vattavada | 10.1777° N, 77.2538° E | *T. virens* | Idukki | OQ851985 |
| ZGC T28 | Pulluramappara | 11.4058° N, 76.0396° E | *Trichoderma* spp. | Kozhikode | - |
| ZGC T30 | Adimali | 10.0115° N, 76.9528° E | *Trichoderma* spp. | Idukki | - |
| ZGC T32 | Kallarkuty | 9.9819° N, 77.0003° E | *T. asperllum* | Idukki | OQ843023 |
| ZGC T35 | Koorachundu | 11.5408° N, 75.8447° E | *T. asperllum* | Kozhikode | OQ845832 |
| ZGC T36 | Kootalida | 11.4962° N, 75.8113° E | *Trichoderma* spp. | Kozhikode | - |

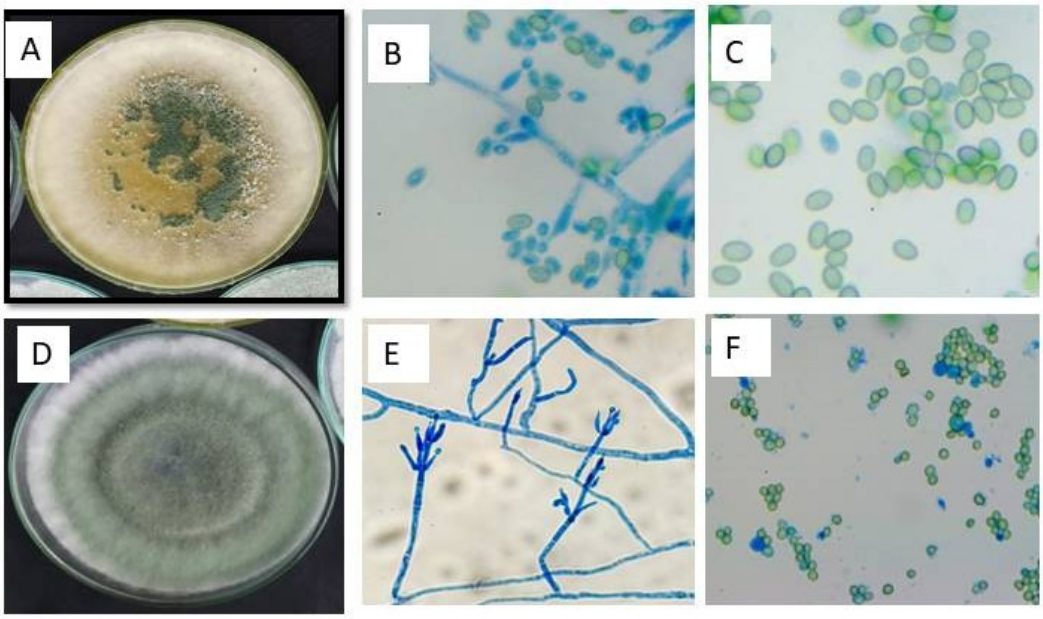


**Figure 1** Characteristics of Selected *Trichoderma* spp.
A; Colony morphology of *Trichoderma ressei* on PDA, B; Phialides with conidiophore, and C; Conidia of *T. ressei*. D; Colony morphology of *Trichoderma virens* on PDA, E; conidiophore with Phialides and conidia and F; Conidia of *T. virens*.

## Dual plate technique

All ten isolates of *Trichoderma* spp. exhibited antibiotic potential against *P. deliense* by inhibiting its mycelial growth (Fig. 2). The isolates showed a percentage of inhibition ranging from 67.77-99.19 (Table 2). The isolate showed the maximum PI of 99.19% was ZGC T20 collected from Adhavanad, Malappuram district, followed by ZGC T17 (87.78%), *T. virens* collected from Kallanode, Kozhikode district of Kerala, and ZGC T30 showed 80.96% inhibition. The remaining seven isolates *viz*., ZGC T35, ZGC T36, ZGC T32, ZGC T10, ZGC T28, ZGC T16 and ZGC T23 showed more than 65% of inhibition. All isolates of biocontrols showed good growth and covered on the mycelium of the pathogen within 5-6 days of incubation. The growth of the antagonist over the pathogen was scored using the modified Bell's Scale (Table 3). ZGC T20 and ZGC T10 showed antibiosis and competition during this study respectively.

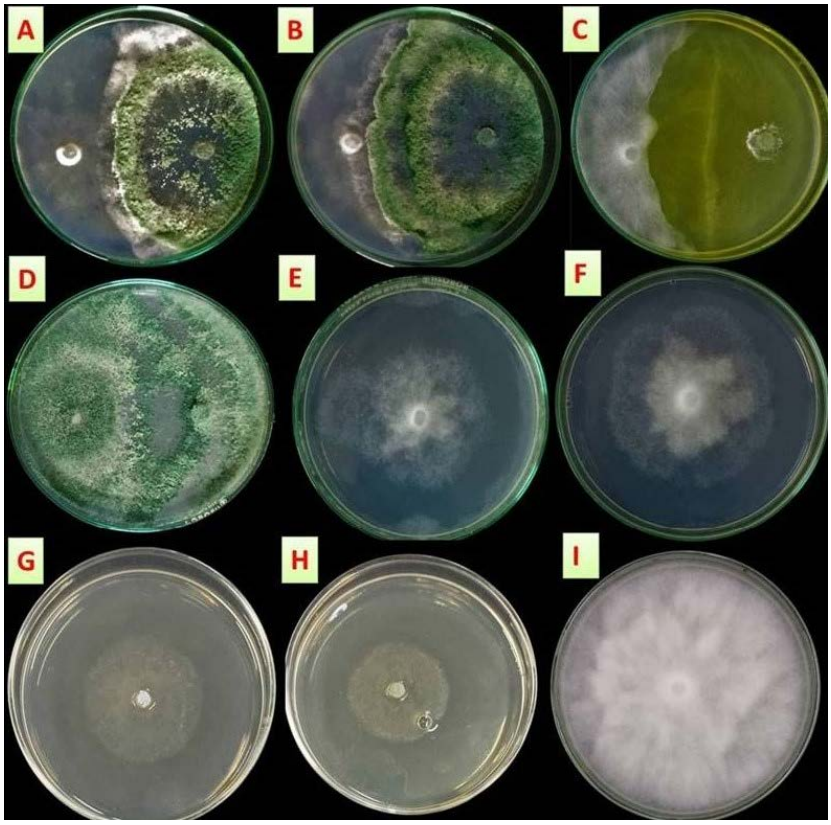


**Figure 2** In- vitro Evaluation Dual plate assay.
A; ZGC T36, B; ZGC Tl7, C; ZGC T20, D; Complete overgrowth of biocontrol, E & F Effect of volatile metabolites of ZGC T16 & ZGC T36 respectively, G& H effect of nonvolatile metabolites of ZGC T20 & ZGC Tl7 respectively, I; Control.

**Table 2** Antagonistic effect of *Trichoderma* against *P. deliense* - *in vitro*- evaluation.

| Isolate No | Inhibition percentage (%) | |
|---|---|---|
| | Dual | Non-Volatiles |
| ZGC T10 | 67.53 ± 0.43$^{g}$ | 17.80 ± 0.03$^{h}$ |
| ZGC T16 | 77.90 ± 1.30$^{d}$ | 39.56 ± 0.39$^{a}$ |
| ZGC T17 | 87.78 ± 0.37$^{b}$ | 27.44 ± 0.42$^{f}$ |
| ZGC T20 | 99.20 ± 0.19$^{a}$ | 30.29 ± 0.62$^{d}$ |
| ZGC T23 | 73.33 ± 0.00$^{ef}$ | 21.14 ± 0.70$^{g}$ |
| ZGC T28 | 74.07 ± 0.37$^{e}$ | 3.95 ± 0.93$^{i}$ |
| ZGC T30 | 80.86 ± 0.21$^{c}$ | 3.58 ± 0.77$^{i}$ |
| ZGC T32 | 67.90 ± 0.21$^{g}$ | 28.80 ± 0.16$^{e}$ |
| ZGC T35 | 72.34 ± 0.43$^{f}$ | 32.51 ± 0.40$^{c}$ |
| ZGC T36 | 77.41 ± 1.96$^{d}$ | 37.21 ± 0.56$^{b}$ |
| LSD (5%) | 1.35 | 0.95 |
| C V (%) | 1.02 | 2.30 |

**Effect of non-volatile metabolites**

The *Trichoderma* isolates showed variations in the PI of nonvolatile metabolites ranging from 0.00 – 65.55% at 10%, 25%, and 50% of concentrations (Table 2). ZGC T20 (65.55%) and ZGC T17 (53.95%) showed high percentage inhibition on dual plating and were found to be more effective by this method at 50 % concentration. The highest inhibitions shown by other isolates were ZGC T36 (53.82%), and ZGC T16 (35.67%). ZGC T23 and ZGC T35 showed 15.43% inhibition against *P. deliense*. At 25% of concentrations, ZGC T36, ZGC T17, ZGC T16 and ZGC T20 showed 52.96, 39.50, 26.29, and 17.25 percent inhibition against pathogen, respectively (Table 2 & Fig. 2).

**Table 3** Ratings of selected isolates of *Trichoderma* spp. on *P. deliense* (Bell's Scale, 1981).

| Isolates | 72 h | 96 h | 121 h | 144 h |
|---|---|---|---|---|
| ZGC T10 | S4 | S4 | S3 | S3 |
| ZGC T16 | S3 | S2 | S1 | |
| ZGC T17 | S2 | S1 | | |
| ZGC T20 | S1 | | | |
| ZGC T23 | S2 | S1 | | |
| ZGC T28 | S3 | S3-S2 | S2 | S1 |
| ZGC T30 | S2 | S2 | S1 | |
| ZGC T32 | S3 | S2 | S1 | |
| ZGC T35 | S2 | S1 | | |
| ZGC T36 | S2 | S1 | | |

**S1**- 100% overgrowth, **S2**-75% overgrowth,
**S3**- 50% overgrowth, **S4**-locked at the point of contact.

## Effect of volatile metabolites

All the isolates showed variation in the production of volatile metabolites, and PI ranged from 3.5 to 39.55 (Table 4). The highest inhibition was shown by the isolate ZGC T16 (39.55%) collected from Kallanode, followed by ZGC T36 (37.20%) from Kootalida both from Kozhikode District, Kerala. ZGC T30 (3.5) and ZGC T28 (3.95) showed the least percent inhibition against *P. delicense* (Fig. 2). Thetwo isolates: ZGC T20 and ZGC T17 appeared as promising isolates of *Trichoderma*. They can be used for their further evaluation under a pot culture study.

**Table 4** Antagonistic effect of *Trichoderma* against *P. deliense-in vitro*-evaluation using Volatile metabolites.

| Isolates | 10% | 25% | 50% | Mean |
|---|---|---|---|---|
| ZGC T10 | $0.00^{p}$ | $1.60^{o}$ | $5.31^{l}$ | $2.30^{g}$ |
| ZGC T16 | $0.00^{p}$ | $26.30^{g}$ | $35.68^{e}$ | $20.66^{d}$ |
| ZGC T17 | $2.22^{n}$ | $39.51^{d}$ | $53.95^{b}$ | $31.89^{b}$ |
| ZGC T20 | $0.00^{p}$ | $17.29^{h}$ | $65.56^{a}$ | $27.62^{c}$ |
| ZGC T23 | $0.00^{p}$ | $2.22^{n}$ | $15.44^{i}$ | $5.89^{e}$ |
| ZGC T28 | $0.00^{p}$ | $0.00^{p}$ | $0.00^{p}$ | $0.00^{h}$ |
| ZGC T30 | $0.00^{p}$ | $4.57^{m}$ | $10.00^{k}$ | $4.86^{f}$ |
| ZGC T32 | $0.00^{p}$ | $0.00^{p}$ | $14.69^{j}$ | $4.90^{f}$ |
| ZGC T35 | $0.00^{p}$ | $2.22^{n}$ | $15.44^{i}$ | $5.89^{e}$ |
| ZGC T36 | $30.12^{f}$ | $52.96^{c}$ | $53.82^{b}$ | $45.64^{a}$ |
| Mean | $3.23^{c}$ | $14.67^{b}$ | $26.99^{a}$ | |

LSD value for Samples = 0.30
LSD value for Percentage = 0.16
LSD value for Interaction = 0.52
CV= 2.11%

*Trichoderma ressei* (ZGC T 20): The colonies grow quickly on PDA at room temperature 28 ± 2 ◦C without aerial cottony mycelium, and they took less than three days to fully colonize the 90mm Petri plate. They spread intense yellow pigmentation on the culture plate and yellowish-green conidia clusters at the center of the plate. Microscopic examination, the conidiophores are sparingly branched, phialides are cylindrical or slightly inflated with an average length of 5-8 µm, and the conidia are smooth-walled, pale green in color and oblong or ellipsoidal with an average length of 3-5 µm (Fig. 1).

*Trichoderma virens*; Whitish to light green colony colour observed initially but gradually became grass green, later with soft or leathery mycelia. The conidiophores were erect, smooth, and penicillately branched; asymmetrical branches were singly or vertically arranged at different levels, phialides were flask-shaped, with coverage toward the main branch, emphasizing the penicillate branching. Phialospores were sub-globose to elliptical and smooth-walled (Fig. 1).

## Greenhouse evaluation

Under greenhouse conditions, a reduction of soft rot of ginger disease was observed by tested isolates (ZGC T20 and ZGC T17) compared to the infected control (positive control-Fig. 3). The disease incidence of each treatment under greenhouse conditions was evaluated. According to our study, *T. ressei* and *T. virens* treated plants showed 0 and 18 percent disease incidence respectively (Table 5). Control plants treated with *P. deliense* without *Trichoderma* showed 100% disease incidence. The greenhouse studies revealed that, *Trichoderma ressei* has got high efficiency in inhibiting the soft rot caused by *P. deliense*.

## Discussion

The investigation on the role of *P. deliense* in rotting and further crop loss in ginger leads to the need to develop an integrated management strategy, which can reduce the intensity of rotting by controlling P. deliense and reducing the use of hazardous agrochemicals in the fields.

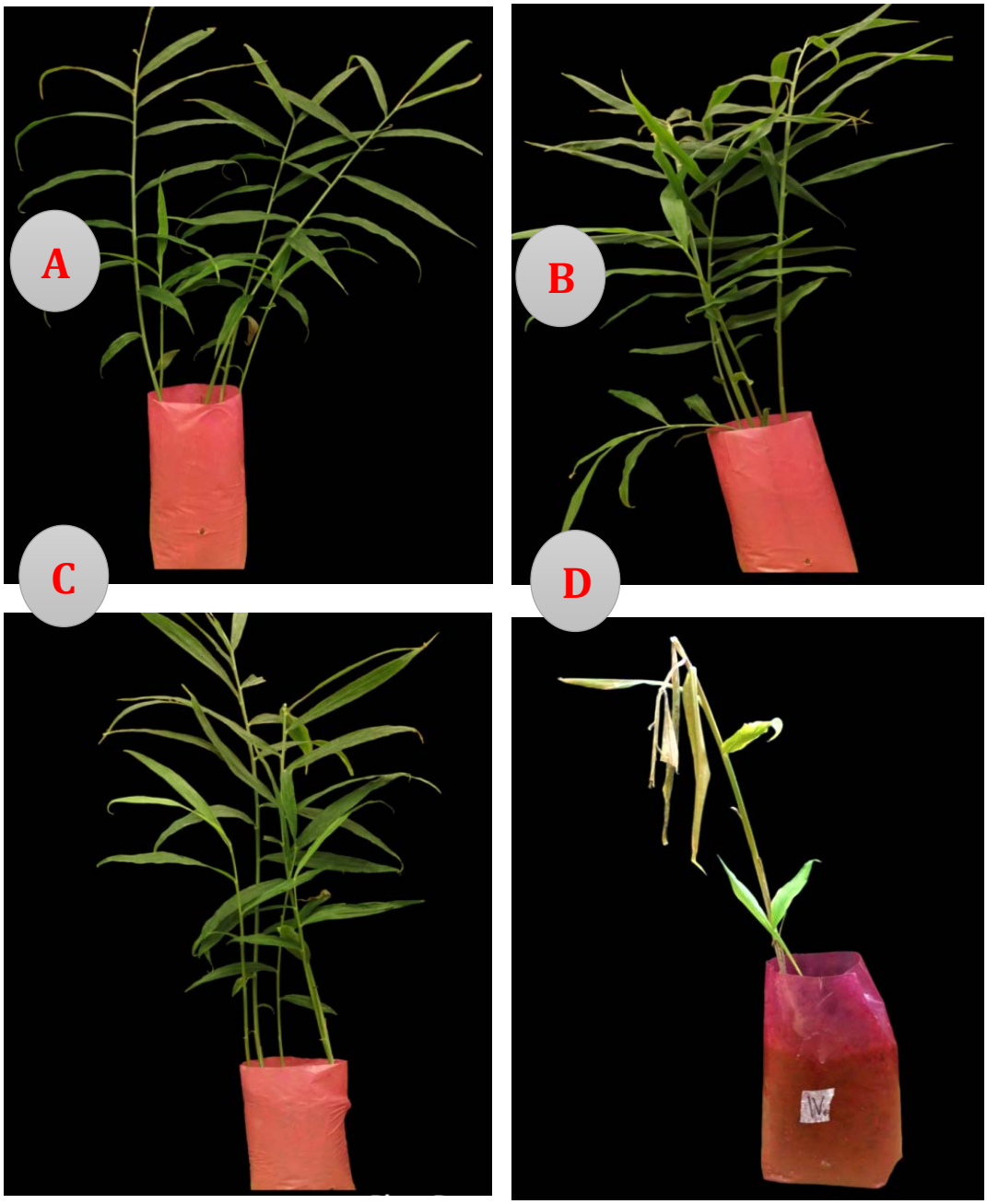


**Figure 3** Green house evaluation.
A; Absolute control, B; Treatment with *T. ressei*, C; Treatment with *T. virens*, D; Control (*Pythium deliense* alone).

In the present study, indigenous *Trichoderma* isolates from different parts of the ginger cultivating areas of Kerala were tested for their efficacy against *P. deliense* for the management of rhizome rot disease. The study involved preliminary screening of the isolates by three methods i.e.: dual plating, volatile metabolites' activity, and nonvolatile metabolites' activity. From the screening tests, shortlisted the efficient *Trichoderma* isolates

then used for greenhouse studies against *P. deliense*. *Trichoderma* isolates show mycoparasitism, and only one isolate (ZGC T20) showed complete inhibition on *P. deliense* during dual plating, revealing the potential of this *Trichoderma* spp. to be used as a biocontrol agent for the management of the disease. Several studies have reported the antagonistic potential of *Trichoderma* isolates against phytopathogens like *Pythium* (Fajola *et al*. 1975; Sivan *et al*. 1984; Howell 1991, Howell 2002; Aswhani *et al*. 2011: Rajan and Gupta 2012). *Trichoderma* isolates showing more than 85% inhibitory effect under *in vitro* were included for greenhouse evaluations and the results clearly showed that *T. ressei and T. virens* are effective in inhibiting the pathogen thereby reducing the intensity of soft rot disease in ginger. Three *Trichoderma* isolates (ZGC T20, ZGC T17 and ZGC T30) showed more than 80 percent inhibition against *P. deliense* in the dual culture plate method. Recently Pavitra *et al*. (2022) reported that, *Trichoderma virens* showed inhibition with 75.88 and 71.76 percent against *P. aphanidermatum* and *P. myriotylum* respectively. Subila and Bhai (2021) revealed that, *Trichoderma harzianum* and *Streptomyces albulus* showed 100% inhibition against root rot of pepper caused by *P. deliense* under *in vitro* conditions. In the present study, we have examined the ability of volatile and nonvolatile metabolites produced by *Trichoderma* isolates against *P. deliense* and many studies confirmed the results (Claydon *et al*. 1987; Rathore *et al*. 1992; Howell 2002; Khalid and Abdel 2017; Kuzmanovoska *et al*. 2018).

**Table 5** Evaluation of *Trichoderma* isolates on soft rot incidence in ginger – greenhouse study.

| Treatment | Disease incidence (%) |
|---|---|
| *T. ressei* | $0.00 \pm 0.00^{c}$ |
| *T. virens* | $18.70 \pm 5.04^{b}$ |
| Treatment with *Pythium* alone (positive control) | $100.00 \pm 0.00^{a}$ |
| Absolute control | $0.00 \pm 0.00^{c}$ |
| LSD (5%) | 4.74 |
| CV (%) | 8.46 |

Since the probability value is less than 0.05, the test is found to be significant at 5% level of significance.

Bhagat and Pan (2010) screened the antagonistic ability of 12 isolates of *Trichoderma* spp. *in vitro* against *R. solani* Kuhn. causing root and collar rot of French bean (*Phaseolus vulgaris* L.) by dual culture tests and production of volatile and nonvolatile antibiotics and it was found that all the isolates showed better inhibition on the mycelial growth of *R. solani*. Greenhouse evaluation supports this finding that *T. ressei* is more effective than *T. virens* against *P. deliense* and reveals the efficiency of the potential isolates in demolishing the effects of *P. deliense*. Monteiro *et al*. (2014) studied *Trichoderma ressei* on their mycoparasitism against *Pythium ultimum*. They reported G-alpha protein GNA1 signaling and suggested that the production of some CWDEs during mycoparasitism by *T. ressei* against *P. ultimum*, was mediated by GNA1 activity or cAMP levels. Muhamed and Haggag (2010) indicated that mutation and protoplast fusion techniques successfully enhance the antagonistic effects of *Trichoderma* species such as *T. koningii* and *T. ressei*, against several fungal plant pathogens. Soil application of *T. harzianum* bio-formulations and ginger seed treatment with onion and garlic extracts was more effective against soft rot disease. It improved growth and yield parameters in a pot culture study conducted by Dohroo *et al*. (2012).

Therefore, our study also suggested that soil application of T. ressei and T. virens could be effectively used as a management strategy or reduce the intensity of soft rot of ginger.

## Statement of Conflicting Interests

The authors declare that they have no competing interests.

## Authors' contributions

Anju John V; Conception, Design of work, writing, Interpretation of data

Vafa A Latheef; Conception, Reviewing and editing, Formal analysis

Rajan P. P.; Conception, Methodology, Supervision,

K. P. Rajesh; Reviewing and editing

All authors read and approved the final manuscript

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# اثربخشی جدایه‌های بومی تریکودرما در برابر *Pythium deliense* Meurs مرتبط با پوسیدگی نرم زنجبیل

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**چکیده:** زرد شدن و پوسیدگی ریزوم‌های زنجبیل یک نگرانی شدید در بسیاری از مناطق کشت زنجبیل در کرالا است، جایی‌که *Pythium deliense* اخیراً به‌عنوان یک پاتوژن از ریزوسفر ریزوم‌های پوسیده آلوده ظاهر شده است، که بیماری‌زا بودن آن توسط فرضیه‌های Koch ثابت شده است. مطالعه حاضر پتانسیل آنتاگونیستی ده جدایه *Trichoderma* spp. جدا شده از ریشه زنجبیل سالم را در برابر *P. deliense* که باعث پوسیدگی نرم زنجبیل به روش صفحه دوگانه می‌شود، ارزیابی می‌کند. اثربخشی متابولیت‌های فرار و غیرفرار تولید شده توسط گونه‌های آنتاگونیست *Trichoderma.* تحت شرایط in vitro در برابر پاتوژن مورد بررسی قرار گرفت. در بین ده جدایه، هشت جدایه مانند ZGC T16 ، ZGC T17، ZGC T20، ZGC T23، ZGC T28، ZGC T30، ZGC T35، و ZGC T36 مهار رشد میسلیوم، بالای ۷۰ درصد را در آزمون دو پلیت نشان دادند. بیش‌ترین درصد بازدارندگی توسط ZGC T20 (۹۹/۱۹ درصد) و پس از آن ZGC T17 (۸۷ درصد)، به‌ترتیب با عنوان *Trichoderma ressei* و *Trichoderma virens*، تحت کشت دوگانه و مهار متوسط به‌دلیل متابولیت‌های فرار (۳۹/۵۵ - ۳/۵ درصد) نشان داده شد. متابولیت‌های غیرفرار تولید شده توسط ZGC T20 (65.55%) ، ZGC T17 (53.95%)، و ZGC T36 (53.82%) کارایی نسبتاً کم‌تری نشان دادند. آن‌هایی‌که بالقوه (ZGC T20 & ZGC T17) بیش‌تر تحت مطالعه کشت گلدان و شرایط گلخانه در داخل بدن مورد ارزیابی قرار گرفتند. این مطالعه نشان داد که *T. ressei* و *T. virens* کارایی بالایی در جلوگیری از پوسیدگی نرم زنجبیل ناشی از *P. deliense* دارند.

**واژگان کلیدی:** بیوکنترل، زنجبیل، *Pythium deliense*، پوسیدگی نرم، تریکودرما