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Research Article

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Posted Date: October 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11148693/v1

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Additional Declarations: No competing interests reported.

Upregulation of chitinase and β-1,3-glucanase enzymes and modulation of biochemical defense response by fungal endophytes against *Rhizoctonia solani* in cotton

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Abstract

Rhizoctonia solani is an important soil-borne pathogen of cotton, causing damping-off, root infection, and poor seedling establishment. The use of beneficial fungal endophytes as seed biopriming agents offers a promising approach for enhancing host defense and reducing dependence on use. In this study, selected fungal endophytes were evaluated to induce biochemical and molecular defense responses against *R. solani* in cotton. Seed biopriming with *Diaporthe longicolla*, *Trichoderma sulphureum*, and *Daldinia eschscholtzii* substantially strengthened the defense status of cotton against *R. solani*. Among these, *D. eschscholtzii* produced the most pronounced accumulation of soluble proteins, sugars, reducing sugars, phenolics and total antioxidants, together with enhanced catalase activity at both 15 and 30 DAS. *T. sulphureum* was particularly effective in activating phenylalanine ammonia-lyase, while its peroxidase activity was highest at 30 days. At the molecular level, early defense activation was evident from strong induction of chitinase and β-1,3-glucanase transcripts. Chitinase expression increased approximately 8.5-, 16-, and 26-fold following treatment with *D. longicolla*, *T. sulphureum*, and *D. eschscholtzii*, respectively, at 15 days after sowing, whereas β-1,3-glucanase reached approximately 9-fold induction with *D. eschscholtzii*. Transcript accumulation generally declined at 30 days, indicating a stronger early molecular response. Overall, *D. eschscholtzii* consistently elicited the broadest biochemical and molecular defense response, while *T. sulphureum* showed notable activation of phenylpropanoid- and peroxidase-associated defenses. Seed biopriming with fungal endophytes enhanced cotton defense against *R. solani* through coordinated modulation of biochemical defense mechanisms and pathogenesis-related gene expression.

Keywords: Biocontrol, damping off, root rot, pathogenesis-related proteins, plant–microbe interactions, Seed biopriming

Introduction

Cotton (*Gossypium* spp.) is an important commercial and natural fibre crop, contributing significantly to the agricultural and textile sectors. India cultivates all four recognized cultivated cotton species and has the world's largest cotton-growing area, with 114.82 lakh hectares under cultivation in 2025–26, accounting for about 38% of the global cotton area. India ranks second in cotton production and consumption, with an estimated production of 290.91 lakh bales (4.95 million tonnes) and domestic consumption of 328 lakh bales (5.58 million tonnes) during 2025–26 (PIB, 2026). Cotton is affected by several diseases, among which damping-off caused by *R. solani* is an important constraint during early crop establishment. The disease can cause losses ranging from 3 to 90% under favourable conditions (Singh and Verma, 1988). Management of soil-borne diseases includes summer ploughing, field sanitation, resistant varieties, biocontrol agents and fungicide drenching (Madhavi and Bhattiprolu, 2011). However, excessive reliance on chemical fungicides can adversely affect soil health and beneficial microorganisms and may promote pathogen resistance. Therefore, biological control is increasingly preferred as an eco-friendly and cost-effective approach for sustainable disease management (Deacon and Berry, 1993). Endophytes are living organisms that may be fungi, bacteria or actinomycetes, which live inside plant tissues without causing any apparent diseases and with more of beneficial effects to their host plant (Pablo *et al*., 2015). Among them, endophytic fungi are widely distributed among plants and exhibit considerable diversity in species composition (Elsalam *et al.,* 2025; Potshangbam *et al.,* 2017). Studies have also demonstrated substantial diversity of endophytic fungi associated with cotton (Ramos *et al.,* 2013; Li *et al.,* 2014). These fungi are considered promising biocontrol resources due to their ability to suppress plant pathogens and insect pests, promote host plant growth, and enhance plant defense and resistance (Backman and Sikora, 2008; Hassani *et al.,* 2018).

Fungal endophytes are particularly important because of their diverse functional activities, including the production of secondary metabolites and protection of host plants against biotic and abiotic stresses (Kusari *et al*., 2012). Their antagonistic activity against plant pathogens may occur through direct mechanisms, such as antibiosis mediated by diffusible and volatile compounds, competition for nutrients and space, and mycoparasitism, as well as indirect mechanisms involving the induction of host defence and promotion of plant growth (Gao *et al*., 2010). In cotton, seed biopriming with fungal endophytes has been shown to enhance plant growth and prime biochemical and antioxidative defence responses, as evidenced by increased soluble protein and sugar contents and elevated catalase and peroxidase activities (Verma *et al*., 2022). Such endophyte-mediated priming may prepare plants for a more effective defence response upon pathogen perception, which involves the activation of reactive oxygen species (ROS), secondary metabolites and pathogenesis-related (PR) proteins (Bolwell *et al*., 2001). Chitinase and β-1,3-glucanase are key hydrolytic enzymes involved in microbial antagonism and plant defense, owing to their ability to hydrolyze chitin and β-glucans, major structural components of fungal cell walls. Endophytic fungi can stimulate these defense responses in plants; notably, *Fusarium solani* CEF-559 enhanced the expression of chitinase and β-1,3-glucanase in cotton following *Verticillium dahliae* challenge (Wei *et al*., 2019). However, the potential of fungal endophytes to enhance cotton resistance against *R. solani* remains relatively underexplored. Therefore, the present investigation was undertaken to elucidate the role of selected fungal endophytes in inducing defense responses in cotton against *Rhizoctonia solani*.

Material and methods

Experimental site and plant material

The experiments were conducted at the ICAR–Central Institute for Cotton Research (CICR), Department of Plant Pathology, Pot house, Nagpur, Maharashtra, India. Cotton cultivar LRA-5166, which is susceptible to major soil-borne diseases of cotton, was used as the experimental material for pot-house studies.

Fungal endophytes used in the study

A total of 72 fungal endophytes isolated from healthy cotton tissues were initially screened for their antagonistic activity against *Rhizoctonia solani* using the dual-culture technique. The isolates were characterized based on their cultural, morphological, and molecular characteristics. Based on the results of the preliminary *in vitro* screening, *Diaporthe longicolla*, *Trichoderma sulphureum*, and *Daldinia eschscholtzii* exhibited 39.02%, 43.82%, and 76.20% inhibition of *Rhizoctonia solani*, respectively. These three promising fungal endophytes were selected for subsequent *in vivo* evaluation.

Pathogen culture and inoculum preparation

The virulence of *Rhizoctonia solani* was evaluated using a pathogenicity assay. Sorghum seeds were soaked in sterile water for 24 h and autoclaved at 121°C and 15 psi for 15 min on three consecutive days. Under aseptic conditions, 5-8 agar discs (10 mm diameter) from actively growing pathogen margins were inoculated into conical flasks containing sterilized sorghum seeds. The cultures were incubated at 27°C for 10 days for mass multiplication of the pathogen (Upadhyay and Mukhopadhyay, 1986).

The resulting inoculum was thoroughly mixed with sterilized potting mixture at 3% (w/w) and filled into pots (Jinantana and Sariah, 1998). The inoculated soil was incubated for 5 days under glasshouse conditions to facilitate pathogen establishment. Surface-sterilized cotton seeds were then sown, with three replications per treatment and five plants per replication. Plants were maintained at approximately 80% relative humidity using a humidifier. Disease development was monitored periodically, and symptomatic plants were selected for pathogen re-isolation. The pathogen was re-isolated on PDA from infected tissues and its identity was confirmed based on cultural characteristics, thereby fulfilling Koch's postulates and confirming pathogenicity (Baird *et al*., 2000).

In vivo evaluation of potential fungal endophytes in pot cultures against *R. solani*

Seeds of cotton cultivar LRA-5166 were surface sterilized by immersion in 1% sodium hypochlorite (NaOCl) for 2 min, followed by treatment with 70% ethanol for 2 min, and subsequently rinsed three times with sterile distilled water to remove residual sterilants, with slight modification of the method described by Miranda *et al.,* 2023.

The selected fungal endophytes were grown on PDA plates for 10 days. Conidial suspensions were prepared (Jaber and Enkerli, 2016) by adding sterile distilled water containing 0.01% Tween-80 to the fungal cultures and gently scraping the mycelial growth with a sterile glass rod to release the conidia. The suspension was filtered through sterile muslin cloth and adjusted to 1×10^7 spores mL^{-1}. Surface-sterilized cotton seeds were soaked separately in the conidial suspensions of individual endophytes for 2 hr under aseptic conditions. Seeds soaked

in sterile distilled water for the same duration served as the untreated control. Following treatment, seeds were removed from the suspensions and air-dried overnight under sterile conditions before sowing.

Effect of fungal endophyte biopriming on seed germination

Seed germination for bioprimed seeds was recorded on the seventh day after sowing. Germination percentage was calculated using the following equation (Mondo *et al*., 2019).

$$Germination\ percentage\ (\%) = \frac{Total\ number\ of\ seeds\ sown}{Number\ of\ seeds\ germinated} \times 100$$

Estimation of total soluble protein

The Bradford method was used to estimate total soluble protein (Bradford, 1976). After homogenising fresh seedling tissue (0.1 g) in 1 mL of phosphate buffer (pH 7.2), it was centrifuged at 10,000 rpm for 10 min at 4°C. The protein extract was made from the supernatant. The sample combinations contained 10 µL protein extract, 990 µL distilled water, and 2 mL Bradford reagent, whereas a reagent blank had 1 mL distilled water and 2 mL Bradford reagent. After 10 min of room temperature incubation, the mixes' absorbance at 595 nm was measured. The standard was bovine serum albumin (BSA), and the standard curve was used to calculate the protein content.

Estimation of total soluble sugars

Using the Anthrone technique (Thimmaiah, S. R. 2012), the total soluble sugars were calculated. In a boiling water bath, the sample was hydrolysed with hydrochloric acid, cooled, and neutralised with sodium carbonate (Na_2CO_3). Analysis was done using the clear supernatant. Freshly made Anthrone reagent was combined with 1 mL of extract, boiled in a boiling water bath for around 10 min, and then cooled. A spectrophotometer was used to measure absorbance at 630 nm. The standard curve was prepared using glucose, and the total soluble sugar content was computed using the standard curve.

Estimation of reducing sugar

Nelson-Somogyi's approach was used to estimate reducing sugars (Nelson 1944). Eighty percent ethanol was used to homogenise samples (0.1 g) of bio-primed and control plants. After centrifuging the extract at 10,000 g for 15 min at room temperature, the homogenates were kept in a water bath at 8x0°C to remove any remaining supernatant. Alkaline copper tartrate and arsenomolybdate reagent were used colorimetrically at a wavelength of 620 nm to quantify reducing sugars. Using glucose as a standard, a graph was created to determine the concentration of decreasing sugar.

Total antioxidant activity

According to Benzie and Strain (1996), the Ferric Reducing Antioxidant Power (FRAP) assay was used to measure total antioxidant activity. After homogenising 100 mg of fresh seedling tissue in 5 mL of cooled phosphate buffer (50 mM, pH 7.0), the mixture was centrifuged at 10,000 rpm for 10 min at 4°C. 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ produced in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ were combined to create the FRAP reagent. After combining 100 µL of the supernatant with 3 mL of FRAP reagent, the mixture was incubated for 10 min at 37°C in the dark. At 593 nm, absorbance was measured. The

standard curve was prepared using $FeSO_4 \cdot 7H_2O$, and antioxidant activity was expressed using the standard curve.

Phenylalanine ammonia lyase activity

The Brueske (1980) method was used to estimate PAL activity. After homogenising fresh tissue (0.5 g) in 1 mL of 0.01 M borate buffer (pH 8.8) in a cold environment, the tissue was centrifuged for 20 min at 10,000 × g. The source of the enzyme was the supernatant. 1 mL of enzyme extract, 1 mL of borate buffer, and 1 mL of 0.15 M L-phenylalanine made up the reaction mixture, which was incubated for sixty min at 38°C. After adding 0.5 mL of 1 M trichloroacetic acid and incubating at 37°C for 5 min, the reaction was stopped. At 270 nm, the amount of trans-cinnamic acid produced was measured using spectrophotometry. A standard curve made with trans-cinnamic acid was used to calculate PAL activity, which was represented as μg trans-cinnamic acid released min^{-1} mg^{-1} protein.

Catalase activity

In accordance with Chance and Maehly (1955), catalase activity was measured by tracking the breakdown of $H2O_2$ at 240 nm. 5 mL of ice-cold phosphate buffer (50 mM, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) was used to homogenise fresh tissue (0.5 g). The homogenate was centrifuged for 10 min at 4°C at 10,000 rpm. The reaction mixture included 0.5 mL of 30 mM $H2O_2$, 0.5 mL of enzyme extract, and 2 mL of 100 mM phosphate buffer (pH 6.8). For 4 min, the absorbance drop at 240 nm was measured every 60 sec. The extinction coefficient of $H2O_2$ ($\varepsilon = 39.4\ M^{-1}\ cm^{-1}$) was used to calculate catalase activity, which was then reported as μmol $H2O_2$ degraded min^{-1} mg^{-1} protein.

Peroxidase activity

Peroxidase activity was computed in compliance with (Hammerschmidt *et al.* 1982). Fresh tissue (0.5 g) was homogenised in 5 mL of ice-cold 0.1 M phosphate buffer (pH 6.0) at 4°C. The tissue was then centrifuged at 2,000 × g for 10 min at 4°C. The supernatant was the source of the enzyme. The reaction mixture consisted of 3.0 mL of 0.05 M pyrogallol, 100 μL of enzyme extract, and 0.5 mL of 1% $H2O_2$. For 80 sec, absorbance was measured at 430 nm every 20 sec, and peroxidase activity was expressed in mL^{-1} enzyme units.

Total phenolic content

According to Mallick and Singh (1980), the total phenolic content was calculated. To reduce phenolic chemical oxidation, fresh leaf tissue (0.25 g) was extracted using 5 mL of 80% ethanol and heated for 10 min. After cooling, the extract was macerated with 80% ethanol to a final volume of 5 mL and centrifuged for 5 min. 1 mL of supernatant, 1 mL of Folin reagent, and 1 mL of 20% sodium carbonate were combined, and the volume was increased to 10 mL using distilled water. At 650 nm, absorbance was measured. Standards ranging from 200 to 1000 ppm were prepared using catechol.

Gene Expression studies for selected endophyte treated seeds

RNA extraction and cDNA synthesis

To quantify the transcript abundance of the two genes *i.e*. β-1,3-glucanase and chitinase post inoculation of selected endophytes against R. *solani* at 15 and 30 DAS. Prior to RNA extraction, frozen leaf samples were crushed with liquid nitrogen and ground to fine powder using pestle and mortar. Total RNA from leaf tissues from 15 DAS and 30 DAS was extracted using Spectrum Plant Total RNA kit (Sigma-Aldrich, USA) as per manufacturer's protocol. To quantify the transcript abundance of the target genes, the total RNA each from treated and control samples will be isolated. 1 µg total RNA was quantifed by using Nanodrop spectrophotometer and reverse transcribed into complementary DNAs (cDNAs) within a 20µl reaction volume using GoScript Reverse Transcription System (Promega, USA) as directed by the manufacturer.

Quantitative Reverse Transcription PCR (qRT-PCR)

Primers were designed for the genes was subjected to RT-PCR using cDNA samples for gene expression studies. RT-PCR conditions were 95 °C for 3 min, 95 °C for 40 sec, 60 °C for 40 sec, 72 °C for 40 sec with 35 cycles followed by 72 °C for 7 min final extension. Similar conditions were simulated for qPCR experiments with an additional step for melt curve segment. Raw Ct value from three biological replicates in each treatment combination were used to calculate fold change. Significance of relative gene expression of each target gene was calculated using ANOVA.

Statistical analysis

The data pertaining to plant growth promotion and antioxidative enzyme activities were subjected to statistical analysis. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the appropriate level of significance, with fungal endophytes considered as treatments. Statistical analysis was performed using the Web Agri Stat Package (WASP v2.0).

Results

Fungal endophyte-mediated enhancement of cotton seed germination

When cotton seeds were bioprimed with specific fungal endophytes, the germination responses under the *R. solani* challenge varied (S1, Table 3). T6, which included *D. eschscholtzii*-bioprimed seeds placed in untreated soil, had the highest germination rate (91%). T3 (88%), which involved sowing *D. eschscholtzii*-bioprimed seeds in *R. solani*-inoculated soil, came next. Germination rates for treatments T2 and T5 were 86%, followed by T4 (84%) and T1 (82%). The negative control (T8; untreated seeds in untreated soil) showed 75% germination, whereas the positive control (T7; *R. solani*-inoculated soil) showed the lowest germination (67%). Under *R. solani* challenge, *D. eschscholtzii* biopriming generally maintained increased seed germination.

Biochemical defense responses induced by fungal endophytes against *R. solani* in cotton was observed 15 and 30 DAS. The experiment was conducted with following treatment as shown in table 3.

Biochemical defense responses induced by fungal endophytes against *Rhizoctonia solani* pathogens in cotton

Effect of endophytes on protein content against *R. solani*

Protein content varied significantly among treatments at 15 DAS, with the highest content recorded in T3 (12.233 ± 0.339), followed by T1 (9.452 ± 0.458) and T2 (7.733 ± 0.125). Among the remaining treatments, T7 (6.395 ± 0.426) and T6 (5.857 ± 0.095) recorded higher protein contents, whereas T5 (5.386 ± 0.252), T8 (5.114 ± 0.278), and T4 (4.790 ± 0.193) showed lower values. At 30 DAS, protein content increased in most treatments, with T3 recording the highest value (12.253 ± 0.296), followed by T1 (10.843 ± 0.135) and T2 (9.800 ± 0.223). Moderate protein contents were observed in T4 (8.438 ± 0.134), T6 (7.791 ± 0.624), and T7 (7.754 ± 0.147), while T5 (5.900 ± 0.405) and T8 (5.115 ± 0.278) recorded the lowest values (Table 4.0.) Overall, T3 consistently exhibited the highest protein content at both stages, followed by T1 and T2, whereas T8 recorded the lowest values. The increase from 15 to 30 DAS suggests enhanced protein accumulation in endophyte-treated plants under *R. solani* challenge (Fig.1.).

Effect of endophytes in sugar content against *R. solani*

Sugar content varied significantly among treatments at 15 DAS, with the highest value recorded in T3 (2.060 ± 0.006), followed by T1 (1.910 ± 0.002) and T2 (1.380 ± 0.011). T6 (1.260 ± 0.009) and T7 (1.260 ± 0.009) recorded comparable values, followed by T5 (1.240 ± 0.008), T4 (1.030 ± 0.009), and T8 (1.020 ± 0.013). At 30 DAS, T3 recorded the highest sugar content (2.007 ± 0.050), followed by T2 (1.823 ± 0.043) and T1 (1.653 ± 0.023). Moderate values were observed in T6 (1.073 ± 0.020), T4 (1.067 ± 0.049), T5 (1.053 ± 0.038), and T7 (1.017 ± 0.035), whereas T8 recorded the lowest value (0.867 ± 0.020). Overall, T3 consistently recorded the highest sugar content at both stages, while T8 recorded the lowest. The higher sugar accumulation in endophyte-treated plants indicates enhanced metabolic activity under *R. solani* challenge (Fig.2.).

Effect of endophytes in reducing sugars content against *R. solani*

Reducing sugar content varied significantly among treatments at 15 DAS, with the highest value recorded in T3 (0.413 ± 0.009), followed by T2 (0.317 ± 0.009) and T1 (0.210 ± 0.006). Among the remaining treatments, T6 (0.253 ± 0.009) recorded a higher value, followed by T4 (0.200 ± 0.023), T7 (0.197 ± 0.007), T5 (0.193 ± 0.009), and T8 (0.157 ± 0.003), which recorded the lowest value. At 30 DAS, reducing sugar content increased across all treatments, with T3 recording the highest value (0.570 ± 0.012), followed by T2 (0.527 ± 0.015) and T1 (0.483 ± 0.009). Among the remaining treatments, T6 (0.397 ± 0.007) recorded the highest value, followed by T5 (0.360 ± 0.012), T7 (0.350 ± 0.017), and T4 (0.343 ± 0.009). Overall, T3 consistently recorded the highest reducing sugar content at both stages, followed by T2 and T1, whereas T8 recorded the lowest value. The increase in reducing sugar content from 15 to 30 DAS indicates enhanced carbohydrate metabolism in endophyte-treated plants under *R. solani* challenge (Fig.3.).

Effect of endophytes in phenol content against *R. solani*

At 15 DAS, the highest total phenol content was recorded in T3 (22.560 ± 0.160), followed by T1 (19.213 ± 0.315) and T2 (18.307 ± 0.243). Among the remaining treatments, T4 (15.453 ± 0.035) and T6 (15.280 ± 0.372) showed comparatively higher values, followed by T7 (13.613 ± 0.581), T5 (13.600 ± 0.583), and T8 (12.893 ± 0.486). At 30 DAS, T3 recorded the highest phenol content (26.253 ± 0.559), followed by T2 (22.733 ± 0.368) and T1 (21.160 ± 0.370). Moderate values were observed in T5 (18.813 ± 0.424), T6

(17.267 ± 1.018), and T7 (17.240 ± 0.633), whereas T8 (14.627 ± 0.218) and T4 (13.173 ± 0.449) recorded lower values. Overall, T3 consistently exhibited the highest total phenol content at both stages, followed by T1 and T2, while T8 recorded the lowest value. The increased phenol accumulation indicates enhanced induction of phenolic compounds and biochemical defense in endophyte-treated plants against *R. solani* (Fig.4.).

Effect of endophytes in antioxidant content against *R. solani*

The effect of fungal endophytes on antioxidant accumulation in cotton seedlings challenged with *R. solani* was evaluated at 15 and 30 DAS, and the results are presented in Table 8.0. Antioxidant content differed significantly among treatments at both stages. At 15 DAS, values ranged from 5531.27 to 11220.00, with the highest accumulation recorded in T3 (11220.00 ± 133.72), followed by T1 (9910.00 ± 203.39) and T2 (9632.53 ± 109.08). Among the remaining treatments, T6 (6580.00 ± 23.85), T5 (6326.27 ± 27.09), T7 (6236.27 ± 21.90), and T4 (6121.27 ± 20.75) recorded intermediate values, while T8 (5531.27 ± 191.70) showed the lowest accumulation. At 30 DAS, antioxidant content increased across all treatments, ranging from 6076.25 to 11706.25. T3 recorded the highest value (11706.25 ± 208.93), followed by T2 (9950.00 ± 16.82) and T1 (9328.75 ± 58.94). Among the remaining treatments, T7 (7055.00 ± 118.47), T4 (6867.38 ± 19.42), T6 (6827.50 ± 18.41), and T5 (6620.00 ± 17.50) showed intermediate values, whereas T8 (6076.25 ± 28.17) recorded the lowest accumulation. Overall, T3 consistently exhibited the highest antioxidant accumulation at both stages, while T8 recorded the lowest, indicating enhanced antioxidant defense in endophyte-treated plants under *R. solani* challenge (Fig.5.).

Effect of endophytes in PAL content against *R. solani*

At 15 DAS, the highest PAL activity was recorded in T2 (2.554 ± 0.018), followed by T3 (2.363 ± 0.026) and T1 (2.216 ± 0.018). Among the remaining treatments, T6 (1.154 ± 0.130), T5 (1.042 ± 0.119), T4 (1.029 ± 0.030), and T7 (0.700 ± 0.052) recorded comparatively lower activities, while T8 (0.529 ± 0.073) showed the lowest value. At 30 DAS, T2 recorded the highest PAL activity (3.042 ± 0.070), followed by T3 (2.913 ± 0.163) and T1 (2.854 ± 0.079). Moderate activity was observed in T5 (1.558 ± 0.114), T7 (1.479 ± 0.085), T4 (1.325 ± 0.033), and T6 (1.175 ± 0.069), whereas T8 (0.783 ± 0.051) recorded the lowest value. Overall, T2 consistently exhibited the highest PAL activity at both stages, followed by T3 and T1, while T8 recorded the lowest activity. These results indicate that endophytic fungal biopriming enhanced PAL-mediated biochemical defense in cotton seedlings challenged with *R. solani* (Fig.6.).

Effect of endophytes in catalase content against *R. solani*

At 15 DAS, the highest catalase activity was recorded in T3 (2.417 ± 0.071), followed by T1 (1.960 ± 0.029) and T2 (1.773 ± 0.007). Among the remaining treatments, T7 (0.963 ± 0.024), T4 (0.853 ± 0.022), T5 (0.707 ± 0.039), and T8 (0.673 ± 0.009) recorded comparatively lower activities, while T6 (0.610 ± 0.015) showed the lowest value. At 30 DAS, catalase activity increased markedly, with T3 recording the highest value (3.277 ± 0.044), followed by T2 (2.523 ± 0.128) and T1 (2.413 ± 0.067). Moderate activity was observed in T5 (1.783 ± 0.020), T6 (1.723 ± 0.082), T7 (1.390 ± 0.032), and T4 (1.297 ± 0.033),

whereas T8 (0.543 ± 0.094) recorded the lowest value. Overall, T3 consistently exhibited the highest catalase activity at both stages, followed by T1 and T2, while T8 recorded the lowest activity at 30 DAS. The enhanced catalase activity indicates improved antioxidant defense in endophyte-treated cotton seedlings challenged with *R. solani* (Fig.7.).

Effect of endophytes in peroxidase content against *R. solani*

At 15 DAS, the highest peroxidase activity was recorded in T3 (34.488 ± 0.577), followed by T1 (29.013 ± 0.353) and T2 (28.150 ± 0.406). Among the remaining treatments, T4 (23.738 ± 1.285), T7 (22.963 ± 1.524), T6 (22.692 ± 0.852), T5 (21.858 ± 0.301), and T8 (21.433 ± 0.457) showed comparatively lower activities. At 30 DAS, T2 recorded the highest peroxidase activity (33.529 ± 0.798), followed by T1 (30.858 ± 0.334) and T3 (28.218 ± 0.426). Moderate activities were observed in T6 (21.608 ± 1.048), T7 (20.446 ± 2.945), T4 (19.396 ± 0.488), and T5 (19.058 ± 0.176), whereas T8 (17.025 ± 0.310) recorded the lowest value. Overall, T3 exhibited the highest peroxidase activity at 15 DAS, while T2 recorded the highest at 30 DAS. T1, T2, and T3 consistently showed higher peroxidase activity than the remaining treatments, indicating enhanced peroxidase-mediated defense in cotton seedlings challenged with *R. solani* (Fig.8.).

Endophyte-mediated modulation of Pathogenesis-Related genes (Chitinase and β-1,3-Glucanase) expression in cotton during *R. solani* challenge

Relative expression of chitinase (CHI19) gene in cotton

Endophyte treatment and sample duration had a significant impact on chitinase expression (Fig.9). Individual endophytes increased chitinase expression to ~2.0–4.4-fold at 15 DAS, but their combination with P3 significantly increased transcript accumulation to ~8.3-fold (E1 + P3), ~16-fold (E2 + P3), and a maximum of ~28-fold in E3 + P3. Most treatments showed a reduction in expression at 30 DAS, although E3 + P3 maintained the highest transcript abundance (~5.5-fold), followed by E2 + P3 (~1.2-fold). The strongest and longest-lasting chitinase response was often triggered by E3 + P3, and the substantial induction at 15 DAS suggested an early activation of chitinase-mediated defence after *R. solani* exposure.

Relative expression of glucanase (GLU 42) gene in cotton

Endophyte-mediated biopriming and R. solani challenge resulted in significant early activation of β-1,3-glucanase (Fig.10.). At 15 DAS, individual endophytes increased GLU expression by ~1.1-4.6-fold, with E3 having the greatest expression (~4.6-fold). Combining with P3 increased transcript accumulation by approximately 2.8-fold in E2 + P3 and up to ~9.2-fold in E3 + P3. At 30 DAS, GLU expression decreased significantly in most treatments. However, E2 + P3 (~1.4-fold) and E3 + P3 (~1.3-fold) maintained higher transcript abundance than the other treatments. Overall, E3 + P3 induced the highest early β-1,3-glucanase response. The substantial induction at 15 DAS indicates an early activation of β-1,3-glucanase-mediated defence following *R. solani* exposure.

DISCUSSION

Rhizoctonia solani is an important soil-borne necrotrophic pathogen of cotton causing damping-off, root and hypocotyl infection, and severe seedling establishment problems (Fulton & Bollenbacher, 1959). Since the

pathogen attacks below-ground tissues and efficiently colonizes host tissues, protection requires coordinated activation of multiple defense mechanisms. In the present study, seed biopriming with fungal endophytes markedly altered defense-related biochemical responses and defense-gene expression in cotton challenged with *R. solani*, indicating activation and/or priming of multiple host defense pathways.

Protein accumulation differed markedly among treatments. At both 15 and 30 DAS, *Daldinia eschscholtzii* + *R. solani* (T3) recorded the highest protein content, followed by *Diaporthe longicolla* + *R. solani* (T1) and *Trichoderma sulphureum* + *R. solani* (T2), while the untreated control (T8) recorded the lowest. Protein content increased from 15 to 30 DAS in most treatments. The higher protein accumulation in endophyte-treated plants may indicate enhanced metabolic activity associated with defense and stress responses. This is consistent with the proteomic analysis of cotton challenged with *R. solani*, which identified 174 differentially accumulated proteins associated with ROS homeostasis, phenylpropanoid metabolism, carbohydrate metabolism and pathogenesis-related responses (Zhang *et al*., 2017). However, total protein content cannot be considered direct evidence of individual defense proteins.

Reducing sugar content showed a similar pattern, with T3 recording the highest values at both 15 and 30 DAS, followed by T2 and T1, whereas T8 recorded the lowest. Reducing sugars increased with plant age, particularly in endophyte-treated plants, suggesting enhanced availability or mobilization of soluble carbohydrates during pathogen challenge. Carbohydrates provide substrates and energy for cellular metabolism and defense-associated processes, and changes in carbohydrate metabolism are known to occur during plant responses to *R. solani*.

Phenolic accumulation was strongly enhanced in endophyte-treated plants, with T3 recording the highest total phenol content at both 15 and 30 DAS, followed by T2 and T1, while untreated plants showed considerably lower accumulation. Phenolics contribute to plant defense through antimicrobial activity and cell-wall strengthening. Enhanced phenolic accumulation in cotton following treatment with beneficial endophytic microorganisms has been associated with increased defense enzyme activities and resistance against *R. solani* (Rajendran & Samiyappan, 2008), supporting the stronger phenylpropanoid-associated defense observed in the present study.

PAL activity further supported activation of the phenylpropanoid pathway. T2 recorded the highest PAL activity at both 15 and 30 DAS, followed by T3 and T1, whereas the untreated control showed the lowest activity. PAL catalyses the entry reaction of phenylalanine into the phenylpropanoid pathway and contributes to the biosynthesis of phenolics and other defense-associated metabolites. Increased PAL activity has been associated with resistance against *R. solani,* while inhibition of PAL reduces phenolic accumulation and resistance (Taheri & Tarighi, 2011). Thus, the high PAL activity in T2 together with increased phenolic accumulation indicates enhanced phenylpropanoid-mediated defense.

Total antioxidant content was markedly higher in endophyte-treated plants, with T3 recording the highest values at both 15 and 30 DAS, followed by T1 and T2, whereas the untreated control recorded the lowest. Enhanced antioxidant capacity is important during R. solani infection because pathogen-induced ROS function in defense signaling but can cause oxidative damage when excessive. The higher antioxidant content in T3 therefore suggests improved regulation of pathogen-induced oxidative stress. Similar enhancement of antioxidant defense

following endophytic-fungus treatment against R. solani has been reported, including increased CAT, GPX, SOD and APX activities (Daroodi *et al*., 2021).

Catalase activity showed the same trend, with T3 recording the highest activity at both 15 and 30 DAS, followed by T1 and T2, while the untreated control showed much lower activity. As CAT regulates hydrogen peroxide and contributes to cellular redox homeostasis, its enhancement indicates stronger antioxidant defense during pathogen challenge. Endophytic bacterial treatment of cotton against *R. solani* has similarly been reported to enhance catalase, peroxidase and polyphenol oxidase activities and improve disease resistance (Selim *et al*., 2017).

Peroxidase activity also varied considerably among treatments. At 15 DAS, T3 showed the highest activity, followed by T1 and T2, whereas at 30 DAS T2 recorded the highest activity, followed by T1 and T3. All three endophyte treatments maintained higher peroxidase activity than the pathogen and untreated controls. Peroxidases participate in ROS regulation and oxidative polymerization associated with lignification and cell-wall strengthening. The stronger early response in T3 and later response in T2 therefore indicate temporal activation of peroxidase-mediated defense. Enhanced peroxidase activity associated with endophytic treatment and resistance to *R. solani* has also been reported in cotton (Rajendran & Samiyappan, 2008).

The biochemical responses were further supported by defense-gene expression. CHI19 showed strong induction in endophyte-treated plants challenged with *R. solani*. At 15 DAS, expression was approximately 8.5-fold in E1+P3, 16-fold in E2+P3 and 26-fold in E3+P3, compared with approximately 1.2-fold in the pathogen control. Thus, *D. eschscholtzii* produced the strongest early CHI19 response, representing more than a twenty-fold stronger expression level than the pathogen control. Chitinases contribute to antifungal defense through degradation of chitin-containing fungal structures. In cotton, *Trichoderma virens* endochitinase expression enhanced resistance to *R. solani,* accompanied by increased defense-related gene expression, ROS production and pathogenesis-related enzyme activity (Kumar *et al*., 2009). The strong CHI19 induction in E3+P3 therefore indicates rapid activation of chitinase-associated antifungal defense.

GLU42 showed a similarly strong early response. At 15 DAS, expression was approximately 0.7-fold in the pathogen control, 1.0-fold in E1+P3, 2.8-fold in E2+P3 and 9.0-fold in E3+P3, with T3 showing the strongest induction. At 30 DAS, expression declined but remained higher in endophyte-treated plants than in the pathogen control, with approximately 1.4-fold in E1+P3, 1.2-fold in E2+P3 and 1.3–1.4-fold in E3+P3. The strong early GLU42 induction, particularly in T3, suggests rapid activation of antifungal defense. Enhanced β-1,3-glucanase activity has been reported in cotton treated with endophytic bacteria against *R. solani*, together with increased chitinase, peroxidase, PAL and phenolic responses (Rajendran & Samiyappan, 2008). Increased β-1,3-glucanase expression has also been associated with reduced R. solani disease development in transgenic rice (Pathania *et al.,* 2022).

Overall, seed biopriming with the three fungal endophytes enhanced multiple defense-associated responses of cotton against *R. solani. D. eschscholtzii* (T3) showed the most consistent and pronounced response, recording the highest protein, reducing sugar, phenol, total antioxidant and catalase contents, the highest early peroxidase activity, and the strongest early induction of CHI19 and GLU42. *T. sulphureum* (T2) showed particularly strong PAL activity and the highest peroxidase activity at 30 DAS, whereas *D. longicolla* (T1) consistently maintained

enhanced defense responses compared with the pathogen and untreated controls. Thus, the endophytes stimulated a coordinated defense response involving phenylpropanoid metabolism, phenolic accumulation, antioxidant regulation, ROS-associated enzymes and antifungal defense genes. This coordinated response is consistent with induced systemic resistance and defense priming, whereby beneficial microorganisms enhance plant readiness for subsequent pathogen attack (Pieterse *et al.,* 2014). The superior biochemical and early molecular responses of T3 suggest that *D. eschscholtzii* was the most effective of the tested endophytes in activating defense mechanisms against *R. solani* in cotton.

Conclusion

This study shows that fungal endophyte-mediated seed biopriming can effectively boost cotton defence against *R. solani* by coordinating biochemical and molecular defence responses. The increased accumulation of phenolics, antioxidants, and defense-related enzymes, together with the significant induction of CHI19 and GLU42, indicates the activation of complementary host defence pathways. *D. eschscholtzii* had the most prominent overall defence response of the tested endophytes, making it a promising bioagent for further development as a biopriming-based method for long-term management of *R. solani* in cotton.

Statements and Declarations:

Acknowledgements

This research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India. The authors are thankful to the Director, ICAR-Central Institute for Cotton Research, Nagpur for his support. The first author is thankful to ICAR, New Delhi and ICAR-IARI, New Delhi for the fellowship.

Funding

This work was supported by ICAR-Central Institute for Cotton Research and ICAR-IARI Academic Hub, Nagpur, India.

Author Contribution:

BSV: Investigation, Methodology, Validation, writing – original draft; NSH: Conceptualization, Data curation, Project administration, Resources, Writing – review & editing; PV: Methodology, Validation, Data curation, Formal analysis; JD: Methodology, Validation, Software, Formal analysis; DTN: Resources, Writing – review & editing; SPG: Project administration, Resources; SHT: Resources, Writing – review & editing; SSK: Formal analysis, writing draft; MDM: Assistance during investigation, Methodology; GTB: Funding acquisition, Project administration.

Data Availability: The datasets generated during and/or analysed during the current study are provided in the manuscript.

Competing interests: The authors declare that there are no known competing interests in the publication

Ethics approval and consent to participate: Not applicable

Consent for publication: Obtained from all the authors

Disclosure of potential conflict of interest: No potential conflict of interest

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Figure legend:

Fig 1. Effect of endophyte bio-priming on total soluble protein content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 2. Effect of endophyte bio-priming on total sugar content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 3. Effect of endophyte bio-priming on total reducing sugar content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 4. Effect of endophyte bio-priming on total phenol content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 5. Effect of endophyte bio-priming on total antioxidant content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 6. Effect of endophyte bio-priming on total PAL content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 7. Effect of endophyte bio-priming on total catalase content in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* +

untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 8. Effect of endophyte bio-priming on total peroxidase in cotton seedlings at 15 and 30 DAS against challenge inoculation with *Rhizoctonia solani*. Where, T1= (*D. longicolla* + *R. solani)*, T2= (*T. sulphureum* + *R. solani)*, T3= (*D. eschscholtzii* + *R. solani)*, T4= (*D. longicolla* + untreated soil), T5= (*T. sulphureum* + untreated soil), T6= (*D. eschscholtzii* + untreated soil), T7= Positive control, T8= Negative control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 9. Relative expression of the CHI19 (chitinase) gene in cotton under selective endophytic fungal-pathogen treatments at 15 and 30 DAS. E1: *D.longicolla*, E2: *T.sulphureum*, E3: *D. eschscholtzii,* P1: *R. solani*, Untreated: Control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 10. Relative expression of the GLU-42 (glucanase) gene in cotton under selective endophytic fungal-pathogen treatments at 15 and 30 DAS. E1: *D.longicolla*, E2: *T.sulphureum*, E3: *D. eschscholtzii,* P1: *R. solani*, Untreated: Control. Different letters above the error bars indicate statistically significant difference (P≤0.05).

Fig 1.

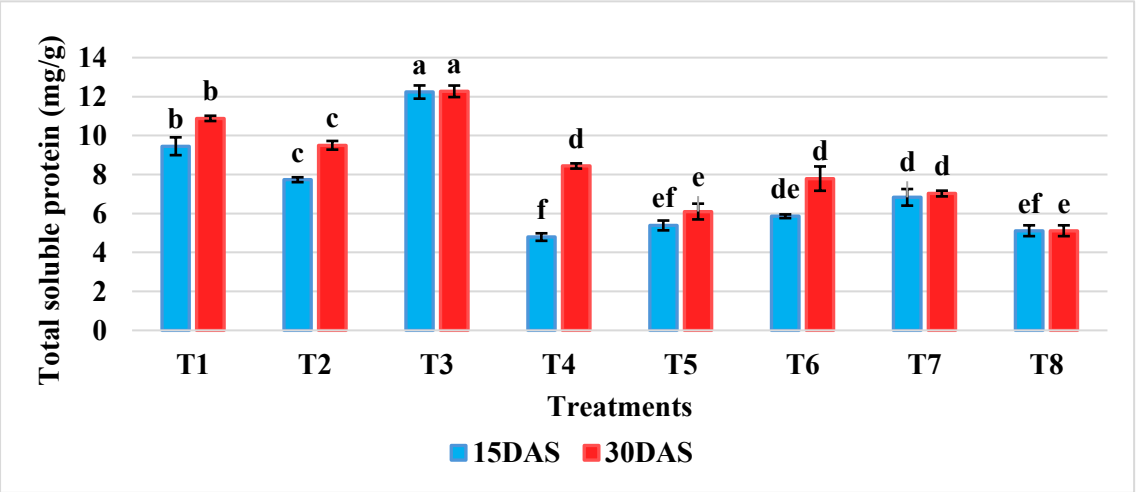
Total soluble protein (mg/g)
14
12
10
8
6
4
2
0
b
b
c
c
a
a
f
d
ef
e
de
d
d
d
ef
e
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15DAS
30DAS

Fig 2.

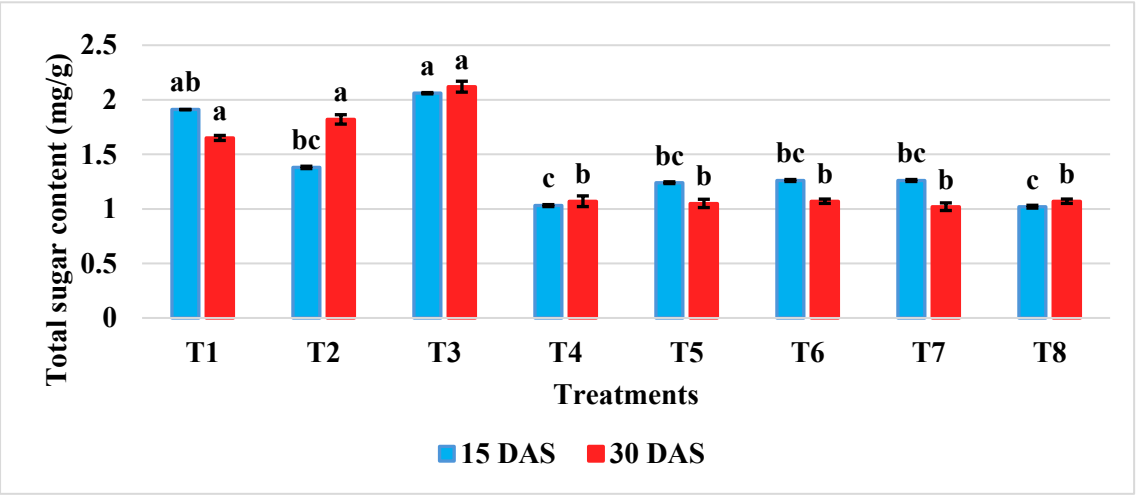
Total sugar content (mg/g)
2.5
2
1.5
1
0.5
0
ab
a
bc
a
a
a
c
b
bc
b
bc
b
bc
b
c
b
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15 DAS
30 DAS

Fig 3.

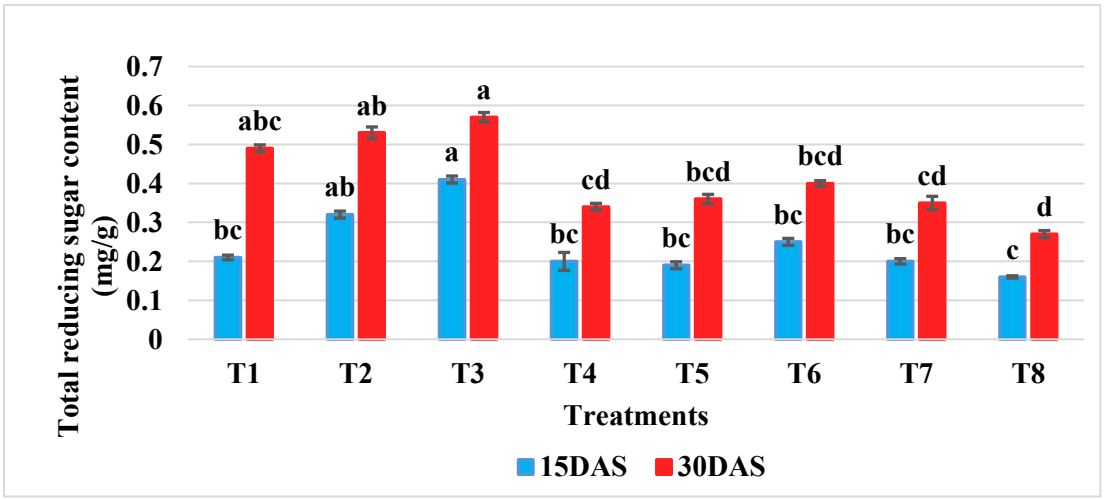
Total reducing sugar content (mg/g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
bc
abc
ab
ab
a
a
bc
cd
bc
bcd
bc
bcd
bc
cd
c
d
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15DAS
30DAS

Fig 4.

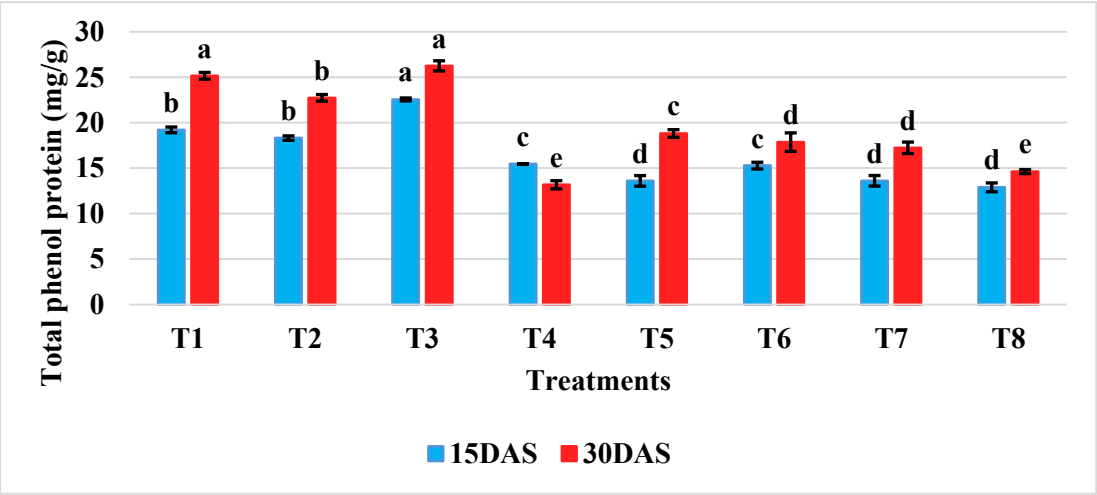
Total phenol protein (mg/g)
30
25
20
15
10
5
0
b
a
b
b
a
a
c
e
d
c
c
d
d
d
d
e
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15DAS
30DAS

Fig 5.

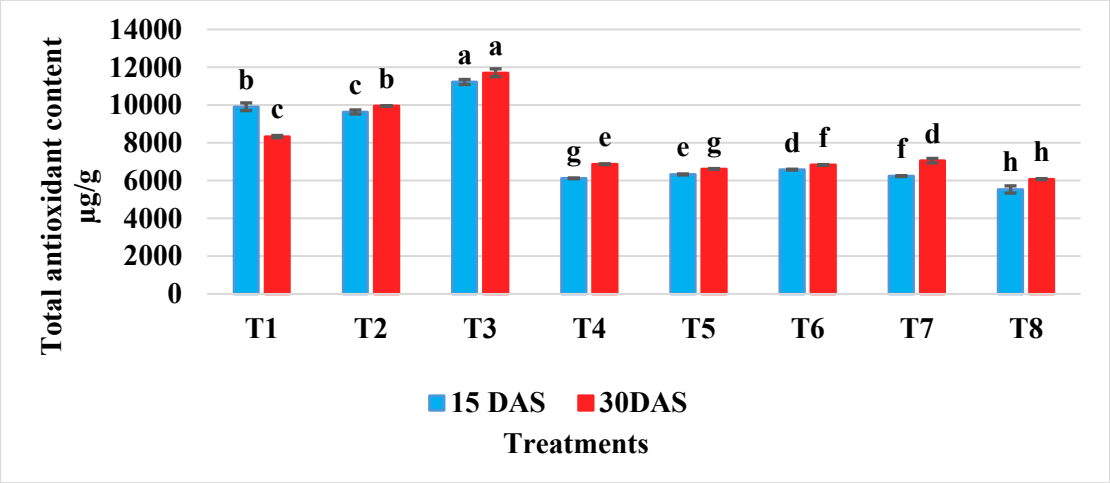
Total antioxidant content µg/g
14000
12000
10000
8000
6000
4000
2000
0
b c
c b
a a
g e
e g
d f
f d
h h
T1
T2
T3
T4
T5
T6
T7
T8
15 DAS
30DAS
Treatments

Fig 6.

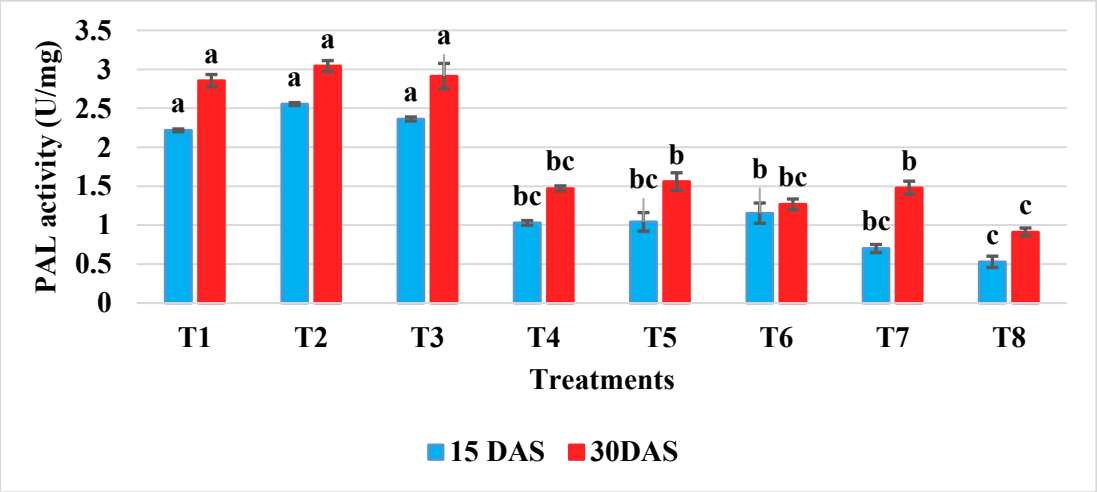
PAL activity (U/mg)
3.5
3
2.5
2
1.5
1
0.5
0
a
a
a
a
a
a
bc
bc
bc
b
b
bc
bc
b
c
c
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15 DAS
30DAS

Fig 7.

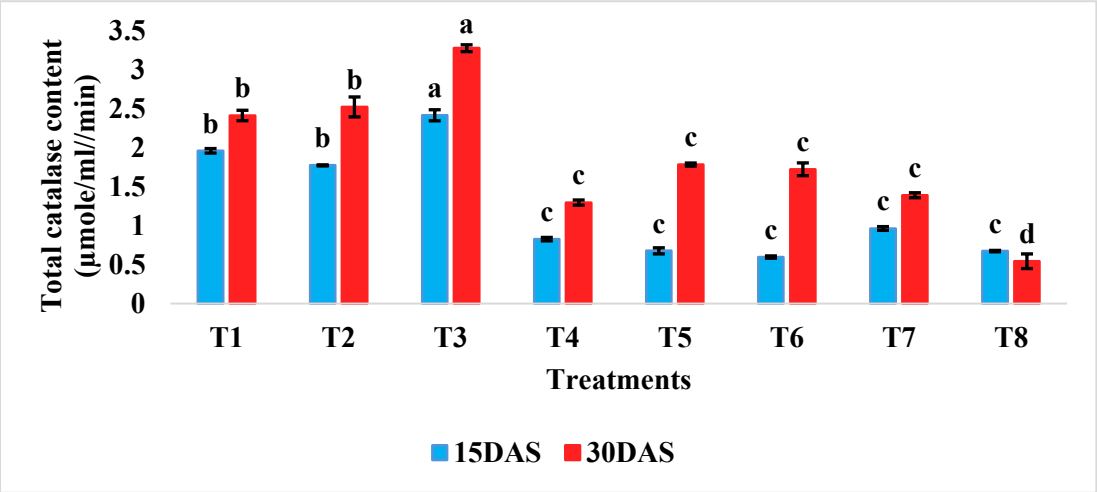
Total catalase content (µmole/ml//min)
3.5
3
2.5
2
1.5
1
0.5
0
b
b
b
b
a
a
c
c
c
c
c
c
c
c
c
d
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15DAS
30DAS

Fig 8.

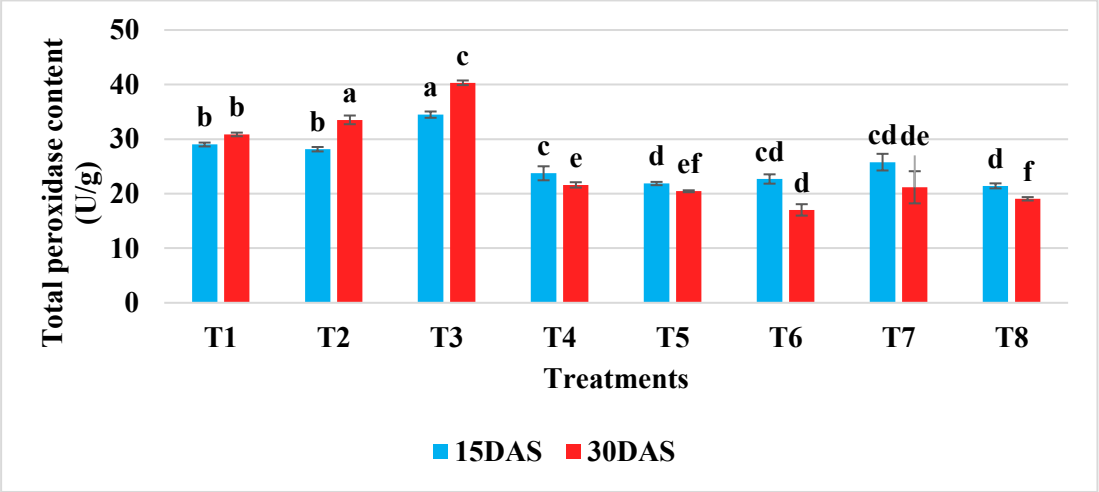
Total peroxidase content (U/g)
50
40
30
20
10
0
b b
b a
a c
c e
d ef
cd d
cd de
d f
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
15DAS
30DAS

Fig 9.

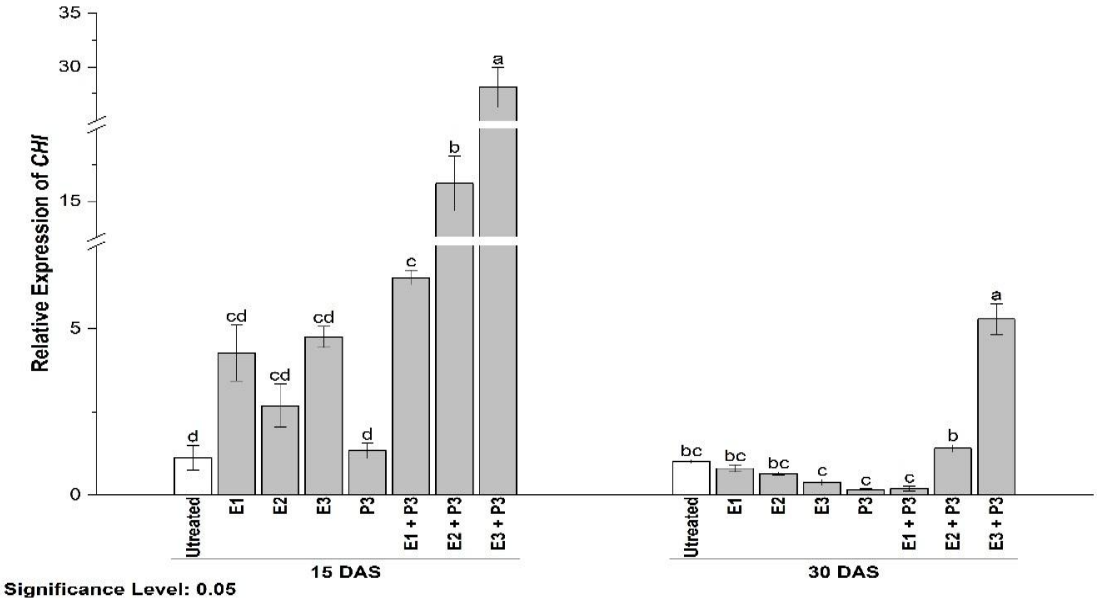
Relative Expression of CHI
35
30
15
5
0
d
cd
cd
cd
d
c
b
a
bc
bc
bc
c
c
c
b
a
Utreated
E1
E2
E3
P3
E1 + P3
E2 + P3
E3 + P3
15 DAS
30 DAS
Significance Level: 0.05

Fig 10.

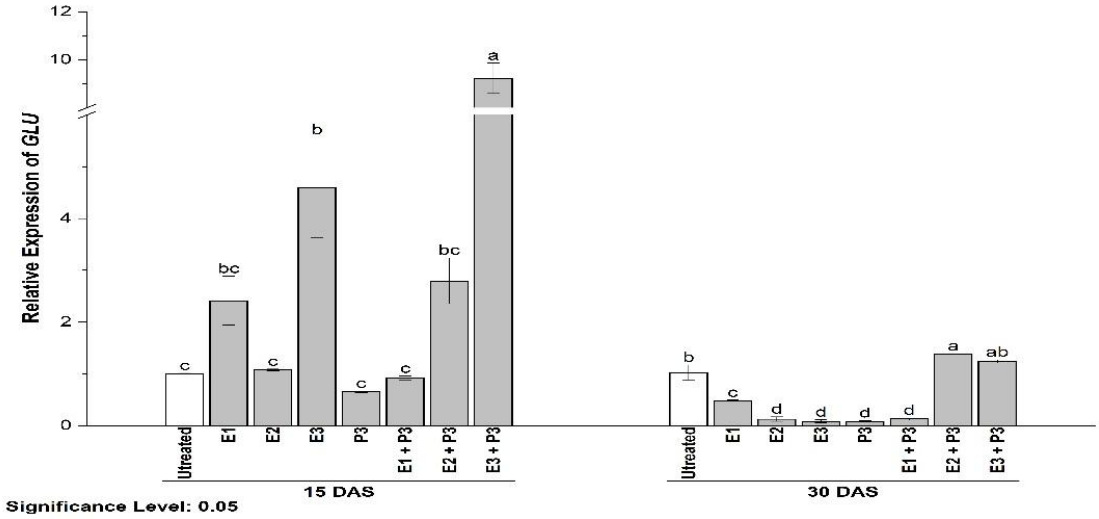
Relative Expression of GLU
12
10
4
2
0
c
bc
c
b
c
c
bc
a
b
c
d
d
d
d
a
ab
Utreated
E1
E2
E3
P3
E1 + P3
E2 + P3
E3 + P3
15 DAS
30 DAS
Significance Level: 0.05

Table 1.0. Selected fungal endophytes of cotton and their GenBank accession numbers

Isolate No.	**Identity**	**Source**	**Accession No.**
FEC-9L	*Diaporthe longicolla*	Cotton leaf	PZ653129
FEC-21L	*Trichoderma sulphureum*	Cotton leaf	PZ832357
FEC-68R	*Daldinia eschscholtzii*	Cotton root	PZ827179

Table 2.0. Pathogenesis-Related genes targeted for gene expression analysis by qRT-PCR

S.No.	Genes	Primer Sequence
1.	Endo 1,3-β- glucanases *qGh GLU42*	5'- GAGTTAGAGAGACACTTTGGGTTG -3' F 5'- AAAAAGACCAAATTTCCCAGGCTT -3'R
2.	Chitinase *q GhCHI 19*	5'- GGATTCGTGGCTATTTCTTT-3' F 5'- GAACTACTACTACTGCTACTTG-3'R
3.	*q GhACTIN 4*	5'- TTGCAGACCGTATGAGCAAG-3' F 5'- ATCCTCCGATCCAGACACTG-3' R

Table 3.0. Experimental treatments for evaluating endophyte-mediated defense against *R. solani* in cotton.

S.No.	Treatment No.	Treatments
1.	T1	Seed bioprimed with *D. longicolla* + *R. solani*
2	T2	Seed bioprimed with *T. sulphureum* + *R. solani*
3.	T3	Seed bioprimed with *D. eschscholtzii* + *R. solani*
4.	T4	Seed bioprimed with *D. longicolla* + untreated soil
5.	T5	Seed bioprimed with *T. sulphureum* + untreated soil
6.	T6	Seed bioprimed with *D. eschscholtzii* + untreated soil
7.	T7	Positive control (inoculated with *R. solani* alone)
8.	T8	Negative control (untreated seed in untreated soil)

Table 4.0. Effect of endophyte biopriming on protein accumulation in cotton seedlings challenge inoculated with *R. solani* at 15 and 30DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla* + *R. solani*	9.452 ± 0.458	10.843 ± 0.135
T2	*T. sulphureum* + *R. solani*	7.733 ± 0.125	9.800 ± 0.223
T3	*D. eschscholtzii* + *R. solani*	12.233 ± 0.339	12.253 ± 0.296
T4	*D.longicolla* + Untreated soil	4.790 ± 0.193	8.438 ± 0.134
T5	*T. sulphureum* + Untreated soil	5.386 ± 0.252	5.900 ± 0.405
T6	*D. eschscholtzii* + Untreated soil	5.857 ± 0.095	7.791 ± 0.624
T7	Untreated soil + *R. solani* inoculated soil	6.395 ± 0.426	7.754 ± 0.147
T8	Untreated seed in untreated soil	5.114 ± 0.278	5.115 ± 0.278
	C.D.	0.898	1.028
	SE(m)	0.293	0.336
	SE(d)	0.415	0.474
	C.V.	7.135	6.848

Table 5.0. Effect of endophyte biopriming on sugar accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla*+ *R. solani*	1.91 ± 0.002	1.653 ± 0.023
T2	*T. sulphureum*+ *R. solani*	1.38 ± 0.011	1.823 ± 0.043
T3	*D. eschscholtzii* + *R. solani*	2.06 ± 0.006	2.007 ± 0.050
T4	*D.longicolla*+ Untreated soil	1.03 ± 0.009	1.067 ± 0.049
T5	*T. sulphureum*+ Untreated soil	1.24 ± 0.008	1.053 ± 0.038
T6	*D. eschscholtzii* + Untreated soil	1.26 ± 0.009	1.073 ± 0.020
T7	Untreated soil + *R. solani* innoculated soil	1.26 ± 0.009	1.017 ± 0.035
T8	Untreated seed in untreated soil	1.02 ± 0.013	0.867 ± 0.020
	C.D.	0.025	0.117
	SE(m)	0.008	0.038
	SE(d)	0.011	0.054
	C.V.	4.173	4.995

Table 6.0. Effect of endophyte biopriming on reducing sugars accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ± S. E	**Mean ± S. E**
T1	*D.longicolla*+ *R. solani*	0.210 ± 0.006	0.483 ± 0.009
T2	*T. sulphureum*+ *R. solani*	0.317 ± 0.009	0.527 ± 0.015
T3	*D. eschscholtzii* + *R. solani*	0.413 ± 0.009	0.570 ± 0.012
T4	*D.longicolla*+ Untreated soil	0.200 ± 0.023	0.343 ± 0.009
T5	*T. sulphureum*+ Untreated soil	0.193 ± 0.009	0.360 ± 0.012
T6	*D. eschscholtzii* + Untreated soil	0.253 ± 0.009	0.397 ± 0.007
T7	Untreated soil + *R. solani* innoculated soil	0.197 ± 0.007	0.350 ± 0.017
T8	Untreated seed in untreated soil	0.157 ± 0.003	0.273 ± 0.009
	C.D.	0.035	0.043
	SE(m)	0.011	0.014
	SE(d)	0.016	0.02
	C.V.	6.323	4.574

Table 7.0. Effect of endophyte biopriming on phenol accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla*+ *R. solani*	19.213 ± 0.315	25.160 ± 0.370
T2	*T. sulphureum*+ *R. solani*	18.307 ± 0.243	22.733 ± 0.368
T3	*D. eschscholtzii* + *R. solani*	22.560 ± 0.160	26.253 ± 0.559
T4	*D.longicolla*+ Untreated soil	15.453 ± 0.035	13.173 ± 0.449
T5	*T. sulphureum*+ Untreated soil	13.600 ± 0.583	18.813 ± 0.424
T6	*D. eschscholtzii* + Untreated soil	15.280 ± 0.372	17.267 ± 1.018
T7	Untreated soil + *R. solani* innoculated soil	13.613 ± 0.581	17.240 ± 0.633
T8	Untreated seed in untreated soil	12.893 ± 0.486	14.627 ± 0.218
	C.D.	1.079	1.744
	SE(m)	0.352	0.569
	SE(d)	0.498	0.805
	C.V.	3.727	5.081

Table 8.0. Effect of endophyte biopriming on antioxidant accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla*+ *R. solani*	9.910 ± 0.203	8.329 ± 0.059
T2	*T. sulphureum*+ *R. solani*	9.633 ± 0.109	9.950 ± 0.017
T3	*D. eschscholtzii* + *R. solani*	11.220 ± 0.134	11.706 ± 0.209
T4	*D.longicolla*+ Untreated soil	6.121 ± 0.021	6.867 ± 0.019
T5	*T. sulphureum*+ Untreated soil	6.326 ± 0.027	6.620 ± 0.018
T6	*D. eschscholtzii* + Untreated soil	6.580 ± 0.024	6.828 ± 0.018
T7	Untreated soil + *R. solani* innoculated soil	6.236 ± 0.022	7.055 ± 0.118
T8	Untreated seed in untreated soil	5.531 ± 0.192	6.076 ± 0.028
	C.D.	0.352	0.266
	SE(m)	0.117	0.089
	SE(d)	0.166	0.126
	C.V.	2.641	1.942

Table 9.0. Effect of endophyte biopriming on PAL accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla*+ *R. solani*	2.216 ± 0.018	2.854 ± 0.079
T2	*T. sulphureum*+ *R. solani*	2.554 ± 0.018	3.042 ± 0.070
T3	*D. eschscholtzii* + *R. solani*	2.363 ± 0.026	2.913 ± 0.163
T4	*D.longicolla*+ Untreated soil	1.029 ± 0.030	1.325 ± 0.033
T5	*T. sulphureum*+ Untreated soil	1.042 ± 0.119	1.558 ± 0.114
T6	*D. eschscholtzii* + Untreated soil	1.154 ± 0.130	1.175 ± 0.069
T7	Untreated soil + *R. solani* innoculated soil	0.700 ± 0.052	1.479 ± 0.085
T8	Untreated seed in untreated soil	0.529 ± 0.073	0.783 ± 0.051
	C.D.	0.218	0.272
	SE(m)	0.071	0.089
	SE(d)	0.101	0.125
	C.V.	8.509	8.125

Table 10.0. Effect of endophyte biopriming on catalase accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	Treatment	15DAS	30 DAS
		Mean ±S. E	Mean ±S. E
T1	*D.longicolla*+ *R. solani*	1.960 ± 0.029	2.413 ± 0.067
T2	*T. sulphureum*+ *R. solani*	1.773 ± 0.007	2.523 ± 0.128
T3	*D. eschscholtzii* + *R. solani*	2.417 ± 0.071	3.277 ± 0.044
T4	*D.longicolla*+ Untreated soil	0.853 ± 0.022	1.297 ± 0.033
T5	*T. sulphureum*+ Untreated soil	0.707 ± 0.039	1.783 ± 0.020
T6	*D. eschscholtzii* + Untreated soil	0.610 ± 0.015	1.723 ± 0.082
T7	Untreated soil + *R. solani* innoculated soil	0.963 ± 0.024	1.390 ± 0.032
T8	Untreated seed in untreated soil	0.673 ± 0.009	0.543 ± 0.094
	C.D.	0.104	0.225
	SE(m)	0.034	0.073
	SE(d)	0.048	0.104
	C.V.	4.731	6.794

Table 11.0. Effect of endophyte biopriming on peroxidase accumulation in cotton seedlings challenge innoculated with *R. solani* at 15 and 30 DAS

Treatment No.	**Treatment**	**15DAS**	**30 DAS**
		Mean ±S. E	**Mean ±S. E**
T1	*D.longicolla*+ *R. solani*	29.013 ± 0.353	30.858 ± 0.334
T2	*T. sulphureum*+ *R. solani*	28.150 ± 0.406	33.529 ± 0.798
T3	*D. eschscholtzii* + *R. solani*	34.488 ± 0.577	28.218 ± 0.426
T4	*D.longicolla*+ Untreated soil	23.738 ± 1.285	19.396 ± 0.488
T5	*T. sulphureum*+ Untreated soil	21.858 ± 0.301	19.058 ± 0.176
T6	*D. eschscholtzii* + Untreated soil	22.692 ± 0.852	21.608 ± 1.048
T7	Untreated soil + *R. solani* innoculated soil	22.963 ± 1.524	20.446 ± 2.945
T8	Untreated seed in untreated soil	21.433 ± 0.457	17.025 ± 0.310
	C.D.	2.284	3.467
	SE(m)	0.746	1.132
	SE(d)	1.055	1.601
	C.V.	5.057	8.249

Table 12.0. Experimental treatment combinations used for q-PCR analysis of chitinase and β-1,3-glucanase gene expression in cotton against *R. solani*

S.No.	Treatment	Treatment name
1.	Untreated	Untreated seed in untreated soil
2.	E1	*D.longicolla*+Untreated soil
3.	E2	*T. sulphureum* +Untreated soil
4.	E3	*D. eschscholtzii* + Untreated soil
5.	P3	Untreated soil + *R. solani* innoculated soil
6.	E1+P3	*D.longicolla*+ *R. solani*
7.	E2+P3	*T. sulphureum* + *R. solani*
8.	E3+P3	*D. eschscholtzii* + *R. solani*

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