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Biocontrol potential of native *Trichoderma* spp. against anthracnose of great millet (*Sorghum bicolour* L.) from Tarai and hill regions of India

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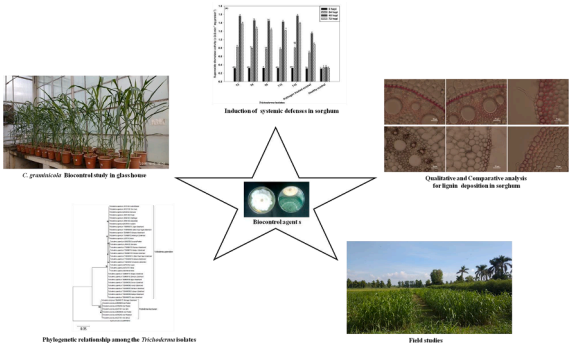
HIGHLIGHTS

- *T. asperellum* is an effective biocontrol agent against *Colletotrichum graminicola.*
- *T. asperellum* reduces anthracnose disease in sorghum crops under glass house and field condition.
- *T. asperellum* triggers systemic defenses against *Colletotrichum graminicola* in sorghum plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Sorghum anthracnose, caused by *Colletotrichum graminicola*, is a destructive disease, and increasing dependency on chemical fungicides for its control has serious environmental concerns since sorghum is fed to cattle. Thus there is a need to develop effective bio-pesticide for biological control of *C. graminicola*. Since *Trichoderma* is a proven biocontrol agent against plant pathogens, exploring the greater diversity that exists in *Trichoderma,* could be of notable economic significance in terms of disease control. To harness the hidden potential of *Trichoderma* strains against *C. graminicola*, a study was undertaken with 20 *Trichoderma* spp. isolated from 40 rhizospheric soil samples. Dual plate antagonism assay indicated the potential of T3, T4, T6, T15, and T19 isolates of *Trichoderma* against *C. graminicola*, with T3 isolate showing maximum (76.47%) mycelial growth inhibition. Molecular characterization based on the sequence analysis of ITS-rRNA and *tef-1α* genes identified these isolates as *Trichoderma asperellum* and *Trichoderma harzianum.* Under the glasshouse condition, biopriming of seed with *Trichoderma* spp. had significantly decreased the percent disease index to 32.92% and helped improve plant growth-promoting attributes compared to untreated control. Seed biopriming with T3 isolate exhibited higher antioxidant enzyme activities in terms of superoxide dismutase (36.63%), peroxidase (43.59%), and polyphenol oxidase

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(40.96%) at 48 h after pathogen inoculation. In most treatments, lignifications were highest in the epidermis, endodermis protophloem, metaphloem, pericycle, and protoxylem of sorghum roots after the 15th-day pathogen inoculation, indicating the strengthening of defense mechanism. To further evaluate, a field experiment was conducted for two consecutive years (Kharif season of 2018 and 2019) to test the best five isolates (T3, T4, T6, T15, and T19) against sorghum anthracnose. Lowest percent disease index of 54.90% and 53.68% and AUDPC value of 740.87 and 751.67 was reported from *T. asperellum* T3 bioprimed plants in the year 2018 and 2019, respectively, at 75 days after sowing. *T. asperellum* T3 isolate showed a significant increase in sorghum yield up to 22.22% and 27.29% higher than untreated control during the 2018 and 2019 Kharif season, respectively. This study indicated that *T. asperellum* T3 could be a potential biocontrol agent for managing the anthracnose of sorghum.

1. Introduction

Sorghum (*Sorghum bicolour* L. Moench) is grown over 90 countries and ranks as the 5th most staple cereal crop worldwide (Vasanthakumari and Shivanna, 2014; Rincent et al., 2017; Ajeigbe et al., 2018; Mundia et al., 2019). India is the 5thlargest producer of sorghum with a growing area of 11 million hectares (Mundia et al., 2019). While sorghum may withstand harsh conditions, its productivity is affected by a wide range of biotic and abiotic stresses resulted in annual yield losses of >70 percent (Ngugi et al., 2002; Woldesemayat and Ntwasa, 2018).

Sorghum is susceptible to foliar diseases widespread across all sorghum-growing regions worldwide. Sorghum anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wilson is the important foliar disease of sorghum and widespread in South America, America, Pakistan, China, India, and eastern Africa, causing a significant loss in productivity (Valério et al., 2005; Thakur, 2007; Bunker et al., 2019). In severe anthracnose epiphytotic symptoms, yield reductions were estimated up to 50% on susceptible cultivars (Gwary et al., 2002; Ngugi et al., 2002).

As sorghum is a significant fodder crop and is fed to the cattle, it is essential to avoid chemicals to control this destructive disease. The development of resistant varieties is the cheapest and feasible method for managing plant diseases. Still, due to the emergence of more virulent races of pathogens, the resistance breaks down (Ali and Warren, 1992; Casela and Ferreira, 1995; Bressan and Figueiredo, 2003; Valério et al., 2005). Therefore, increased emphasis on the use of biological control for managing the diseases of plants is justified. Consequently, the utilization of bacterial and fungal biocontrol agents that are eco-friendly and economical can be relied upon to offset the issues mentioned above (Keswani et al., 2014, 2019b; Singh et al., 2019). *Trichoderma* spp. is the most explored and investigated fungal biocontrol agent accounting for more than 60% of the registered biofungicides (Keswani et al., 2013). *Trichoderma* plays a vital role in managing plant diseases (Ramírez-Valdespino et al., 2019) along with growth-promotion.

These fungi protect the plant from harmful plant pathogens by various mechanisms such as inducing resistance (Gajera et al., 2013; Keswani et al., 2014), mycoparasitism (Vinale et al., 2008; Wisecaver et al., 2014;), antibiosis by secondary metabolites (Keswani et al., 2019b) enzymes (Elad, 2000), competition (Verma et al., 2007; Singh et al., 2019), volatile secondary metabolites (Benítez et al., 2004; Keswani et al., 2014, 2019b).

Based on DNA and phylogenetic studies' sequence analysis, 290 species of *Trichoderma* have been identified and characterized (du Plessis et al., 2018). Since *Trichoderma* spp. are highly diverse, molecular studies based on single gene sequences such as the ITS region are insufficient to determine identity and phylogeny (Kullnig-Gradinger et al., 2002). Several studies showed that the *tef-1α* gene has enough variability of sequences within the fungal species and among the fungal genera and used an efficient genetic, molecular marker for phylogenetic studies in fungal taxonomy (Alhawatema et al., 2019). Also, the *tef-1α* gene sequence has been used to evaluate the relatedness within 160 strains of dermatophyte species, indicating that *tef-1α* is a candidate for DNA barcoding to distinguish species' isolates within a genus (Mirhendi et al., 2015). Therefore, for precise molecular identification, the sequence analysis of ITS1 and ITS2 region and the *tef-1α* gene sequence analysis is a promising tool for phylogenetic studies (Samuels et al., 2002; Kubicek et al., 2008; Sun et al., 2016).

Considering the importance of sorghum as important grain and fodder crop in the national economy and the destructive nature of anthracnose of sorghum and less availability of resistant sources and lack of information about the biocontrol of sorghum anthracnose and defense mediated induction by *Trichoderma* spp. against *C. graminicola* in sorghum crops are less available. *Trichoderma* antagonistic ability and ecology are greatly affected by the texture and properties of soil and various ecological zones (Zachow et al., 2009; Körmöczi et al., 2013). Uttarakhand has been one of India's northwestern states, consisting of valleys and hill districts with subtropical monsoon climate. The Uttarakhand's Tarai and hill region (Udham Singh Nagar, Haridwar, Dehradun, and Nainital) geo-climate situations are responsible for creating different niches for various forms of life (Sati, 2012). A few studies have been conducted to reveal the factors responsible for the abundance, diversity, biocontrol activity, and growth of native *Trichoderma* species in the hilly region (Tomah et al., 2020).

The biocontrol mechanisms and genetic diversity need to be studied from the less-exploited locations. Studies on the mechanisms involved in the activation of plant defense responses induced by *Trichoderma asperellum* and *Trichoderma harzianum* against *Colletotrichum graminicola* in sorghum plants are not available. There is less evidence of the impact of *Trichoderma asperellum* and *Trichoderma harzianum* on the management of anthracnose of sorghum and grain yield in field trials subtropical agro-ecosystems. Hence, the current experiment was performed to establish the *Trichoderma* species diversity in Uttrakhand soils and to evaluate the antagonistic potential of the most effective *Trichoderma* species isolates for the management sorghum anthracnose under glasshouse and field condition.

To achieve this aim, we screened different *Trichoderma* isolates from sorghum rhizospheric soil collected from different Uttarakhand regions to understand the biocontrol-plant-pathogen tripartite interaction (*Trichoderma* spp.-*Sorghum bicolor*-*Colletotrichum graminicola*) with objectives: i) molecular characterization of *Trichoderma* isolates based on the ITS1 and ITS4 and *tef-1α* gene sequences; ii) *In vitro* screening of *Trichoderma* isolates for their antagonistic potential against *C. graminicola*; iii) Effect of *Trichoderma* isolate on disease incidence, growth parameters, and yield components, and defense enzymatic activities in sorghum plants grown under glasshouse condition; iv) Effect of *Trichoderma* isolate on disease severity, growth parameters and yield components in sorghum plants grown under field conditions for two consecutive years. The data will allow the potent *Trichoderma* isolates to develop an active biocontrol agent to manage anthracnose of sorghum.

2. Materials and methods

2.1. Fungal strains and chemicals

The 40 samples of rhizospheric soil from healthy sorghum plants from various regions of Uttarakhand were collected. Out of total isolations, 20 isolates were identified as *Trichoderma* is based on morpho-cultural characteristics. All these isolates were maintained on Potato Dextrose Agar medium (Hi-Media M096) slants and stored at 4 °C for

further investigation. Molecular biology grade chemicals were obtained from Genetix Biotech Asia Pvt. Ltd., and authentic standards of enzymes and analytical grade chemicals, and solvents have been from Sigma–Aldrich, St. Louis, USA, and Merck, Mumbai, India, respectively.

Here, the diseased samples of sorghum showing anthracnose symptoms were obtained from sorghum pathology block, G.B. Pant University at Agriculture and Technology, Pantnagar, Uttarakhand, latitude, and longitude (29.00N, 79.48E) respectively. The diseased leaf samples were taken to the sorghum laboratory for confirmation of pathogen by microscopic examination. Isolation of *C. graminicola* (C1) from infected leaves of sorghum crop showing anthracnose symptoms was done using the tissue isolation method as per given by Valério et al. (2005). Pure culture of *C. graminicola* was maintained on potato dextrose agar (PDA) medium at room temperature (27 ± 1 °C). Molecular identification of *Colletotrichum graminicola* using the ITS region was done as per the method described by Photita et al. (2005). The ITS sequence was submitted to NCBI, and the accession number of the C1 isolate of *C. graminicola* was MT742843.

2.2. Molecular characterization of Trichoderma isolates

The *Trichoderma* isolates were cultured on potato dextrose agar medium for 4 days at 28 °C for DNA isolation. After incubation, 3 to 4 mycelial plugs of bioagent were inoculated in 250 mL conical flask containing 100 mL PD broth and incubated in a shaker (150 rpm) at 28 °C. After 6 days of growth, the mycelial mat was filtered through Whatman filter paper 42. It was washed by centrifugation at 10000 rpm at 4 °C for 20 min with sterile distilled water and then powdered in a pestle mortar with liquid nitrogen.

2.2.1. DNA extraction by CTAB method

The genomic DNA was extracted from 1 g of ground mycelia of *Trichoderma* isolates using the CTAB method given by Doyle and Doyle (1990). Quantification and assessment of the quality of genomic DNA were checked using spectrophotometer by recording absorbance at 245 nm.

2.2.2. Amplification and purification of PCR products

The 100 ng of genomic DNA was used for amplification and sequencing of the *Trichoderma* isolates, using the universal primer ITS1 (5′TCCGTAGGTGAACCTGCGG 3′) and ITS4 (3′TCCTCCGCTTAT TGATATGC5′) (White et al., 1990) and *tef-1α* genes specific forward primer EF1728F (5′CATCGAGAAGTTCGAGAAGG3′) and reverse primer EF1986R (5′TACTTGAAGGAACCCTTACCGG3′) (Carbone and Kohn, 1999).

PCR was performed in 25 µl volume containing 1X Taq Buffer with KCl (2.5 µl), 200 µM dNTP (0.5 µl), 1.5 mM $MgCl_2$ (1.5 µl), 10 pM forward primer (1 µl), 10 pM reverse primer (1 µl), 1U/reaction Taq polymerase (0.2 µl) and 1X nuclease-free water (17.3 µl) (Genetix brand) and 100 ng genomic DNA (1 µl). ITS region of *Trichoderma* isolates was amplified under conditions: 95 °C for initial denaturation for 2 min followed by 40 denaturation cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min followed by a final extension at 72 °C for 10 min. Amplified products (10 µl) were separated from 1.2% agarose gel containing ethidium bromide (0.5 µg/mL) by gel electrophoresis performed at 2 V cm^{-1} of the gel length until the bands were resolved and visualized, documented, and photographed in the gel-documentation unit (Bio-Rad, Philadelphia, PA, United States). Gel extraction kit (Sure extract spin PCR clean/ Gel extraction kit, Genetix brand) was used to purify both regions (ITS1 and ITS4), and *tef-1α* amplified PCR products.

2.2.3. Phylogenetic analysis

All amplicons were bidirectionally sequenced through the Sanger sequencing method (Eurofins Pvt. Ltd) to get the maximum length. The sequences were analyzed and assembled by using Bio Edit Version7.0.5. Multiple sequence alignment was done through CLUSTAL W (Thompson et al., 1994) using MEGA6 software for all the sequences of ITS1 and ITS4 and *tef-1α* regions.

The aligned ITS1 and ITS4 gene sequences of all the *Trichoderma* isolates used for BLAST interface analysis in TrichoOKey (http://www.isth.info) and NCBI database (http://blast.ncbi.nlm.gov) and Tricho-BLAST program for *tef-1α* gene sequences and the similar match was confirmed through identity percentage. After identification, the aligned sequences were submitted to NCBI GenBank and DDBJ database Japan to obtain the accession number for all the sequences of the 20 *Trichoderma* isolates of ITS (1 and 4), and *tef-1α* regions. Using the Maximum Likelihood method, the molecular phylogenetic analysis was determined. The evolutionary history or consensus tree was inferred using Mega6.06 software using the Maximum Likelihood test with Tamura Nei Model (Tamura et al., 2013). Nodal robustness was tested using the bootstrap method, and the phylogenetic robustness was concluded with a replication of 1000. The Cladogram was designed to explain the relationship between the isolates and the various *Trichoderma* species under study.

The gene sequences were submitted to NCBI GenBank and given accession number was obtained of 20 *Trichoderma* isolates for ITS1 and ITS4 sequences, and their geographical coordinates for *T. asperellum*-T1-MH809172 (30.44N, 77.73E), T2-MH809173 (30.41N, 77.78E), T3-MH809174 (29.22N, 78.92E), T4-MH809175 (28.92N, 78.92E), T5-MH809176 (29.99N, 78.20E), T7-MH809178 (30.08N, 78.17E), T8-MH809179 (28.87N, 79.52E), T9-MH809180 (29.21N, 79.13E) T10-MH809181 (29.18N, 79.05E), T11-MH806364 (28.87N, 79.59E), T12-MH806365 (30.17N, 78.11E), T13-MH806366 (29.14N, 79.52E), T14-MH806367 (30.36N, 77.86E), T15-MH806368 (29.16N, 79.06E), T16-MH806369 (29.24N, 78.91E), T17-MH806370 (30.41N, 77.80E), T18-MH806371 (29.30N, 78.81E), T19-MH806372 (30.14N, 78.11E), and T20-MH806373 (28.92N, 79.48E); and for *T. harzianum* -T6-MH809177 (29.00N, 79.48E). The *tef-1α* gene sequences for these *Trichoderma* isolates were submitted in the Genbank and given accession numbers was obtained for *T. asperellum* T1-MK967709, T2-MK967710, T3-LC489991, T4-LC488384, T5-MK882522, T7-MK864272, T8-LC488385, T9-LC489992, T10-LC483819, T11-LC489993, T12-MK967712, T13-MK967711, T14-MK864273, T15-MK850420, T16-LC489994, T17-MK864271, T18-MK862120, T19-LC489995, and T20-MK864270; and for *T. harzianum* T6-LC489996. Details regarding the geographical coordinates, location, country, and other informations are given in Table 1. To illustrate the root condition, the *Hypomyces subiculosus* (MH858053) was chosen as an outgroup.

Reference Genbank accession numbers with their geographical coordinates, isolates name, and other details were obtained from NCBI, and that reference sequences were used to construct phylogeny for comparison for ITS (1 and 4), and *tef-1α* regions were described in Supplementary Table 2A and 2B.

2.3. In vitro screening of Trichoderma isolates for their antagonistic potential against Colletotrichum graminicola

The 20 *Trichoderma* isolates were screened for their antagonism against *C. graminicola* by inoculating the test pathogen *C. graminicola*, and *Trichoderma* isolates at the opposite edges of Petri plates (Morton and Stroube, 1955). *C. graminicola* showed slow growth in a culture, so the test pathogen *C. graminicola* was inoculated 3 days earlier than *Trichoderma* isolates. The dual culture assay was performed in 3 replications, and observations were reported on the 6^{th} day. The *C. graminicola* mycelial growth area was calculated.

Percent inhibition of the pathogen over control was calculated by adopting the formula given by (Mokhtar and Aid, 2013): MGI (%) = (C − T) × 100/C where MGI = Percent mycelial growth inhibition, C = Area of *C. graminicola* mycelial growth in the absence of *T. asperellum* and T = Area of *C. graminicola* mycelial growth in dual culture with *T. asperellum*.

Table 1
Identification, Origin, GenBank accession numbers and isolation details of the different *Trichoderma* isolates.

Isolate code	Area (Uttarakhand)	GPS location		Location	Host	Source of isolation	GenBank accession no.		Identity of isolates	NAIMCC accession number
		Latitude	Longitude				ITS1 and ITS4	*tef-1α*		
T1	Herbertpur (Dehradun)	30.44N	77.73E	India	Sorghum	Rhizospheric soil	MH809172	MK967709	*Trichoderma asperellum*	NAIMCC-F-03921
T2	Chharba (Dehradun)	30.41N	77.78E	India	Sorghum	Rhizospheric soil	MH809173	MK967710	*T. asperellum*	NAIMCC-F-03922
T3	Kashipur (Udham Singh Nagar)	29.22N	78.92E	India	Sorghum	Rhizospheric soil	MH809174	LC489991	*T. asperellum*	NAIMCC-F-03923
T4	Sitarganj (Udham Singh Nagar)	28.92N	79.69E	India	Sorghum	Rhizospheric soil	MH809175	LC488384	*T. asperellum*	Yet to be submitted
T5	Haripur Kalan (Haridwar)	29.99N	78.20E	India	Sorghum	Rhizospheric soil	MH809176	MK882522	*T. asperellum*	NAIMCC-F-03924
T6	Pantnagar (Udham Singh Nagar)	29.00N	79.48E	India	Sorghum	Rhizospheric soil	MH809177	LC489996	*T. harzianum*	Yet to be submitted
T7	Chidderwala (Dehradun)	30.08N	78.17E	India	Sorghum	Rhizospheric soil	MH809178	MK864272	*T. asperellum*	NAIMCC-F-03925
T8	Sutuiya (Udham Singh Nagar)	28.87N	79.52E	India	Sorghum	Rhizospheric soil	MH809179	LC488385	*T. asperellum*	NAIMCC-F-03926
T9	Kishanpur (Udham Singh Nagar)	29.21N	79.13E	India	Sorghum	Rhizospheric soil	MH809180	LC489992	*T. asperellum*	NAIMCC-F-03927
T10	Noorpur (Udham Singh Nagar)	29.18N	79.05E	India	Sorghum	Rhizospheric soil	MH809181	LC483819	*T. asperellum*	NAIMCC-F-03928
T11	Kichha (Udham Singh Nagar)	28.87N	79.59E	India	Sorghum	Rhizospheric soil of sorghum	MH806364	LC489993	*T. asperellum*	NAIMCC-F-03929
T12	Doiwala (Dehradun)	30.17N	78.11E	India	Sorghum	Rhizospheric soil of sorghum	MH806365	MK967712	*T. asperellum*	NAIMCC-F-03930
T13	Haldwani (Nainital)	29.14N	79.52E	India	Sorghum	Rhizospheric soil of sorghum	MH806366	MK967711	*T. asperellum*	NAIMCC-F-03931
T14	Haripur (Dehradun)	30.36N	77.86E	India	Sorghum	Rhizospheric soil	MH806367	MK864273	*T. asperellum*	NAIMCC-F-03932
T15	Sultanpur (Udham Singh Nagar)	29.16N	79.06E	India	Sorghum	Rhizospheric soil	MH806368	MK850420	*T. asperellum*	NAIMCC-F-03933
T16	Kunda (Udham Singh Nagar)	29.24N	78.91E	India	Sorghum	Rhizospheric soil	MH806369	LC489994	*T. asperellum*	NAIMCC-F-03934
T17	Chharba (Dehradun)	30.41N	77.80E	India	Sorghum	Rhizospheric soil	MH806370	MK864271	*T. asperellum*	NAIMCC-F-03935
T18	Bahadurpur (Udham Singh Nagar)	29.30N	78.81E	India	Sorghum	Rhizospheric soil	MH806371	MK862120	*T. asperellum*	NAIMCC-F-03936
T19	Jhabrawala (Dehradun)	30.14N	78.11E	India	Sorghum	Rhizospheric soil	MH806372	LC489995	*T. asperellum*	NAIMCC-F-03937
T20	Deoria (Udham Singh Nagar)	28.92N	79.48E	India	Sorghum	Rhizospheric soil of sorghum	MH806373	MK864270	*T. asperellum*	NAIMCC-F-03938

Table 2
Effect of seed biopriming with *Trichoderma* isolates on growth promotion and disease reduction under glass-house condition at 45 days of sowing.

Isolates	Root length(cm)	Shoot length(cm)	Fresh Shoot weight(g)	Fresh root weight(g)	Dry root weight(g)	Dry shoot weight(g)	Percent Disease Index(PDI)	Reduction in PDI over pathogen inoculated control (%)
T3	19.00 ± 0.5a*	132.00 ± 1.73a	58.00 ± 1.16a	8.81 ± 0.58a	1.28 ± 0.01a	8.36 ± 0.05a	31.20 ± 0.82** (33.93)	31.87
T4	15.00 ± 0.58bc	110.00 ± 1.16c	28.33 ± 3.76c	7.40 ± 0.13bc	0.59 ± 0.01d	3.43 ± 0.13d	33.56 ± 0.31b (35.38)	26.70
T6	12.67 ± 0.88 cd	109.33 ± 1.76c	30.67 ± 1.76c	6.91 ± 0.27c	0.87 ± 0.01c	5.14 ± 0.22c	34.35 ± 0.55b (35.86)	24.98
T15	14.33 ± 1.20c	114.00 ± 2.08c	43.67 ± 0.88b	6.73 ± 0.28c	0.90 ± 0.02c	6.84 ± 0.20b	33.44 ± 0.80b (35.31)	32.92
T19	17.17 ± 0.73ab	125.00 ± 1.16b	45.33 ± 2.03b	8.33 ± 0.38ab	1.12 ± 0.03b	7.32 ± 0.29b	30.71 ± 0.92c (33.63)	32.92
Pathogen inoculated control	8.97 ± 0.27e	96.57 ± 0.64d	16.00 ± 0.58d	4.12 ± 0.37d	0.20 ± 0.02f	2.10 ± 0.06e	45.79 ± 0.85a (42.56)	
Uninoculated untreated Control	10.67 ± 0.88de	107.67 ± 4.33c	20.67 ± 1.76d	6.37 ± 0.36c	0.31 ± 0.04e	2.51 ± 0.24e	–	

*Values given in column are the average of three replications followed by standard deviation. ** Values given in column are the average of five replications followed by standard deviation. Values with different alphabetical (a–e) superscripts within a column are significantly different ($P \leq 0.05$) using Duncan's multiple range tests (DMRT), Values in parenthesis are arcsine transformed values

2.4. Inoculum preparation for treatment of sorghum plants with selected bioagents

The washed sorghum seeds were soaked in water for 12 h. After soaking, seeds were boiled for 20 to 25 min to soften the grains; cooked sorghum seeds were spread in the blotter paper to decrease the moisture content and to cool down. After that 50 g of seeds were put in the 250 mL Erlenmeyer flasks, and then the flasks containing sorghum seeds were autoclaved at 15 lbs psi for 1 h at 121 °C. The autoclaved seeds were cool down and then inoculated with 3 to 4 mycelial plugs cut with the help of

flame sterilized cork borer from the edge of 3 days old actively grown culture of *Trichoderma* isolates and incubated at 28 °C in a BOD incubator for 2 weeks. The flasks were shaken vigorously after 7 days of inoculation to avoid clumping and break the mycelial mat. After 14 days of incubation, the sorghum seeds colonized by *Trichoderma* isolates were air-dried in open shade and then ground with a mixer grinder. The pure powder of bioagents was obtained when the powder was passed through 50 to 80 mesh sieves simultaneously. The talc powder and the fine powder of *Trichoderma* isolates cultured in sorghum seeds were mixed at 1:2 proportions and air-dried in the shade for 3 days at room temperature. Tapioca starch @5g/kg was added to the mixture. The final *Trichoderma* inocula were adjusted in the formulation 5×10^8cfu/g and then packed in polythene bags at room temperature (25 ± 2 °C) and further used for pot experiments.

The conidial suspension of *Colletotrichum graminicola* was prepared by scrapping all the conidia from the culture after three days and suspended in deionized water containing 0.02% tween 20. The final pathogen concentration was adjusted to 1×10^6 conidia L^{-1}, as suggested in earlier reports (Muimba Kankolongo and Bergstrom, 2011). Conidial suspension of *C. graminicola* (10^6 conidia L^{-1}) was sprayed on 25 days old plants in the evening time with a hand operated sprayer to develop anthracnose symptoms. The plants untreated with *Trichoderma* isolates and *C. graminicola*, but treated only with water were considered healthy controls. *C. graminicola*, inoculated plants were considered as pathogen inoculated control.

2.5. Pot preparation and plant material

The 5 kg capacity plastic pots were filled with sterilized autoclaved soil and kept in the glasshouse. Surface-sterilized healthy seeds of PC-23 (susceptible variety of sorghum) were sown in 30 cm plastic pots. The glasshouse trial comprised 7 treatments- Uninoculated untreated control, pathogen inoculated control, T3, T4, T6, T15, and T19. *Trichoderma* isolates used in the experiment were T3, T4, T6, T15, and T19. The 10 seeds were sown in each pot, and after germination, only 1 healthy plant was maintained in each pot. The 5 replications of each treatment were maintained and set up in a completely randomized design. The pathogen was applied at 25 days after sowing.

2.5.1. Different modes of application of bioagents

For seed biopriming, sorghum seeds were surface sterilized with 1% sodium hypochlorite, for 1 min followed by 2-times washing with sterilized water. Surface disinfected sorghum seeds were presoaked in water for 24 h. Presoaked seeds were treated with the talc-based formulation of *Trichoderma* isolates having 5×10^8cfu/g @10 g/kg of seeds in a 2% gum arabic solution as suggested in earlier reports (Lewis et al., 1991). The seeds were incubated for 48 h at 25 °C. Total 10 sorghum seeds were sown in each pot. After germination, only 1 plant per pot was maintained in all the pots under glasshouse conditions.

2.5.2. Sampling of leaves for biochemical test and determination of antioxidant activities

For each biochemical assay, samples were collected from the individual treatments of *Trichoderma* treated plants and plants inoculated with or without pathogen at regular intervals at different post-inoculation times, viz. 0 h, 24 h, 48 h, and 72 h to determine defense enzymes induction in response to *Trichoderma* isolates in sorghum plants under glasshouse conditions. Samples were stored at −80 °C. Biochemical data, including enzyme assays of superoxide dismutase, peroxidase, and polyphenol oxidase, were recorded with the spectrophotometer. SOD was estimated by the method described (Beauchamp and Fridovich, 1971). Polyphenol oxidase (PPO) activity was estimated by the method described (Mayer et al., 1966). Peroxidase activity was determined by the standard method (Hammerschmidt et al., 1982).

2.5.3. Observation of plant growth parameters and disease severity under glasshouse condition after seed biopriming with Trichoderma isolates

Plant growth parameters, viz. shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were recorded after 45 days of sowing. Disease severity was recorded after post-inoculation by measuring the percentage of leaf area infected. Percent disease index (PDI) was recorded based on a 1–9 disease intensity scale for anthracnose of sorghum proposed by All India Coordinated Sorghum Improvement Project as described below:

1-Highly resistant (No symptoms), 2-Resistant (1–5% intensity), 3-Resistant (6–10% intensity), 4-Moderately resistant (11–20% intensity), 5-moderately resistant (21–30% intensity), 6-Susceptible (31–40% intensity), 7-Susceptible (41–50% intensity), 8-Highly Susceptible (51–75% intensity), 9-Highly Susceptible (76–100% intensity).

The disease scale score was converted to percent disease index using the following formula (Wheeler, 1969):

PDI (%) = Σ (Scale × Amount of Plants)/ (Maximum grade × Total number of plants)

2.5.4. Qualitative assay for lignifications

Trichoderma isolates treated plants were harvested 15 days after *C. graminicola* inoculation. Hand-cut transverse sections of roots were stained with 1% phloroglucinol saturated aqueous solution in 20% HCl and mounted on a slide and fixed with 95% (v/v) ethanol and observed in the compound light microscope (Nikon, Japan). The development of pink colour indicated lignin deposition (Guo et al., 2001).

2.6. Biological control assays under field conditions

The field experiment was conducted in the randomized block design with 5 replications in the Kharif season of 2018 and 2019. The potent *Trichoderma* isolates –T3, T4, T6, T15, and T19 were used for field evaluation based on glasshouse results. Susceptible variety of sorghum PC23 was planted at the sorghum pathology block, Livestock research centre, GBPUA&T in three rows of 6 m length with a sub-plot of 2 × 2 m^2.The spacing size was 45 × 15 cm, seed @15 kg/hectare was sown at a depth of 3–4 cm. N in the form of urea, phosphorous was added in the form of single super phosphate (P_2O_5) and K in the form of Potash Muriate (K_2O) at 120, 50, and 40 kg/hectare respectively.

Nitrogen, as urea, was applied in 2 split doses, half as a basal dose with potash and phosphorous, and the second half was top-dressed after 40 days after sowing. Presoaked surface disinfected sorghum seeds were treated with *Trichoderma* talc-based product having 5×10^8 cfu/g @10 g/kg of seeds in 2% gum arabic solution. The seeds were incubated for 48 h at 25–28 °C. *Trichoderma* isolates adhered to the seed; grow on the seed surface to form a protective layer all around the seed coat under moist condition. After 90 days after sowing, the observations were recorded regarding root length, shoot length, stem diameter, and green fodder yield. The percent disease index (PDI) was recorded after sowing at 30, 45, 75, and 90 days. The percent disease index was recorded on a 1–9 scale proposed by All India Coordinated Sorghum Improvement Project before the emergence of the boot leaf and up to 90 days after sowing. In the glass-house experiment, the disease rating scale is given.

2.7. Statistical analysis

Data from laboratory and glasshouse experiments data were analyzed by using a one-way analysis of variance (Trillas et al., 2006) and Duncan's multiple range test (DMRT) using Statistical Product and Service Solution (SPSS) version 16.0 software Developed by SPSS Inc., now IBM SPSS. The power of the test at a 5% level of significance ($\alpha = 0.05$) has been calculated based on the existing setup using R version 3.6.2 (2019-12-12) – Copyright (C) 2019. The Statistical Computing Platform R-Foundation: x86 64-w64-mingw32 / x64 (64-bit) was used.

The values in vitro test (dual culture assay) shown in figures as the mean of three replications ± standard deviation and glasshouse

experiments were shown in figures as the mean of three replications ± standard deviation. In contrast, percent disease index values were shown in figures as the mean of five replications ± standard deviation in the glass-house experiment and the whole experiment was repeated twice. The values were shown in figures as the mean of five replications ± standard deviation in the field experiments. The treatment mean values were compared with Duncan's multiple range tests at $P \leq 0.05$ significance level.

3. Results

In the current investigation, 20 isolates of *Trichoderma* isolated from different Uttarakhand regions were identified based on internal transcribed spacer (ITS1 and ITS4) gene regions, and translation elongation factor-1 α (*tef-1α*) gene sequences and the details of geographical coordinates and Genbank accession number were given in Table 1.

3.1. Molecular identification of Trichoderma isolates by using internal transcribed region (ITS1 and ITS4) and tef-1α gene

Molecular identification of 20 isolates of *Trichoderma* was based on DNA sequence analysis to confirm species identity, which was earlier done by morphological parameters solely. The ITS regions (ITS1 and ITS4 primer) and the region represents the small fragment of the *tef-1α* gene, the smallest intron between the 5th and 6th exon (EF1-728F and EF1-986R primer) for all 20 *Trichoderma* isolates has been amplified. The amplicon size of 650 bp for ITS region and 315 bp of *tef-1α* gene observed in all the isolates was sequenced and analyzed using BLASTn programme. Sequence lengths ranging from 447 to 697 bp for the ITS and 336–363 bp for *tef-1α* gene were obtained by bidirectional sequencing of forward and reverse primer and used for blast search. All the sequences of the ITS and *tef-1α* gene were multiple aligned with MUSCLE software. After the BLAST search on the NCBI GenBank database, based on the ITS and *tef*-1α gene sequences analysis confirmed that 19 isolates belonged to *T. asperellum* (T1, T2, T3, T4, T5, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, and T20) with 100% similar identity and one isolate T6 belonged to *T. harzianum* with 99.83% similar identity. After identification, *Trichoderma* isolates were deposited on National Agriculturally Important Microbial Culture Collection (NAIMCC) (Table1).

3.2. Phylogenetic relationship based on ITS rRNA and tef-1α gene sequences

The phylogenetic analysis based on ITS1 and ITS4 and *tef-1α* sequences revealed that 19 isolates (T1, T2, T3, T4, T5, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, and T20) belong to *T. asperellum*, whereas isolate T6 belonged to *T. harzianum*. Nodal robustness was targeted by the bootstrap method. The numerical value presented in the node indicates the bootstrap value.

Based on the bootstrap values, the 20 *Trichoderma* spp. isolates could be divided into two separate clades based on ITS1 and ITS4 gene sequences. The *T. asperellum* isolates T1, T2, T11, and T18 clustered with Delhi, Punjab, Karnataka, Kerala, Andhra Pradesh, Tamil Nadu, and Uttar Pradesh isolates with a bootstrap value of 62%. The T7 isolate of *T. asperellum* clustered with Karnataka and Arunachal Pradesh isolates with a bootstrap value 62%, whereas isolatesT3, T8, T10, T17, and T19 clustered with Gujarat and Kerala isolates with a bootstrap value 62%. *T. asperellum* isolates T4, T5, T9, T12, T13, T14, T15, and T16 of Uttarakhand were clustered as distinct clades, bootstrap value of 99%. *T. harzianum* (T6) isolate of Pantnagar, Uttarakhand as a distinct clade with a bootstrap value of 100%, respectively (Fig. 1).

Based on the bootstrap values, the 20 *Trichoderma* spp. isolates could be divided into two separate clades based on *tef-1α* gene sequences. The *Trichoderma asperellum* isolates T1, T2, T3, T7, T8, T9, T10, T12, T13, T14, T16, T17, T19, and T20 clustered with Karnataka, Andhra Pradesh,

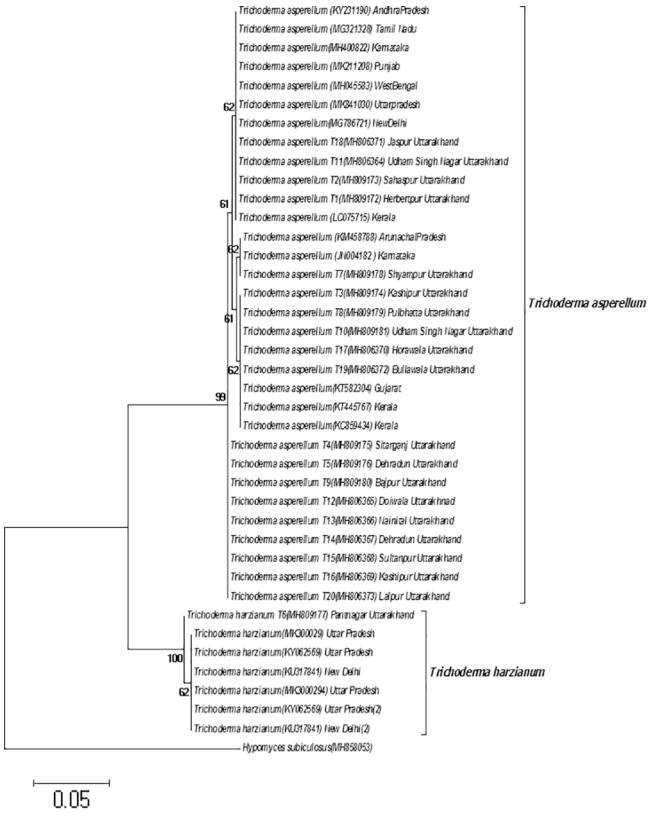

Fig. 1. Phylogenetic relationship among the isolates of *Trichoderma*. Cladogram was constructed using 650 bp ITS sequences according to Maximum likelihood method. Nodal robustness was tested by bootstrap method. Bootstrap replication was 1000. Numerical value presented in the node indicates bootstrap value. Sequences used for this comparison were obtained under the following NCBI GenBank accession numbers: NCBI Genbank accession numbers for *Trichoderma asperellum* (MG786721, MK841030, MH045583, MK211208, MH400822, KY231190, MG321328, KT582304, KT445767, LC075715, KM458788, KC859434, and JN004182) and *Trichoderma harzianum* (MK300029, KY062569, KU317841, MK3000294, KY062569, KU317841, and MH858053) were used as reference sequences. To demonstrate the situation of the root the *Hypomyces subiculosus* (MH858053) were chosen as an outgroup. Bar 0.05 substitutions per site.

Kerala, and Uttar Pradesh isolates with a bootstrap value of 83%. T4 and T11 clustered as a distinct clade with a bootstrap value of 45%. *T. asperellum* isolates T3, T10, T15, and T18 clustered as a distinct clade with a bootstrap value of 38%. *T. harzianum* T6 isolate of Pantnagar, Uttarakhand were clustered as a distinct clade with a bootstrap value of 100% that differed from other *T. harzianum* isolates of India. *T. asperellum* T5 isolate of Haridwar, Uttarakhand clustered with a distinct clade with a bootstrap value of 99% that differed from other *T. asperellum* isolates of India (Fig. 2).

3.3. In vitro screening of Trichoderma isolates for their antagonistic potential against C. Graminicola

The results showed that all the 20 isolates of *Trichoderma* spp. showed a significant reduction in the mycelial growth of *C. graminicola* at a range of 60.39%-76.47% with a mean value of 66.11%. *T. asperellum* isolate T3 gave maximum mycelial growth inhibition with 76.47% (Fig. 3).

For the variable isolates, the power of the ANOVA test with 60 observations is 0.97, which is almost near to 1 (highest value of power). Thus the findings are reliable enough to draw valid conclusions.

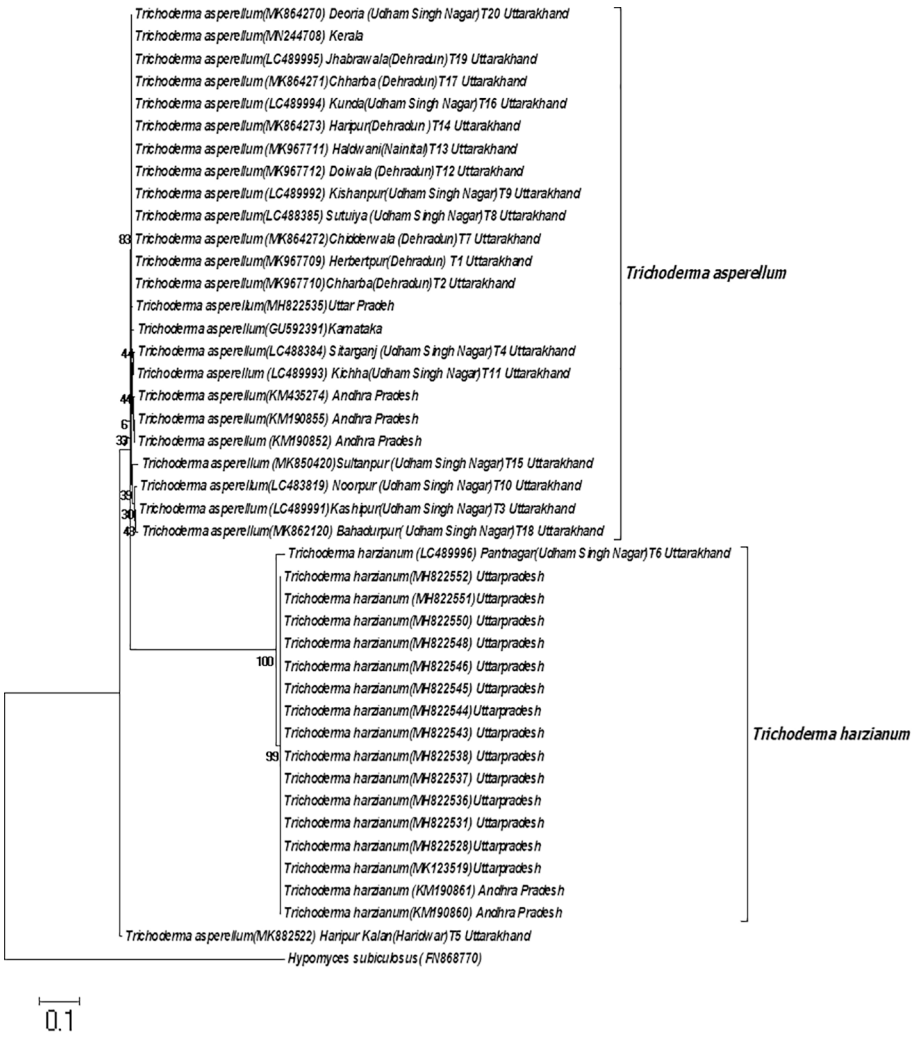

Fig. 2. Phylogenetic relationship among the isolates of *Trichoderma*. Cladogram was constructed using *tef-1α* sequences according to Maximum likelihood method. Bootstrap replication was 1000. Sequences used for this comparison was obtained under the following NCBI GenBank accessions numbers for *Trichoderma asperellum* (MN244708, MH822535, KM190855, GU592391, KM190852, KM435274) and *Trichoderma harzianum* (MH822552, MK348512, MH822551, MH822550, MH822548, MH822546, MH822545, MH822544, MH822543, MH822538, MH822537, MH822536, MH822531, MH822528, MK123519, KM190861, KM190860, and KM190862). To demonstrate the situation of the root the *Hypomyces subiculosus* (MH858053) were chosen as an outgroup. Bar 0.1 substitutions per site.

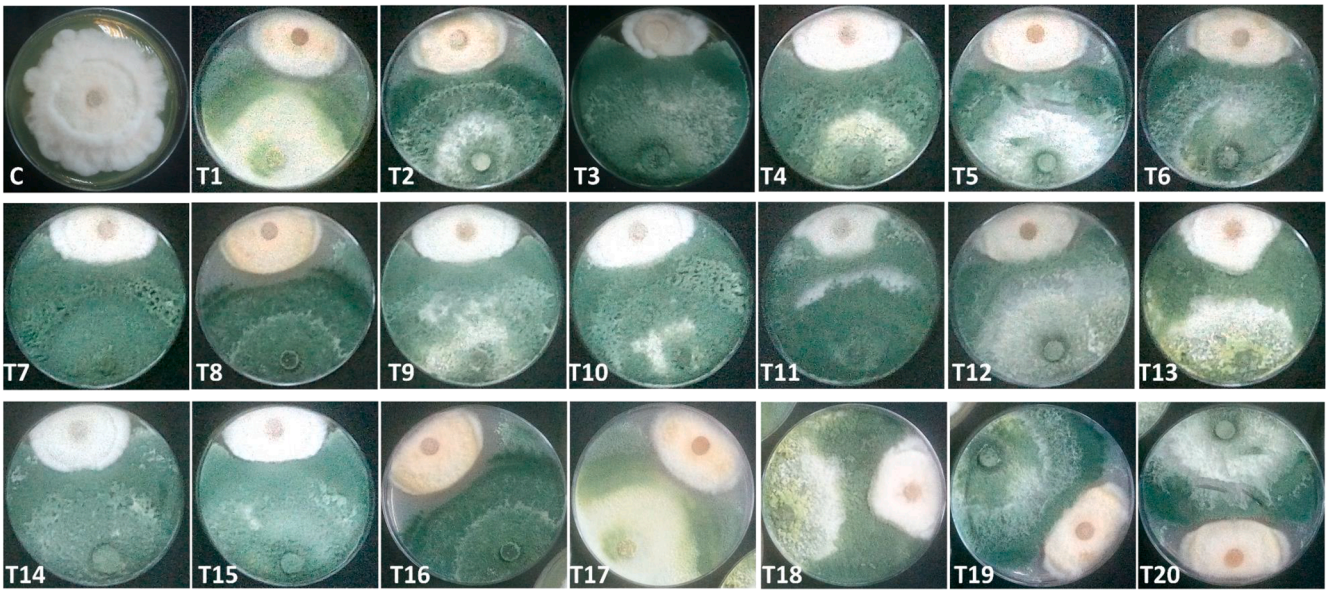

Fig. 3a. Plates showing the antagonistic activity of *Trichoderma asperellum* isolates (T1, T2, T3, T4, T5, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, and T20) and *T. harzianum* isolate (T6) against *Colletotrichum graminicola* (Control) in a dual culture assay under *in vitro* laboratory condition at 28 ± 2 °C and observations were recorded on the sixth day.

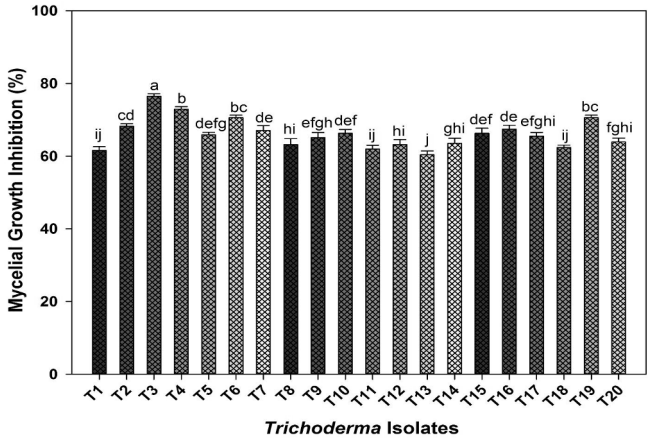

Fig. 3b. Percent inhibition of mycelial growth of *Colletotrichum graminicola* by *Trichoderma* isolates. Results are expressed as average of three replications and error bars indicate standard deviation of the means. Values with different alphabetical (a–j) superscripts in the error bars are significantly different using Duncan's multiple range tests (DMRT) at $P \leq 0.05$.

3.4. Quantification of biochemical defense induces superoxide dismutase activity, peroxidase, and polyphenol oxidase enzymes in sorghum against *Colletotrichum graminicola* under glasshouse conditions

The enzymes (SOD, PPO, and PO) were estimated at 0 h, 24 h, 48 h, and 72 h after *Colletotrichum graminicola* inoculation in 25 days old sorghum plants. In all the treatments, viz. seed biopriming with *Trichoderma* isolates the superoxide dismutase (SOD), polyphenol oxidase (PPO), and peroxidase activities, which were significantly higher as compared to pathogen treated control and untreated unchallenged healthy control. *T. asperellum* T19 isolate showed maximum superoxide dismutase (SOD) activity, which was 36.63% higher than the pathogen inoculated control at 48 h. *T. asperellum* T3 isolate recorded maximum peroxidase and polyphenol oxidase (PPO) activities, which were 43.59% and 40.96% higher than pathogen inoculated control respectively, at 48 h after pathogen inoculation (Fig. 4).

From the current study, it was concluded that the activity of superoxide dismutase, polyphenol oxidase, and peroxidase was at least 0 h after pathogen inoculation, then increased at 24 h after pathogen inoculation and reached the maximum at 48 h after pathogen inoculation, then gradually decreased at 72 h after pathogen inoculation irrespective of all treatments and the untreated unchallenged pathogen showed no increased activity of the enzyme.

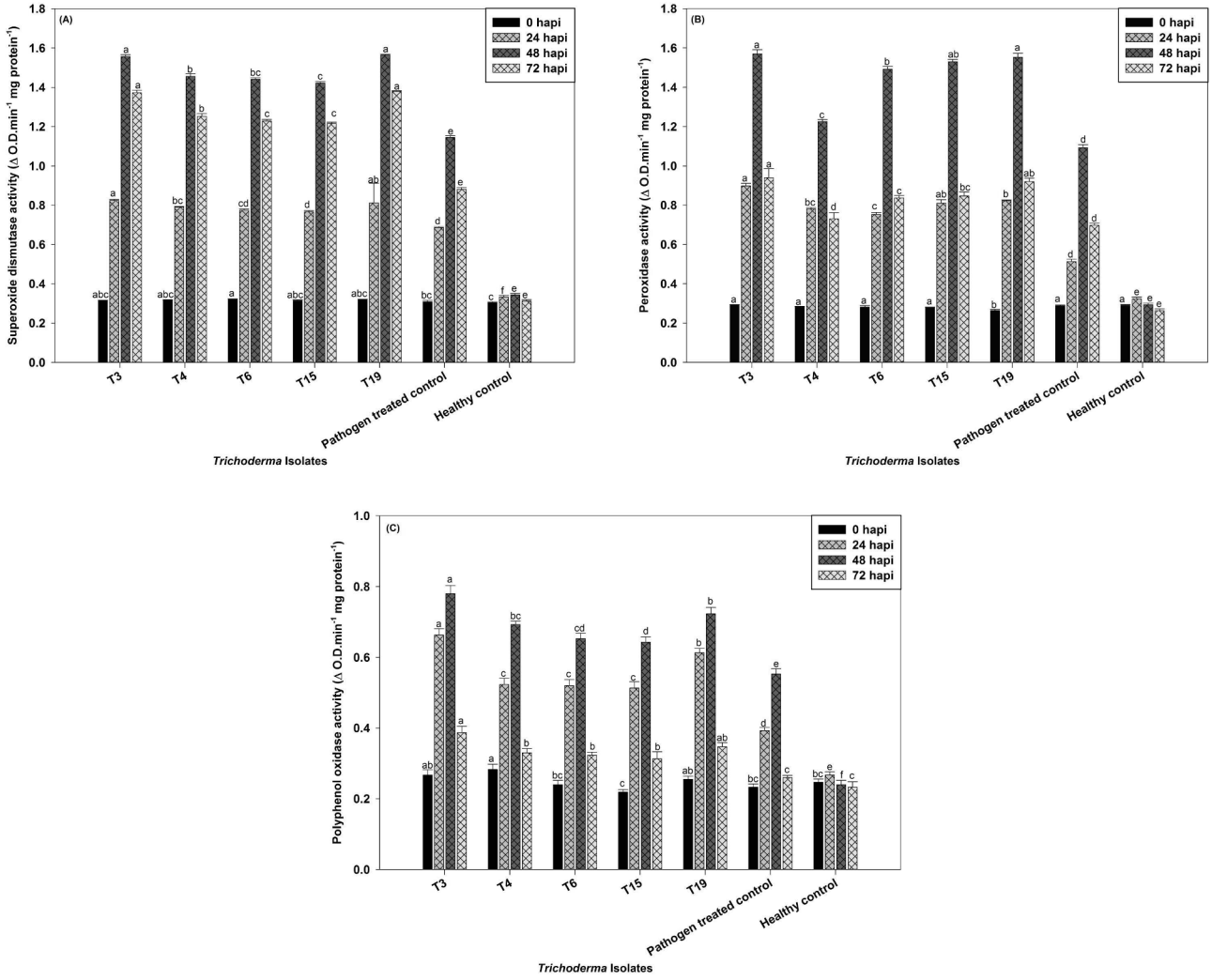

Fig. 4. Changes in Superoxide dismutase (A), polyphenol oxidase (B), and Peroxidase (C) activities in Sorghum leaves after seed biopriming with *Trichoderma* isolates and challenged with *Colletotrichum graminicola* under glass-house conditions and observations were recorded after 25 days of sowing. Results are expressed as average of three replications and error bars indicate standard deviation of the means. Values with different alphabetical (a–f) superscripts in the error bars are significantly different using Duncan's multiple range tests (DMRT) at $P \leq 0.05$.

For all the parameters, power of the ANOVA test was sufficiently large enough (>0.7) to make the findings highly reliable based on the present investigation under glasshouse condition.

3.5. Effect of seed biopriming with Trichoderma isolates on growth promotion and disease reduction under glasshouse condition

We observed that a significant increase in root length, shoot length, fresh shoot, and root weight, dry shoot, and root weight, and disease reduction were recorded in biopriming of seed with different *Trichoderma* isolates, viz. T3, T19, T4, T6, and T15 as compared to control under glasshouse conditions after 45 days after sowing. Among the treatments, the highest root length, shoot length, fresh shoot and root weight, dry shoot, and root weight was recorded in T3 isolate (19.00 cm),(132.00 cm), (58.00 g), (8.81 g), (8.36 g), and (1.28 g) respectively (Table 2).

It is evident from the data that all the isolates of *Trichoderma* reduced the percent disease index over pathogen inoculated control. Minimum PDI was recorded in T19 isolate (30.71%) followed by T3 isolate (31.20%), which was statistically at par with T19 isolate, whereas maximum PDI was recorded in pathogen inoculated control (45.79%) at 45 days after sowing.

For all the parameters, the power of the ANOVA test is sufficiently large enough (>0.7) to make the findings highly reliable based on the present investigation under the glasshouse condition. For the variable percent disease index under the glasshouse experiment, the power of the ANOVA test is sufficiently large enough (>0.7), and thus, the findings are reliable enough to draw valid conclusions.

3.6. Induction of lignin deposition in sorghum roots against pathogen invasion

Lignin deposition in the cell wall provides resistance against the plant pathogen invasion inside the vascular tissues. After 15th-day pathogen inoculation, histochemical staining of the transverse segment of root sorghum tissue treated with T3 *T. asperellum* isolate showed a difference in lignin deposition compared with pathogen inoculated and uninoculated untreated control. Seed biopriming with T3 isolate shows the highest lignifications in most of the root sections, including endodermis, epidermis, endodermis protophloem, metaphloem, pericycle, and protoxylem compared to pathogen challenged sorghum plant and uninoculated untreated healthy control (Fig. 5).

3.7. Field evaluation of Trichoderma isolates on growth promotion and disease reduction

Field experiments were carried out to show the effect of biopriming of seed with *Trichoderma* spp. on root length, shoot length stem diameter, percent disease index, AUDPC, and sorghum infection rate (Fig. 6; Supplementary Data 1) during the 2018 and 2019 Kharif season. Among the treatments, the highest root length (48.72 cm, 51.11 cm), shoot length (308.80 cm, 315.00 cm), and stem diameter (2.12 cm, 2.18 cm) was recorded in T3 isolate during the 2018 and 2019 Kharif seasons, respectively.

The effect of seed biopriming with *Trichoderma* isolates on disease severity was recorded on 30, 45, 60, and 75 days after sowing to check the effect of different treatments on the progress of anthracnose of sorghum. Minimum PDI was recorded in T19 isolate (53.63%) and T3 isolate (53.68%), whereas maximum PDI was recorded in the untreated control (74.84%, 76.91%) at 75 days after sowing. Minimum AUDPC was recorded in T3 isolate (740.87) and T19 isolate (751.38), and in contrast, maximum AUDPC was found in the untreated control (974.95, 1026.27) during the 2018 and 2019 Kharif season, respectively. Suggests that biocontrol activity of *Trichoderma* isolates minimizes disease effect and substantially increases sorghum yield (60.86 tons/hectare and 65.09 tons/hectare) in combination with T3 isolates, which is 22.22 percent and 27.29 percent higher than untreated control (47.33 tons/hectare and 47.32 tons/hectare) during 2018 and 2019 Kharif season, respectively.

Based on the results for 2018 and 2019, it can be seen that the power for the ANOVA test is sufficiently large (>0.8) for 30 days (PDI), 60 days (PDI), 75 days (PDI) and stem diameter, and therefore the ANOVA-based conclusion for these variables is highly reliable. For shoot length, power is also moderately high (0.76 and 0.68, respectively), and hence the result based on the present investigation is competent enough for drawing valid conclusions.

Based on the heat map, *Trichoderma* isolates were grouped into two significant clusters based on different parameters. Among them, all isolates of *Trichoderma* were identified in the same group examined through cluster analysis. Besides, *Trichoderma* (T3 and T19) isolates and *Trichoderma* isolates (T4, T6, and T14) were classified into two subclusters based on clustering analysis. In contrast, clustering between parameters revealed that there are 2 major clusters, Cluster I and Cluster II. Cluster I consists of increased yield percentage, green fodder yield, shoot length, and root duration. In comparison, the rest of the parameter was grouped into Cluster II.

Clustering among *Trichoderma* isolates and various parameters

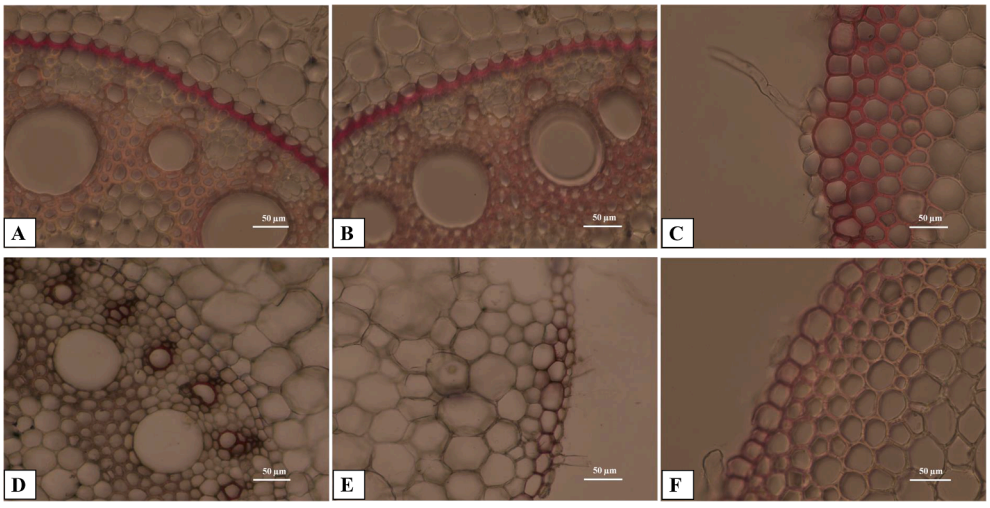

Fig. 5. Qualitative and Comparative analysis for lignin by phloroglucinol staining of root transverse sections after 15 days of pathogen inoculation (A, B, and C) Pathogen + *Trichoderma* (T3 isolate), (D and E) Pathogen treated control, and (F) Untreated control (magnification of image: 400X).

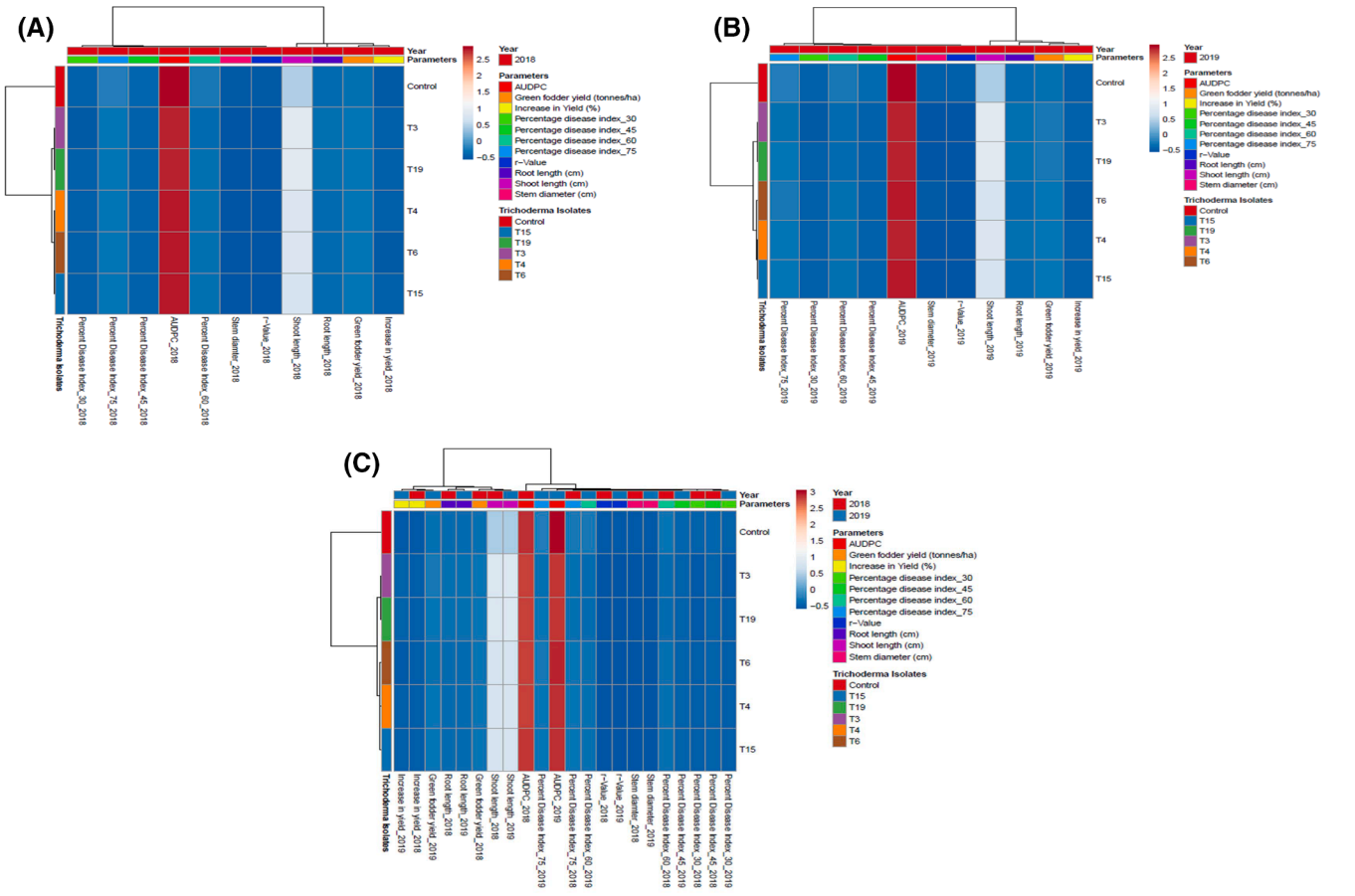

Fig. 6. Heat map showing effect of seed biopriming with *Trichoderma* isolates on growth promotion and disease reduction under field condition of two consecutive years (2018 and 2019) at 90 days after sowing, (A) 2018, (B) 2019, and (C) 2018 and 2019 combined.

exhibited various plant growth parameters, yield, and disease suppression compared to control. Clustering analysis also revealed that *Trichoderma* isolates varied in terms of their efficiency towards disease suppression and improved various plant attributes compared to control.

In the second year (2019), similar trends were observed on disease suppression and plant attributes compared to the previous year (2018). However, the effectiveness of *Trichoderma* isolates for disease suppression, and plant attributes were varied significantly.

4. Discussion

Trichoderma spp. has ubiquitous and found in different ecological niches. Two hundred ninety *Trichoderma* species were characterized until they know (du Plessis et al., 2018). The *Trichoderma* species, however, are likely to increase in rhizospheric soil, but little research has been carried out for the distribution of fungal soil communities from many parts of the country.A potent *Trichoderma* spp. could alleviate the percent disease index and modulation of induced systemic resistance that enhanced the plant immune system.

Primarily the identification of *Trichoderma* species is based on morphological characteristics. We have used a multigene approach in the present study for accurate identification of *Trichoderma* isolates. In the current study, *tef-1α* gene and ITS1 and ITS4 gene sequence analysis were able to identify and differentiate 20 *Trichoderma* isolates. Based on the sequence analysis of *tef-1α* and ITS1 and ITS4 gene, these isolates were divided into 2 species, 19 isolates belonged to *T. asperellum*, and one isolate belonged to *T. harzianum*. Similar results have been reported by Oskiera et al. (2015) where they identified 104 strains of *Trichoderma* based on the sequences of translation elongation factor 1 alpha (*tef-1α*), internal transcribed spacers 1 and 2 regions, as *T. simmonsii, T. harzianum, T. atroviride, T. lentiforme*, and *T. virens*. Thus, the comparative nucleotide sequence analysis of ITS1 and ITS4 and *tef-1α* gene gives better resolution to distinguish different *Trichoderma* species from the sorghum rhizosphere.

Based on the *in vitro* results, the *T. asperellum* and *T. harzianum* isolates showed antagonistic potential against *C. graminicola*. The potent inhibition was shown by *T. asperellum* isolates (T3, T4, T15, and T19) and *T. harzianum* isolate (T6) compared to other isolates in the dual culture assay. The antagonistic potential of *Trichoderma* spp. against *C. graminicola* is well known (Vasanthakumari and Shivanna, 2014; Manzar and Singh, 2020b). *Trichoderma* spp. showed the antagonistic potential, mycoparasitism, production of extracellular enzymes like pectinase, amylase, cellulase, chitinase, and induced systemic resistance against phytopathogens and reduces the incidence and severity of the disease (Atanasova et al., 2013; Vargas et al., 2014; Cherkupally et al., 2017).

Glasshouse assays were performed before field experiments were carried out to evaluate the antagonistic potential of *T. asperellum* isolates T3, T4, T15, and T19, and *T. harzianum* T6 isolate against *C. graminicola* in the sorghum plants. Several investigations had revealed the antagonistic potential of *T. asperellum* against *Pythium myriotylum* in cocoa (Mbarga et al., 2012), *Verticillium dahliae* in olive cultivars (Carrero-Carrón et al., 2016), *Rhizoctonia solani* in cucumber (Trillas et al., 2006) and *Fusarium oxysporum* in tomato (Debbi et al., 2018).

In biotic stress, reactive oxygen species overproduction causes damage to the lipid, carbohydrate, DNA, RNA, and disrupts the plant's healthy metabolic system (Sharma et al., 2012; Keswani et al., 2019a). Seed biopriming with *T. asperellum* isolate T3 reduced the overproduction of reactive oxygen species and H_2O_2 in the leaves and indirectly involved in the production of defense enzymes like superoxide

dismutase, peroxidase, and polyphenol oxidase in the host tissues, which act as scavengers of deleterious ROS. Superoxide dismutase catalyzes the ROS degradation and converts into H_2O_2, superoxide anion O_2^- and hydroxyl radical, this process is called oxidative burst, and these oxidative bursts protect the plant from the deleterious action of ROS and provides disease resistance to plant (Engwa, 2018).

Seed biopriming with *Trichoderma* spp. triggered the defense enzyme activity and induced systemic resistance, which increased the lignification process and increased phenyl propanoid activity to prevent the invasion, penetration, and establishment of phytopathogens within the host tissues (Ben Amira et al., 2018). In the present investigation, seed biopriming with *Trichoderma* isolates (T3, T4, T6, T15, and T19) triggered the superoxide dismutase, peroxidase, and polyphenol oxidase activity as compared to pathogen treated control and untreated unchallenged healthy control and thus restricted the disease progression. Similar findings have been reported by Zehra et al. (2017) where pretreatment of plants with *Fusarium oxysporum lycopersici* + *Trichoderma harzianum* + salicylic acid has resulted in 145.13% and 168.09% higher activity of superoxide dismutase and guaiacol peroxidase as compared to pathogen-inoculated plants respectively. It has been reported that the activation of peroxidase and polyphenol oxidase enzymes plays a vital role in the biological control and provide resistance to plants against plant pathogens, which collaborated with the findings of several other works (Zhang et al., 2001, 2008; Petrov and Van Breusegem, 2012).

Beneficial plant-associated microorganisms directly affect plant growth and indirectly stimulate plant health by altering plant gene expression in the roots, increasing net yield, stem diameter, shoot and root weights, delaying leaf senescence, and decreasing diseases severity. In this study, the disease progression rate in plants pre-treated with *T. asperellum* T3 was slower than in pathogen- infected plants. *Trichoderma* isolates help in promote plant growth parameters and help in disease reduction under glasshouse condition (Nagaraju et al., 2012; Singh and Singh, 2012). They observed the effect of seed biopriming of *Trichoderma* isolates on growth promotion and reduction in disease severity against downy mildew of sunflower. He reported that biopriming of seeds with *T. harzianum* isolates promotes plant height, vegetative and reproductive growth parameters compared to untreated pathogen inoculated control under glasshouse condition. In this research, the biopriming with *Trichoderma* isolates T3 and T19 encouraged lateral root formation. They were depicted from the enhanced accumulation of fresh and dry root and shoot weight under pathogenic conditions.

Our result showed that sorghum plants treated with *Trichoderma* isolates had a high level of lignification and were resistant to anthracnose of sorghum (*C. graminicola*). Our results are similar to Meshram et al. (2019), where they reported that lignification enhanced in *Trichoderma* treated chickpea plants compared to control challenged with *Fusarium oxysporum* f.sp. *ciceris*, as observed in histochemical staining.

Due to fluctuating climatic conditions rarely, controlled glasshouse assays were translated to field conditions. Two field trials were carried out in two consecutive seasons of Kharif 2018 and 2019 to check the efficacy of biocontrol potential of *Trichoderma* isolates (T3, T4, T6, T15, and T19). However, the year of planting turned out to be a factor that affects the yield and disease severity due to variations in climatic conditions (temperature, humidity, and rainfall). However, sorghum seeds bioprimed with T3 isolate showed better control of *C. graminicola* and enhanced plant biomass, grain yield and able to reduce disease severity under field conditions which collaborated with the findings of several other works (Dubey et al., 2011, 2013; Jambhulkar et al., 2015; Manzar and Singh, 2020a; Singh and Singh, 2008).

5. Conclusion

Trichoderma isolates collected from the sorghum rhizosphere from different Uttarakhand regions revealed a predominance of two main species: namely *T. asperellum* and *T. harzianum*. The seed biopriming with *Trichoderma* (T3 isolate) could be used as a biocontrol agent against *C. graminicola*. It also resulted in the enhancement of induce systemic resistance by activation of defense enzymes, high lignifications, promotion of plant growth, and minimize disease severity under the glasshouse and field condition. We conclude from the above observations that a multigene approach is a powerful tool for correctly identifying the *Trichoderma* species. *T. asperellum* T3 isolate can be used as a potent plant growth promoter and biocontrol agent to manage sorghum anthracnose for sustainable crop production. It may serve as an alternative to chemical fungicides to mitigate the harmful impact on the environment, provide the plant with excellent protection, and be environment friendly.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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