Research Square

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Bio-Priming with Endophytic Mycobiome-Derived Penicillium oxalicum and Aspergillus niger Activates Defence Enzymes in Maize and Suppresses Banded Leaf and Sheath Blight Disease

Vineeth Vijayan
ICAR- Indian Agricultural Research Institute
Robin Gogoi
r.gogoiiari@gmail.com

ICAR- Indian Agricultural Research Institute
Aundy Kumar
ICAR- Indian Agricultural Research Institute
Venkatesh Iddumu
ICAR- Indian Agricultural Research Institute

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Abstract

Banded leaf and sheath blight (BLSB), caused by f. sp. , is a major constraint to maize productivity worldwide, causing characteristic banded lesions on leaves and sheaths. To identify effective biocontrol potential of endophytic fungi, the culturable maize mycobiome was surveyed across two agro-climatic regions of India, revealing endophytic genera including , , , , , , , and in Bihar, and , , , and in Delhi. dual-culture assays showed isolates from Bihar inhibited f. sp. mycelial growth by 14.16–47.81%, while isolates from Delhi achieved 24.60–53.14% inhibition. screening identified three promising isolates (RT-L-EPI-9D) and (CTd-C-EPI-5B and CTd-L-EPI-1F) conferring resistant reactions with Percent Disease Incidence (PDI) values of 13.98, 18.37, and 18.59, respectively, at 25 days after inoculation (DAI). Field trials confirmed disease suppression and yield benefits, with RT-L-EPI-9D, CTd-L-EPI-1F, and CTd-C-EPI-5B recording PDI values of 52.93, 61.62, and 60.73 at 20 DAI, alongside yield increments of 31.69%, 32.11%, and 33.79% over control, respectively. Seed bio-priming with these isolates induced a two-phase defence-enzyme response in maize: β-1,3-glucanase and superoxide dismutase peaked rapidly within 24–48 h (up to ~ 5.2-fold), indicating an early induced-resistance burst, whereas catalase, peroxidase, and polyphenol oxidase increased gradually, sustaining elevated activity through 96 h (polyphenol oxidase reaching ~ 8-fold), reflecting prolonged antioxidant defence. Across isolates, , (CTd-C-EPI-5B) consistently drove the strongest and most significant responses across nearly every panel; SOD, β-1,3-glucanase, and PPO in particular followed by (CTd-L-EPI-1F) as a solid second, with (RT-L-EPI-9D) showing comparatively weaker or delayed effects.

1. Introduction

Maize (*Zea mays* L.), a cereal crop domesticated approximately 9,000 years ago in Mexico, has become one of the most widely cultivated plants, serving as a critical source of food, feed, and industrial raw materials across diverse agroecological zones (Ranum et al., 2014). Banded leaf and sheath blight (BLSB) caused by the fungal pathogen *Rhizoctonia solani* f. sp. *sasakii*, is a devastating foliar disease of maize characterized by distinctive banded lesions on leaves and sheaths, predominantly occurring in warm, humid tropical and subtropical regions with temperatures ranging from 25–32°C and relative humidity above 80% (Sharma & Shetty, 2016). The control of BLSB primarily relies on multiple fungicide applications throughout the growing season, which imposes substantial economic burdens on farmers and demands considerable labor investment (Vincelli & Dixon, 2002). The continuous and intensive use of synthetic fungicides has led to the emergence of fungicide-resistant strains of *R. solani*, thereby compromising the effectiveness of chemical control measures and necessitating higher doses or alternative compounds (Hollomon, 2015). Consequently, there is an urgent demand for eco-friendly, non-chemical disease management alternatives that can mitigate yield losses while minimizing environmental contamination and reducing potential health risks to agricultural workers and consumers (Glare et al., 2012). In response to these challenges, contemporary agricultural research worldwide is increasingly focused on identifying sustainable alternatives to conventional chemical fungicides that can simultaneously address the problem of pathogen resistance development and align with stringent food safety regulations (Khallaf et al., 2020). The utilization of biological control agents represents a promising and environmentally sound strategy for managing phytopathogenic diseases, offering effective disease suppression while maintaining ecological balance (Köhl et al., 2019). Moreover, endophytic microorganisms offer significant potential as biocontrol agents due to their inherent capacity to colonize internal plant tissues and establish stable associations with their hosts without causing detrimental effects on plant health.

Endophytes are microbes that inhabit the internal structures of plants, residing there for most or all of their existence while maintaining a harmless relationship with their host (Petrini, 1991; Saikkonen et al., 1998). Fungal endophytes show promise as natural antagonists against plant diseases because they have adapted to utilize identical ecological resources as pathogenic organisms (Silva et al., 2018). These fungi support plant health through multiple

mechanisms: they synthesize compounds that offer protection, enhance the ability of plants to withstand both environmental stresses and biological threats, and stimulate development through hormone production (Rai et al., 2014; Terhonen et al., 2019). These microbes can safeguard plants against fungal diseases through various mechanisms, including direct antagonism such as resource competition, production of antimicrobial compounds, or parasitism of other fungi, as well as indirect protection by triggering the plant defense systems (Latz et al., 2018). Some endophytes, *Colletotrichum* sp., *Aspergillus* sp., and *Annulohypoxylon* sp., have shown greater efficacy than chemical fungicides in controlling pathogens such as *R. solani* (Narayanan et al., 2024). Endophytic *Fusarium verticillioides* suppressed corn smut. Coinoculation of both reduced smut severity and significantly improved plant growth by interfering with initial infection and with pathogen development (Amin et al., 2013). The *in vitro* and *in vivo* assays of the endophytic *Trichoderma koningii* showed that it suppressed the wilt-causing pathogens of maize by reducing the wilting rate significantly (Orole & Adejumo, 2009). *Chaetomium globosum* (isolates Chg-1) associated with *Zea mays* inhibited *Cephalosporium maydis*, causing late wilt disease. It reduced late wilt incidence not only directly by antagonizing the wilt pathogen, but also indirectly by inducing resistance mechanisms in maize plants (Elshahawy & Khattab, 2022). Endophytic *Colletotrichum tofieldiae*, can colonize both maize and other crops, promoting growth and yield (Díaz-González et al., 2020). *Trichoderma asperellum* and *Chaetomium subaffine*, endophytic fungi, significantly improved the growth indices of infected maize plants (Degani et al., 2021).

Seed biopriming activates systemic defense pathways, enabling faster, stronger stress responses and improved plant fitness under pathogen attack. Seed bio-priming with fungi like *Beauveria bassiana*, *Metarhizium anisopliae*, and *Trichoderma* species strengthens defense against insect herbivory (Batool et al., 2024), drought and salinity (Yassin et al., 2024; Taha et al., 2025), and pathogens like *Fusarium* and *R. solani* (Singh et al., 2020; Hasan et al., 2026), while improving germination and growth (El-Nagar et al., 2024). Despite the agronomic importance of maize, its associated microbiome particularly the fungal (mycobiome) component remains comparatively understudied, and the functional role of these endophytic communities in field-level disease management is not well characterized. To address this knowledge gap, the present study was undertaken with the following objectives: (i) to characterize the structure and composition of the endophytic mycobiome associated with maize; (ii) to evaluate the biocontrol potential of the associated fungal isolates against banded leaf and sheath blight disease incited by *R. solani* f. sp. *sasakii*; and (iii) to assess the capacity of the selected isolates to induce bio-priming-mediated defence responses in maize seedlings following seed treatment.

2. Materials and methods

Sample collection and isolation of endophytic fungi

In two key maize-growing regions of India, namely Patna, Bihar and Delhi, samples were meticulously collected for in-depth mycobiome community isolation and analysis. PMH-1 is the hybrid maize variety that was cultivated in those distantly located (about 850km aerial distance) sampling sites named Patna (25° 36' 45.63" N and 85° 9' 31.95" E) and New Delhi (28° 63' 3.31" N, 77.1° 5.25" E). Aseptic and asymptomatic plant tissue samples including leaves, cobs, and roots were brought to the laboratory within the cold containers having 4°C temperature. After aseptically removal of the outer portion of tissues weighing about 100 g each, the samples were homogenized with 10 ml of phosphate-buffered saline (PBS, containing per litre: NaCl 8g; KCl 0.2g; Na_2HPO4 1.44g; KH_2PO_4 0.24g; pH adjusted to 7.4) with 0.1% Tween-20 (PBS-T). After treatments of the tissues, an aliquot was taken, and then serial dilutions of the solution up to 10^{-9} was done. The aliquot (50 µl) from each dilution, specifically 10^{-4}, 10^{-5} and 10^{-6} were then plated onto the enriched media. Inoculated plates were then incubated at 28°C ± 2°C for 15 days.

Molecular identification of fungal endophytes

Molecular identification of fungal isolates was performed through polymerase chain reaction (PCR) amplification of the nuclear ribosomal internal transcribed spacer (ITS) region using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Each PCR reaction was prepared in a total volume of 25 µl, comprising 2–5 µl of fungal genomic DNA template, 2.5 µl of 10× PCR buffer, 0.5 µl of 10 mM dNTP mix, 0.5 µl each of forward and reverse primers, 0.4 µl of Taq DNA polymerase, and 13.6–15.6 µl of sterile Milli-Q water. Thermal cycling was performed using a programmable thermal cycler under the following conditions: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at the optimized temperature for 30 sec, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min. Amplified PCR products were resolved by electrophoresis on a 2% (w/v) agarose gel in 1× TAE buffer, stained with ethidium bromide, and visualized under ultraviolet (UV) illumination. Successfully amplified products were recovered and purified using a commercial PCR purification kit and subsequently submitted for Sanger sequencing.

In-vitro dual culture confrontation assay

The antagonistic potential of the recovered endophytic mycobiome against BLSB disease causing agent, *R. solani* f. sp. *sasakii*, was evaluated using a dual plate confrontation assay. Potato Dextrose Agar (PDA) was selected as the preferred medium based on fungal growth performance. Endophytic isolates exhibiting comparatively higher growth rates on culture plates were selected for antagonist screening. Inoculum of the pathogen and selected endophytes were aseptically placed 40 mm apart on Petri plates (diameter 90 mm) containing 10 ml of fresh PDA, using an inoculation needle. Control plates containing individual inoculum of either the pathogen or the endophyte alone were maintained simultaneously. All plates were incubated at 28°C for seven days in triplicate. Colony diameters of all expanding isolates were measured across their width, and pathogen growth in dual culture was compared against the pathogen-only control to calculate the percentage inhibition of radial growth.

In-vivo bioassay of endophytic isolates against R. solani under controlled conditions

Endophytic isolates were evaluated for their ability to suppress BLSB disease and promote plant growth under controlled conditions using a seed coating approach. Spore suspensions of selected endophytic fungi were prepared in sterile 0.01% Triton X-100 solution and quantified to achieve a concentration of approximately 10^6-10^7 spores/ml. Maize seeds (Vivek QPM-9) were coated with spore suspension of selected endophytic fungi using a synthetic hydrophilic acrylic polymer following a standardized protocol; 100 g of seed was treated with equal volumes (200 µl each) of polymer and spore suspension were mixed, and 35 µl of the mixture was pipetted onto groups of ten seeds, which were gently agitated to ensure uniform coating. Seeds were air-dried for 3–5 min and stored overnight at 4°C for synchronize and uniform germination. Control seeds were coated with a 50:50 mixture of 0.01% Triton X-100 and polymer solution. Following acclimatization to room temperature, three seeds per 1.5-litre pot were planted in potting mix and grown to the V4 growth stage in a controlled growth chamber. A foliar application of spore suspension (10^6–10^7 spores/ml) of endophytic fungi was made prior to pathogen inoculation. Based on the results of dual culture assays, 32 isolates were advanced to *in-planta* testing, with each experiment replicated three times.

Disease assessment and percent disease index

Disease severity was assessed 25 days after application of the endophytic fungi by recording the lesion length of the infected internode in each inoculated plant. The percent disease index (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\text{Lesion lenght (in cm) in the internaode}}{\text{Average lenght of internode (in cm)}} \text{ X } 100$$

Disease reactions were classified using a modified 1–5 rating scale based on the percentage of affected area (Payak and Sharma, 1983), as presented in Table 2.1, with ratings categorized as Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), and Susceptible (S)

Table 2.1
Rating scale (1–5) for banded leaf and sheath blight disease reaction based on infected area for controlled conditions

Disease rating	Area affected (%)	Disease Reaction
1.0	10	R
1.5	11–20	R
2.0	21–30	MR
2.5	31–40	MR
3.0	41–50	MS
3.5	51–60	MS
4.0	61–70	S
4.5	71–80	S
5.0	81–100	S

Field-level screening of endophytic fungi against banded leaf and sheath blight disease of maize

A susceptible maize variety, Vivek QPM-9, was cultivated during the normal season (July to November) of 2021 and 2022 following the standard agronomic practices in the experimental field of ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. The field experiment was laid out in a Randomized Block Design (RBD) with a plot size of 3 m comprising three rows (row length: 2 m; row-to-row spacing: 75 cm). Minimum Ten to thirteen plants per row were maintained, and data were recorded from ten plants per row with three replications per treatment. Mass culture of *R. solani* f. sp. *sasakii* was prepared following the method of Ahuja and Payak (1978). Barley grains were soaked in normal water for 24 hours, drained out the water, and 40 g of soaked grains were transferred into 250 mL Erlenmeyer flasks. The flasks were plugged and autoclaved twice at 121.6°C (15 lb pressure) for 20 minutes. The sterilized grains were inoculated with 5–8 day-old fungal culture and incubated at 27 ± 1°C for 10–15 days, with periodic shaking at 2–3 day intervals to ensure uniform mycelial colonization. Inoculation was performed on 35-day-old maize plants (Growth Stage 5) (Payak and Sharma, 1983) by inserting 3 barley grains colonized by *R. solani* f. sp. *sasakii* between the stalk and leaf sheath at the second or third internode from the soil surface using gloved hands (Ahuja and Payak, 1978). The most effective endophytic fungal species, identified through prior *in vitro* screening, were mass-cultured and applied as biocontrol agents against the pathogen.

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{Total no. of plants assessed x Maximum disease rating}} \text{ x } 100$$

Disease severity and percent disease index (PDI) were recorded 30 days after inoculation (DAI) using 1–5 scale (Table 2.2) proposed by Ahuja and Payak (1983). PDI was calculated mentioned formula. Plants were categorized as Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), or Susceptible (S) based on recorded PDI values.

Table 2.2
Disease intensity based on 1–5 rating scale

Rating scale	Disease severity (%)	PDI	Disease reaction
1.0	Disease on one leaf sheath only, few small non-coalescent lesions present	20.0	Resistant (score: ≤ 2.0) (PDI ≤ 40.0)
1.5	Disease on two sheaths: lesions large and coalescent	30.0	
2.0	Disease up to four sheath; lesions many always coalescent	40.0	
2.5	As in disease rating symptoms of 2.0 + rind discolored with small lesions	50.0	Moderately resistant (Score: 2.1-3.0) (PDI 40.1–60.0)
3.0	Disease on all sheaths except two internodes below ear	60.0	
3.5	Disease up to one inter node below ear shoot, rind discoloration on many internodes with large depressed lesions	70.0	Moderately susceptible
4.0	Disease up to the internode ear bearing the ear shoot but shank not affected	80.0	(Score: 3.1-4.0) (PDI 60.1–80.0)
4.5	Disease on the ear, husk leaves show bleaching, bands and caking among themselves as also silk fibers; abundant fungal growth between and on kernels; kernel formation normal except being lustreless; ear size less than normal; some plants prematurely dead	90.0	Susceptible (Score: ≥ 4.0) (PDI ≥ 80.0)
5.0	In addition to diseases rating symptoms of 4.5, shrinkage of stalk; reduced ear dimensions, wet rot and disorganization of ear; kernel formation absent or rudimentary; prematurely dead plants common; abundants sclerotial production on husk leaves, kernels, ear tips and stalks fibers	100.0	

Yield analysis of endophyte-inoculated plants

To evaluate the economic impact by endophytes application, yield-related parameters were recorded at crop maturity. Data on grain yield per plot were recorded from ten tagged plants per treatment across three replications. Grain yield was expressed in quintals per hectare (Qha^{-1}) following standard conversion. The comparative yield performance between biocontrol-treated plots and the uninoculated control was used to assess the agronomic efficacy of the endophytic fungi in mitigating yield losses caused by *R. solani* f. sp. *sasakii* under field conditions.

Bio-priming-induced defence genes expression in maize

Expression of defense-related genes following the endophytic application on maize was analyzed using quantitative real-time PCR (qRT-PCR). The genes targeted for induced defense expression profiling included superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POA), catalase (CAT), and β-1,3-glucanase. Endophytic fungal isolates demonstrating potential antifungal and BLSB disease-suppressive activity were selected for this assay. Germinated maize seedlings were inoculated with fungal spore suspensions at concentrations of 10^6-10^7 spores mL^{-1} and maintained under controlled conditions. Plant tissue samples were collected at 24, 48, 72, and 96 hours post-inoculation, immediately snap-frozen in liquid nitrogen to arrest cellular activity, and stored at -80°C until further use. Total RNA was extracted from treated seedlings using the PureLinkTM RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, USA) following the instructions of manufacturer. Approximately 100 mg of frozen tissue from individual maize seedlings was homogenized to a fine powder under liquid nitrogen using RNase-free equipment. The homogenate was resuspended in 1 mL of lysis buffer containing β-mercaptoethanol (1:100 ratio), vortexed for 30 seconds, and centrifuged at 12,000 g for 5 minutes at room temperature. All consumables, including Eppendorf

tubes, micropipette tips, homogenizers, and scissors, were treated overnight with freshly prepared 0.1% DEPC solution, dried at 50°C, and autoclaved twice at 121.6 ± 1°C and 15 lb for 20 minutes to ensure an RNase-free environment. RNA purification was performed using spin cartridge-based columns from the same kit. An equal volume of 70% ethanol (prepared in DEPC-treated water) was added to the cell homogenate and mixed thoroughly. The mixture was passed through the spin cartridge and centrifuged sequentially at 12,000 g, followed by washing with Wash Buffer I and twice with Wash Buffer II (in 100% ethanol). The membrane-bound RNA was dried by centrifugation at 12,000 g for 2 minutes and eluted with 30–100 µL of RNase-free water. Purified RNA was stored at -20°C. RNA quantity and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) at 260/280 nm and 260/230 nm absorbance ratios. RNA integrity was verified by electrophoresis on a 2% agarose gel (in DEPC-treated 1X TAE buffer) at 80 V for 45 minutes using a UVITEC Cambridge Fire Reader V10. First-strand cDNA synthesis was performed using the ImProm-IITM Reverse Transcription System (Promega, USA). A 20 µL reaction mixture was prepared comprising total RNA (5 µL), ImProm-IITM 5X reaction buffer (4 µL), $MgCl_2$ (1.5 µL), dNTP mix (1 µL), RNase in ribonuclease inhibitor (40 U/µL), ImProm-II reverse transcriptase (1 µL), random primers (1 µL), and nuclease-free water (4.5 µL). Thermocycling conditions comprised 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min. Synthesized cDNA was diluted 1:5 to 1:10 with RNase-free water prior to use as a PCR template. Gene-specific primers targeting defence-related genes like SOD, B-1, 3 glucanase (PR6), PPO, CAT, PAL and amplicon sizes of 80–200 bp, melting temperatures of 58–62°C, and GC content of 40–60% were given in Table 2.3 and already used for gene-suppression studies of maize (Hamidi et al., 2023). RT-qPCR was performed on a Bio-Rad CFX96 Real-Time PCR Detection System using SYBR Green Master Mix in 20 µL reactions containing 2 µL cDNA template, run in technical triplicates alongside no-template and no-reverse transcriptase controls. Cycling conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec, with a final melt curve analysis from 65–95°C. Reference gene stability was assessed using geNorm and NormFinder, and relative expression was calculated by the 2-ΔΔCt method. Statistical differences were determined by Two-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$) across three independent biological replicates.

Table 2.3
Gene-specific primers targeting defence-related genes used for this study

Gene	primer details (5’- 3’)	Size (bp)
Superoxide dismutase	ZM_SOD (F)5’-AGT CAC CCA CCC CAT CCA AG-3’	146
	ZM_SOD (R) 5’-GTG CGG AGG AAT AGG GAG C-3’	
B-1,3 glucanase (PR6)	ZM_Glucan (F) 5’-ATG GCG AGG CAG GGT GTC-3’	188
	ZM_Glucan (R) 5’-ACG CCG ATG GAT TGG ACT C-3’	
Polyphenol oxidase	ZM_PPO (F) 5’-CGT CCA AGA AGA CCA CCG T-3’	146
	ZM_PPO (R) 5-’ACT GGA CAG GCC GTT GAG CA-3’	
Catalase	ZM_CAT (F) 5’-ACG TGC GCC GAC TTC CTG-3’	118
	ZM_CAT (R) 5’- GAA GAA GAC GGG GAA GTT GTT-3’	
Phenylalanine ammonialyase	ZM_PAL (F) 5’-TCG AAC TGC AAC CGA AAG A-3’	108
	ZM_PAL (R) 5’-CAG CCA GGA TTG CCA GAA TA-3’	

Statistical analysis

The field experiments were conducted in a randomised block design (RBD). All the experiments conducted in laboratory of the Division of Plant Pathology and in the National Phytotron Facility of ICAR-IARI were performed in complete randomized design (CRD). The statistical analysis was done using the online software using analysis of variance (ANOVA) under a Randomized Block Design, and treatment means were compared using Tukey's HSD test at $p \leq 0.05$.

3. Results

Characterisation of endophytic fungi

A total of 32 morphologically distinct culturable endophytic fungal communities were distributed among different organs of maize, such as the leaf, cob, and root of maize. Pure-culture of isolates obtained from different dilutions (10^{-4}, 10^{-5}, and 10^{-6}) of samples from Delhi and Bihar and both sampling sites. Sequences of the fungal ribosomal internal transcribed spacer (ITS) region were BLAST analysed with comparison to the NCBI database. The fungal species identity of the isolates was established. Then isolates were sub-grouped into 32 morphotypes. Samples generated 24 distinct morphotypes from different tissues of maize of Bihar, from which 50 representative isolates were used for identification using ITS of fungi (Table 3.1) and 9 distinct morphotypes from different tissues of maize of Delhi, of which 21 representative isolates were used for identification of endophytic fungi using ITS (Table 3.2).

Identification of endophytic fungi collected from Bihar

Around 23 morphotypes were distributed in different organs of maize, such as the leaf, cobs, and root, and 50 isolates were identified by sequencing the ribosomal ITS region of endophytic fungal colonies. Out of 50 isolates, 15 were from the cob, 21 were from the leaf, and 14 were from the root, specifically obtained. Overall, the endophytic fungal genera such as *Fusarium, Pencillium, Cladosporium, Alternaria, Aspergillus, Sarocladium, Mucor* and *Kalmusia* comprised of the structure of the culturable component of mycobiome.

Table 3.1
Identification of endophytic fungi associated with maize collected from Patna, Bihar

Sl. No.	Isolate Name	Identification of endophytic fungi	Geographical Origin	Tissue	Gen Bank Accession
1	CT-B.S-EPI-35A	*Fusarium neocosmosporillum*	Patna, Bihar	Root	OQ731788 .1
2	CT-C-EPI-22A	*Pencillium sumatrense*	Patna, Bihar	Cob	OQ859979.1
3	CT-C-EPI-22D	*Cladosporium oxysporum*	Patna, Bihar	Cob	OQ859980.1
4	RT-C-EPI-3C	*Alternaria tenuissima*	Patna, Bihar	Cob	OQ863201.1
5	RT-L-EPI-2A	*Fusarium odoratissimum*	Patna, Bihar	Leaf	OQ859981.1
6	RT-L-EPI-4H	*Cladosporium tenuissimum*	Patna, Bihar	Leaf	OQ859982.1
7	CT-L-EPI-6A	*Cladosporium* sp.	Patna, Bihar	Leaf	OQ859983.1
8	CT-L-EPI-16C	*Cladosporium cladosporioides*	Patna, Bihar	Leaf	OQ859984.1
9	CT-C-EPI-22B	*Aspergillus terreus*	Patna, Bihar	Cob	OQ859985.1
10	RT-Bulk-EPI-29A	*Aspergillus terreus*	Patna, Bihar	Root	OQ859986.1
11	RT-Bulk-EPI-29B	*Aspergillus terreus*	Patna, Bihar	Root	OQ859987.1
12	RT-C-EPI-11B	*Aspergillus terreus*	Patna, Bihar	Cob	OQ859988.1
13	RT-C-EPI-11F	*Aspergillus terreus*	Patna, Bihar	Cob	OQ859989.1
14	RT-L-ENDO-44A	*Alternaria alternate*	Patna, Bihar	Leaf	OQ859990.1
15	RT-L-EPI-2F	*Cladosporium cladosporioides*	Patna, Bihar	Leaf	OQ859991.1
16	RT-L-EPI-9C	*Cladosporium xanthochromaticum*	Patna, Bihar	Leaf	OQ859992.1
17	RT-L-EPI-9D	*Aspergillus niger*	Patna, Bihar	Leaf	OQ859993.1
18	RT-L-EPI-9G	*Aspergillus niger*	Patna, Bihar	Leaf	OQ859994.1
19	CT-L-EPI-14C	*Aspergillus terreus*	Patna, Bihar	Leaf	OQ859995.1
20	CT-RS-EPI-42A	*Aspergillus terreus*	Patna, Bihar	Root	OQ860013.1
21	CT-C-EPI-15B	*Aspergillus niger*	Patna, Bihar	Cob	OQ818860.1
22	RT-L-EPI-4F	*Cladosporium tenuissimum*	Patna, Bihar	Leaf	OQ859997.1
23	RT-C-EPI-8D	*Sarocladium strictum*	Patna, Bihar	Cob	OQ859998.1
24	RT-C-EPI-10G	*Mucor fragilis*	Patna, Bihar	Cob	OQ859999.1
25	CT-L-EPI-14D	*Aspergillus niger*	Patna, Bihar	Leaf	OQ860000.1
26	RT-C-EPI-24B	*Penicillium chermesinum*	Patna, Bihar	Cob	OQ860002.1
27	RT-C-EPI-3B	*Alternaria alternata*	Patna, Bihar	Cob	OQ860003.1

Sl. No.	Isolate Name	Identification of endophytic fungi	Geographical Origin	Tissue	Gen Bank Accession
28	RT-L-EPI-2G	*Fusarium proliferatum*	Patna, Bihar	Leaf	OQ860004.1
29	CT-L-ENDO-45A	*Sarocladium implicatum*	Patna, Bihar	Leaf	OQ860005.1
30	CT-C-EPI-15A	*Alternaria alternata*	Patna, Bihar	Cob	OQ860006.1
31	CT-L-EPI-12A	*Alternaria alternata*	Patna, Bihar	Leaf	OQ860007 .1
32	CT-L-EPI-14A	*Fusarium proliferatum*	Patna, Bihar	Leaf	OQ860008.1
33	CT-L-EPI-16A	*Alternaria alternata*	Patna, Bihar	Leaf	OQ860009.1
34	CT-L-EPI-16B	*Aspergillus niger*	Patna, Bihar	Leaf	OQ860010.1
35	CT-L-EPI-20A	*Penicillium expansum*	Patna, Bihar	Leaf	OQ860011.1
36	CT-R-EPI-26A	*Aspergillus* sp.	Patna, Bihar	Root	OQ863202.1
37	CT-RS-EPI-27B	*Aspergillus niger*	Patna, Bihar	Root	OQ860012.1
38	CT-RS-EPI-42A	*Aspergillus terreus*	Patna, Bihar	Root	OQ860013.1
39	RT-BS-EPI-30A	*Curvularia lunata*	Patna, Bihar	Root	OQ860014.1
40	RT-BS-EPI-31A	*Aspergillus terreus*	Patna, Bihar	Root	OQ860015.1
41	RT-C-EPI-8F	*Alternaria alternata*	Patna, Bihar	Cob	OQ860016.1
42	RT-C-EPI-17A	*Fusarium fujikuroi*	Patna, Bihar	Cob	OQ860017.1
43	RT-C-EPI-18B	*Kalmusia italic*	Patna, Bihar	Cob	OQ860018.1
44	RT-C-EPI-24A	*Penicillium chermesinum*	Patna, Bihar	Cob	OQ860019.1
45	RT-L-EPI-2C	*Alternaria* sp.	Patna, Bihar	Leaf	OQ860020.1
46	RT-L-EPI-21A	*Penicillium* sp.	Patna, Bihar	Leaf	OQ860021.1
47	RT-R-EPI-12B	*Cladosporium* sp.	Patna, Bihar	Root	OQ860022.1
48	RT-R-EPI-13A	*Aspergillus terreus*	Patna, Bihar	Root	OQ860023.1
49	RT-RS-EPI-28C	*Aspergillus terreus*	Patna, Bihar	Root	OQ860024 .1
50	RT-RS-EPI-33A	*Aspergillus niger*	Patna, Bihar	Root	OQ860025.1

Identification of endophytic fungi collected from Delhi

Around 9 morphotypes were distributed in different organs of maize, such as the leaf, cob and root, and as many as 21 isolates were identified by sequencing the ribosomal internal transcribed spacer (ITS) region of endophytic fungal colonies. Out of 21 isolates, 7 were from the cob, 8 were from the leaf, and 6 were from the root, specifically obtained. Overall, the endophytic fungal genera such as *Fusarium*, *Penicillium*, *Aspergillus*, *Purpureocillium* comprised of the structure of the culturable component of mycobiome.

Table 3.2
Identification of endophytic fungi associated with maize collected from Delhi

Sl.No.	Isolate Name	Identification of endophytic fungi	Geographical Origin	Tissue	Gen Bank Accession
1	CTd-C-EPI-5B	*Penicillium oxalicum*	Delhi	Cob	OQ732709.1
2	CTd-C-EPI-5C	*Fusarium equiseti*	Delhi	Cob	OQ732710.1
3	CTd-C-EPI-6A	*Penicillium oxalicum*	Delhi	Cob	OQ732711.1
4	CTd-C-EPI-6D	*Penicillium oxalicum*	Delhi	Cob	OQ732712.1
5	CTd-L-EPI-1F	*Penicillium* sp.	Delhi	Leaf	OQ732713.1
6	CTd-L-EPI-2B	*Fusarium equiseti*	Delhi	Leaf	OQ732714.1
7	CTd-L-EPI-2C	*Fusarium equiseti*	Delhi	Leaf	OQ732715.1
8	CTd-L-EPI-6C	*Penicillium oxalicum*	Delhi	Leaf	OQ732716.1
9	CTd-R-ENDO-19A	*Aspergillus fumigatus*	Delhi	Root	OQ732717.1
10	CTd-R-EPI-9B	*Penicillium oxalicum*	Delhi	Root	OQ732718.1
11	CTd-R-EPI-9C	*Aspergillus flavus*	Delhi	Root	OQ732719.1
12	CTd-R-EPI-9E	*Penicillium raperi*	Delhi	Root	OQ732720.1
13	ZTd-b-EPI-15C	*Penicillium* sp.	Delhi	Root	OQ732721.1
14	ZTd-C-EPI-7B	*Penicillium oxalicum*	Delhi	Cob	OQ732722.1
15	ZTd-C-EPI-7E	*Penicillium oxalicum*	Delhi	Cob	OQ732723.1
16	ZTd-C-EPI-8B	*Fusarium equiseti*	Delhi	Cob	OQ732724.1
17	ZTd-L-EPI-3B	*Fusarium cf. incarnatum*	Delhi	Leaf	OQ732725.1
18	ZTd-L-EPI-3C	*Penicillium oxalicum*	Delhi	Leaf	OQ732726.1
19	ZTd-L-EPI-4C	*Fusarium incarnatum*	Delhi	Leaf	OQ732727.1
20	ZTd-R-ENDO-18A	*Purpureocillium lilacinum*	Delhi	Root	OQ732728.1
21	CTd-L-EPI-11B	*Fusarium verticillioides*	Delhi	Leaf	OQ732729.1

In-vitro assay for biocontrol ability of endophytic fungi of Bihar

All isolates obtained from *rabi* maize of Bihar location were evaluated through the dual culture assay. Details inhibition (%) of pathogen (*R. solani* f. sp. *sasakii*) by *Alternaria alternata* in range of 9.22–25.54, *Aspergillus niger* in range of 14.16–47.81, *Aspergillus terreus* in range of 5.32–19.17, different species of *Cladosporium* in range of 5.18–14.45, and different species of *Curvularia*, *Fusarium*, *Kalmusia*, *Mucor*, *Penicillium*, and *Sarocladium* showed the mycelial inhibition below 15 percent. *Aspergillus niger* showed myceial inhibition in the range of 14.16 to 47.81, and the isolate RT-L-EPI-9D was considered the most efficient among those obtained from maize (Table 3.3). The standard radial growth of endophytic fungi was measured at 28°C in PDA medium, which irrespectively showed suppressive effects on the pathogen of maize during the dual culture assay (**Supplementary Fig. 3.1**).

Table 3.3

In-vitro assay for the assessment of biocontrol potential of endophytic fungal isolates of Bihar

Sl. No.	Isolate Name	Identified fungus	Location of collection	Mean of radial growth (mm/day)	Mycelial Inhibition of pathogen (*R. solani* f.sp. *sasakii*) (%)
1	RT-L-ENDO-44A	*Alternaria alternata*	Patna, Bihar	7.92	$9.22^{nopqrst}$
2	RT-C-EPI-8F	*Alternaria alternata*	Patna, Bihar	7.86	20.36^{efghi}
3	CT-L-EPI-16A	*Alternaria alternata*	Patna, Bihar	7.55	22.11^{defg}
4	CT-L-EPI-12A	*Alternaria alternata*	Patna, Bihar	5.80	25.54^{cde}
5	CT-C-EPI-15A	*Alternaria alternata*	Patna, Bihar	6.84	22.51^{cdef}
6	RT-C-EPI-8F	*Alternaria alternata*	Patna, Bihar	6.82	16.77^{ghijkl}
7	RT-C-EPI-3B	*Alternaria alternata*	Patna, Bihar	7.13	11.37^{lmnopq}
8	RT-C-EPI-3C	*Alternaria tenuissima*	Patna, Bihar	7.29	$9.63^{nopqrst}$
9	RT-C-EPI-2C	*Alternaria* sp.	Patna, Bihar	6.88	26.92^{cd}
10	RT-L-EPI-9D	*Aspergillus niger*	Patna, Bihar	3.82	47.817^{a}
11	CT-C-EPI-15B	*Aspergillus niger*	Patna, Bihar	3.63	20.62^{efgh}
12	CT-RS-EPI-27B	*Aspergillus niger*	Patna, Bihar	4.20	27.37^{cd}
13	RT-RS-EPI-33A	*Aspergillus niger*	Patna, Bihar	3.81	33.33^{b}
14	CT-R-EPI-26A	*Aspergillus* sp.	Patna, Bihar	3.67	14.16^{jklmn}
15	CT-RS-EPI-42A	*Aspergillus terreus*	Patna, Bihar	8.08	5.32^{rst}
16	CT-L-EPI-14C	*Aspergillus terreus*	Patna, Bihar	4.73	8.10^{pqrst}
17	CT-C-EPI-22B	*Aspergillus terreus*	Patna, Bihar	4.77	9.78^{nopqrs}
18	RT-Bulk-EPI-29A	*Aspergillus terreus*	Patna, Bihar	4.32	8.15^{opqrst}
19	RT-RS-EPI-34A	*Aspergillus terreus*	Patna, Bihar	3.64	13.84^{jklmno}

Sl. No.	Isolate Name	Identified fungus	Location of collection	Mean of radial growth (mm/day)	Mycelial Inhihition of pathogen (*R. solani* f.sp. *sasakii*) (%)
20	RT-R-EPI-13A	*Aspergillus terreus*	Patna, Bihar	4.65	19.17 ^{fghij}
21	RT-RS-EPI-28C	*Aspergillus terreus*	Patna, Bihar	6.21	15.80^{hijklm}
22	RT-BS-EPI-31A	*Aspergillus terreus*	Patna, Bihar	4.19	14.21^{jklmn}
23	RT-Bulk-EPI-29B	*Aspergillus terreus*	Patna, Bihar	5.56	14.59^{ijklmn}
24	RT-C-EPI-11B	*Aspergillus terreus*	Patna, Bihar	5.77	9.73^{nopqrs}
25	RT-C-EPI-11F	*Aspergillus terreus*	Patna, Bihar	4.86	16.19^{hijklm}
26	RT-L-ENDO-42A	*Aspergillus terreus*	Patna, Bihar	4.36	16.59^{ghijkl}
27	RT-L-ENDO-44A	*Alternaria alternata*	Patna, Bihar	7.92	9.22^{nopqrst}
28	RT-C-EPI-8F	*Alternaria alternata*	Patna, Bihar	7.86	20.36^{efghi}
29	CT-L-EPI-16A	*Alternaria alternata*	Patna, Bihar	7.55	22.11^{defg}
30	CT-L-EPI-12A	*Alternaria alternata*	Patna, Bihar	5.80	25.54^{cde}
31	CT-C-EPI-15A	*Alternaria alternata*	Patna, Bihar	6.84	22.51^{cdef}
32	RT-C-EPI-8F	*Alternaria alternata*	Patna, Bihar	6.82	16.77^{ghijkl}
33	RT-C-EPI-3B	*Alternaria alternata*	Patna, Bihar	7.13	11.37^{lmnopq}
34	RT-L-EPI-2F	*Cladosporium cladosporioides*	Patna, Bihar	2.78	6.45^{qrst}
35	CT-L-EPI-16C	*Cladosporium cladosporioides*	Patna, Bihar	2.77	6.37^{qrst}
36	CT-C-EPI-22D	*Cladosporium oxysporum*	Patna, Bihar	2.74	5.18^{rst}
37	CT-L-EPI-6A	*Cladosporium* sp.	Patna, Bihar	4.14	8.89^{nopqrst}
38	RT-L-EPI-4F	*Cladosporium tenuissimum*	Patna, Bihar	2.45	10.71^{mnopqr}
39	RT-L-EPI-4H	*Cladosporium tenuissimum*	Patna, Bihar	4.25	14.25^{jklmn}

Sl. No.	Isolate Name	Identified fungus	Location of collection	Mean of radial growth (mm/day)	Mycelial Inhihition of pathogen (*R. solani* f.sp. *sasakii*) (%)
40	RT-L-EPI-9C	*Cladosporium xanthochromaticum*	Patna, Bihar	3.37	14.45^{jklmn}
41	RT-BS-EPI-30A	*Curvularia lunata*	Patna, Bihar	5.42	16.07^{hijklm}
42	RT-C-EPI-17A	*Fusarium fujikuroi*	Patna, Bihar	4.74	15.88^{hijklm}
43	CT-B.S-EPI-35A	*Fusarium neocosmosporillum*	Patna, Bihar	3.66	4.72st
44	RT-L-EPI-2G	*Fusarium proliferatum*	Patna, Bihar	4.45	17.64^{fghijk}
45	RT-C-EPI-18B	*Kalmusia italica*	Patna, Bihar	5.10	12.47^{klmnop}
46	RT-C-EPI-10G	*Mucor fragilis*	Patna, Bihar	11.17	19.59^{fghij}
47	CT-L-EPI-20A	*Penicillium expansum*	Patna, Bihar	6.75	20.62^{efgh}
48	RT-L-EPI-21A	*Penicillium* sp.	Patna, Bihar	8.34	27.65^{c}
49	RT-C-EPI-24B	*Penicillium chermesinum*	Patna, Bihar	6.78	16.14^{hijklm}
50	CT-L-ENDO-45A	*Sarocladium implicatum*	Patna, Bihar	9.90	3.67^{t}
		Coeff Var		4.29	18.74
		CD		CD (0.01) = 0.50 CD (0.05) = 0.37	CD (0.01) = 6.378 CD (0.05) = 4.813

In-vitro assay for biocontrol ability of endophytic fungi of Delhi

Fungal isolates of Delhi were assessed for antagonistic activity against *R. solani* f. sp. *sasakii* using dual culture assays. Mycelial inhibition varied among genera: *Aspergillus* isolates exhibited 33.60–37.03% inhibition, *Fusarium* isolates 12.21–23.12%, *Penicillium* isolates 24.60–53.14%, and *Purpureocillium* 25.92%. Among the *Aspergillus* isolates, *A. fumigatus* (CTd-R-ENDO-19A) and *A. flavus* (CTd-R-EPI-9C) demonstrated inhibition rates of 33.60–37.03%. Seven *Penicillium oxalicum* isolates (CTd-C-EPI-5B, ZTd-C-EPI-7B, ZTd-C-EPI-7E, CTd-C-EPI-6A, CTd-L-EPI-6C, ZTd-L-EPI-3C, and CTd-L-EPI-1F) exhibited inhibition ranging from 24.60–53.14%, with CTd-C-EPI-5B and CTd-L-EPI-1F showing the highest antagonistic efficacy among all maize-derived isolates. Antagonistic activity was further evaluated under a temperature gradient with mean inhibition values presented in Table 3.4. Radial growth measurements of endophytic fungi were standardized at 28°C on PDA medium; all tested isolates exhibited suppressive effects against *R. solani* f. sp. *sasakii* under these conditions (**Supplementary Fig. 3.2**).

Table 3.4

In-vitro assay for the assessment of biocontrol potential of endophytic fungi of Delhi, India

Sr. No.	Endophytic Isolates Code	Endophytic fungal species	Mean of radial growth (RG) (mm/day; Temp: 28^0C)	Mycelial growth inhibition (%) of *R. solani* f. sp. *sasakii*) (Temp: 28, 30 and 32^0C)	Least Sign. Diff.
1	CTd-R-ENDO-19A	*Aspergillus fumigatus*	3.47	33.604bc	6.77
2	CTd-R-EPI-9C	*Aspergillus flavus*	3.43	37.03^{b}	6.58
3	ZTd-L-EPI-3B	*Fusarium incarnatum*	3.93	12.217^{h}	7.56
4	CTd-C-EPI-5C	*Fusarium equiseti*	3.66	15.55^{h}	7.55
5	ZTd-C-EPI-8B	*Fusarium equiseti*	3.87	16.09^{h}	7.53
6	CTd-L-EPI-2B	*Fusarium equiseti*	4.17	23.12^{fg}	7.43
7	CTd-L-EPI-2C	*Fusarium equiseti*	4.22	17.68^{gh}	7.51
8	ZTd-L-EPI-4C	*Fusarium incarnatum*	4.14	18.44^{gh}	7.49
9	CTd-L-EPI-11B	*Fusarium verticillioides*	3.49	18.68^{gh}	7.47
10	CTd-C-EPI-5B	*Penicillium oxalicum*	8.32	53.133^{a}	6.25
11	ZTd-C-EPI-7B	*Penicillium oxalicum*	7.23	24.603^{efg}	7.4
12	ZTd-C-EPI-7E	*Penicillium oxalicum*	7.89	32.87^{bcd}	6.93
13	CTd-C-EPI-6A	*Penicillium oxalicum*	6.98	27.95^{cdef}	7.27
14	CTd-L-EPI-6C	*Penicillium oxalicum*	7.33	29.44^{cdef}	7.21
15	ZTd-L-EPI-3C	*Penicillium oxalicum*	8.33	29.997^{cdef}	7.14
16	CTd-L-EPI-1F	*Penicillium* sp.	7.49	53.14^{a}	0.00
17	ZTd-b-EPI-15C	*Penicillium* sp.	9.13	31.203^{bcde}	7.04
18	CTd-R-EPI-9E	*Penicillium raperi*	7.54	26.48^{def}	7.32
19	ZTd-R-ENDO-18A	*Purpureocillium lilacinum*	5.8	25.923^{def}	7.36
		C V	5.49	13.87	
		CD	CD (0.01) = 0.766 CD (0.05) = 0.571	CD (0.01) = 8.525 CD (0.05) = 6.363	

(Dual culture method of in vitro assay tested on PDA media; horizontal radial measures used for mycelial inhibition under temperature gradients (28, 30 and 32^{0}C).

In-planta assays of biocontrol potential of endophytic fungi against BLSB pathogen

A total of 30 endophytic fungal isolates, 8 from Delhi and 22 from Bihar, sourced from leaf (11), cob (9), and root (11) tissues were evaluated for biocontrol efficacy against *Rhizoctonia solani* f. sp. *sasakii* on susceptible maize variety Vivek QPM-9. Selected isolates, pre-screened through *in vitro* antagonistic assays, were applied as seed treatment and foliar spray (10^{-6} spore suspension) to establish endophytic colonization before pathogen inoculation into internodes at V6–V8 stage (35 to 50 days after planting). Under controlled conditions (95% RH, 28–30°C) in the National Phytotron Facility, lesion development was assessed, revealing differential suppression of disease symptoms across isolates and application methods. The percent disease index (PDI) on Vivek QPM-9 inoculated with *R. solani* f. sp. *sasakii* varied significantly among endophytic fungi. *Aspergillus niger* isolates resulted the most effective disease suppression, with PDI values ranging from 13.98–29.89, while *Penicillium* isolates (PDI: 18.37–35.38) and *Aspergillus flavus* (PDI: 32.32) also showed notable biocontrol efficacy. Moderate disease management was achieved by *Alternaria* species (PDI: 54.99–70.47), *Aspergillus terreus* (PDI: 43.53–79.58), *Cladosporium* sp. (PDI: 56.54–74.18), and *Sarocladium implicatum* (PDI: 67.26), while *Purpureocillium lilacinum* (PDI: 58.46) and *Alternaria tenuissima* (PDI: 69.03) exhibited intermediate biocontrol potential across their respective isolates. Based on *in planta* assay results, three isolates, *Aspergillus niger* (RT-L-EPI-9D) from Bihar and *Penicillium oxalicum* (CTd-C-EPI-5B and CTd-L-EPI-1F) from Delhi were selected for further field trials, exhibiting resistant disease reaction with PDI values of 13.98, 18.37, and 18.59, respectively (Table 3.5) (**Supplementary Fig. 3.3**) demonstrating efficient biocontrol potential against BLSB disease in maize.

Table 3.5
Biocontrol potential of endophytic fungi isolated from Delhi and Bihar

Sl. No	Isolate	Scientific name	Organs	Stage of crop	Inter Node	Lesion length	PDI	DSR
1	CT-L-EPI-12A	*Alternaria alternata*	L	V6-V8	9.49	6.05	63.80	16.95
2	RT-C-EPI-8F	*Alternaria alternata*	C	V6-V8	10.33	5.68	54.99	25.77
3	CT-C-EPI-15A	Alternaria alternata	C	V6-V8	6.19	4.36	70.47	10.29
4	RT-C-EPI-3C	Alternaria tenuissima	C	V6-V8	10.51	7.26	69.03	11.73
5	CTd-R-EPI-9C	Aspergillus flavus	R	V6-V8	8.22	2.66	32.32	48.43
6	CTd-R-ENDO-19A	Aspergillus fumigatus	R	V6-V8	8.22	3.27	39.74	41.01
7	CT-C-EPI-15B	Aspergillus niger	C	V6-V8	10.38	3.08	29.63	51.12
8	RT-RS-EPI-33A	Aspergillus niger	R	V6-V8	10.58	3.16	29.89	50.87
9	RT-L-EPI-9D	Aspergillus niger	L	V6-V8	10.80	1.51	13.98	66.78
10	CT-RS-EPI-27B	Aspergillus niger	R	V6-V8	7.19	2.10	29.20	51.56
11	CT-R-EPI-26A	Aspergillus sp.	R	V6-V8	9.81	4.38	44.67	36.09
12	RT-BS-EPI-31A	Aspergillus terreus	R	V6-V8	8.29	5.51	66.45	14.30
13	RT-Bulk-EPI-29B	Aspergillus terreus	R	V6-V8	12.60	6.51	51.65	29.10
14	CT-C-EPI-22B	Aspergillus terreus	C	V6-V8	10.21	4.44	43.53	37.23
15	RT-C-EPI-11F	Aspergillus terreus	C	V6-V8	7.98	6.35	79.58	1.18
16	CT-RS-EPI-42A	Aspergillus terreus	R	V6-V8	10.24	5.25	51.23	29.53
17	RT-L-EPI-2F	Cladosporium cladosporioides	L	V6-V8	8.08	5.35	66.19	14.57
18	CT-L-EPI-6A	Cladosporium sp	L	V6-V8	12.68	7.17	56.54	24.22

(PDI- Percent Disease Index, DRS- Disease Severity Reduction, L-leaf, C-Cob, R-root, Vegetative stage of maize selected for in planta assay: V6-V8)

Sl. No	Isolate	Scientific name	Organs	Stage of crop	Inter Node	Lesion length	PDI	DSR
1	CT-L-EPI-12A	*Alternaria alternata*	L	V6-V8	9.49	6.05	63.80	16.95
2	RT-C-EPI-8F	*Alternaria alternata*	C	V6-V8	10.33	5.68	54.99	25.77
19	RT-L-EPI-9C	Cladosporium xanthochromaticum	L	V6-V8	7.38	5.48	74.18	6.57
20	RT-C-EPI-24A	Penicillium chermesinum	C	V6-V8	9.75	3.45	35.38	45.37
21	CT-L-EPI-20A	Penicillium expansum	L	V6-V8	9.92	3.07	30.91	49.85
22	ZTd-L-EPI-3C	Penicillium oxalicum	L	V6-V8	9.26	3.48	37.58	43.18
23	CTd-C-EPI-5B	Penicillium oxalicum	C	V6-V8	11.05	2.03	18.37	62.39
24	CTd-C-EPI-6A	Penicillium oxalicum	C	V6-V8	8.22	2.79	33.95	46.80
25	CTd-R-EPI-9E	Penicillium raperi	R	V6-V8	9.15	2.24	24.51	56.24
26	RT-L-EPI-21B	Penicillium sp	L	V6-V8	11.86	6.41	54.07	26.69
27	RT-L-EPI-21B	Penicillium sp.	L	V6-V8	11.47	6.39	55.70	25.06
28	CTd-L-EPI-1F	Penicillium sp.	L	V6-V8	11.06	2.05	18.59	62.16
29	ZTd-R-ENDO-18A	Purpureocillium lilacinum	R	V6-V8	8.38	4.89	58.46	22.30
30	CT-L-ENDO-45A	Sarocladium implicatum	L	V6-V8	10.36	6.97	67.26	13.50
	Control (mock)			V6-V8	11.58	9.24	79.77	00.00
	Control (mock)			V6-V8	10.41	8.507	81.74	00.00
		C V			3.32	5.09		
		CD (0.05)			0.52	0.39		

(PDI- Percent Disease Index, DRS- Disease Severity Reduction, L-leaf, C-Cob, R-root, Vegetative stage of maize selected for in planta assay: V6-V8)

Efficacy of selected endophytic fungi under field conditions

Field-level biocontrol efficacy of three endophytic isolates *Aspergillus niger* (RT-L-EPI-9D), *Penicillium oxalicum* (CTd-L-EPI-1F), and *Penicillium oxalicum* (CTd-C-EPI-5B) was evaluated against BLSB disease on Vivek QPM-9 by seed

treatment (S) and combined seed + foliar spray (S + F) applications. Disease severity was assessed at 10 and 20 DAI. The RT-L-EPI-9D resulted PDI values of 41.30 and 52.93 at 10 and 20 DAI, respectively (Table 3.6), with 31.69% yield increment over control **(Supplementary Fig. 3.4)**. CTd-L-EPI-1F recorded PDI of 23.02 and 61.62, yielding 32.11% increment, while CTd-C-EPI-5B showed PDI of 42.22 and 60.73 with 33.79% yield increment. Combined S + F treatment consistently reduced disease severity and increased yield compared to S treatment alone and control, with S + F demonstrating superior biocontrol efficacy under field conditions (Fig. 3.1).

Table 3.6
Effect of selected fungal isolates RT-L-EPI-9D (*Aspergillus niger*), CTd-L-EPI-1F (*Pencillium oxalicum*), and CTd-C-EPI-5B (*Pencillium oxalicum*) on BLSB disease and grain yield of maize (variety Vivek QPM-9) under field conditions

Isolates	**Treatments used for efficacy test**		**1st score**			**2nd Score**				**Increment in Yield (%)**
			Lesion length* (cm)	**Disease Score (1–5 scale)**	**PDI (%)**	**Lesion length (cm)**	**Disease Score (1–5 scale)**	**PDI (%)**	**Yield (Q/ha)**	
Aspergillus niger	RT-L-EPI-9D	S + F	25.23	2.14	41.302	33.7	2.815	52.93	32.69	31.69
Penicillium oxalicum	CTd-L-EPI-1F	S + F	15.235	1.39	23.025	38.205	3.225	61.62	32.115	31.115
Penicillium oxalicum	CTd-C-EPI-5B	S + F	27.19	2.25	42.22	38.1	3.11	60.73	34.79	33.79
Control	Mock	S + F	24.435	2.05	42.105	43.385	3.63	70.34	27.425	-
Aspergillus niger	RT-L-EPI-9D	S	25.655	2.11	41.245	38.025	3.065	61.08	30.22	29.22
Penicillium oxalicum	CTd-L-EPI-1F	S	21.27	1.68	32.835	43.11	3.51	71.03	28.305	27.305
Penicillium oxalicum	CTd-C-EPI-5B	S	30.225	2.56	50.685	52.315	4.29	82.28	27.81	26.81
Control	mock	S	30.815	2.61	50.935	53.13	4.54	83.72	27.77	-

(S-Seed Treatment; S + F-seed treatment + Foliar application)

Bio-priming-induced defence genes in maize seedlings

Seed bio-priming with the three fungal isolates significantly altered the expression of defence-related enzymes in maize seedlings relative to the mock control, with clear differences across genes, time points, and isolates (two-way ANOVA, Bonferroni post-test; $p < 0.05$ to $p < 0.0001$). β-1,3-glucanase activity increased rapidly following priming, peaking at 24 h in PEN2-treated seedlings (~ 5.2-fold change relative to mock, $p < 0.01$), while ASPR-treated seedlings showed a smaller but significant increase at the same time point ($p < 0.05$). PEN1 exhibited its maximum

induction slightly later, at 48 h (~ 2.6-fold, $p < 0.001$). Expression declined across all treatments by 96 h, with PEN2 falling significantly below mock levels ($p < 0.05$). Superoxide dismutase (SOD) followed a similar early-response pattern, with PEN2 producing the highest fold-changes of any enzyme measured, approximately 5.2-fold at 24 h and 3.7-fold at 48 h ($p < 0.05$) substantially exceeding PEN1 and ASPR at these time points (Fig. 3.2). A modest but significant increase was also recorded for PEN2 at 96 h ($p < 0.05$). In contrast, catalase (CAT) activity increased more gradually, with PEN1 and PEN2 reaching peak induction at 48 h (~ 0.8-fold and 0.85-fold, respectively; $p < 0.001$) and remaining elevated at 72 h. ASPR showed a delayed response, with its highest expression recorded at 96 h (~ 0.78-fold). Peroxidase (POX) activity rose significantly from 24 h onward ($p < 0.001$), with PEN1 and PEN2 sustaining elevated levels through 48 h. PEN2 reached its peak at 72 h (~ 2.9-fold), while ASPR showed significant increases only at the later time points, 72 h and 96 h ($p < 0.01$). Polyphenol oxidase (PPO) displayed the largest cumulative induction among all enzymes assayed, increasing progressively across the time course. Significant differences from mock were recorded from 48 h onward for PEN1 and PEN2 ($p < 0.05$ to $p < 0.01$), with PEN2 showing the highest overall induction, reaching approximately 8-fold at 96 h (Fig. 3.2). Across all five enzymes, PEN2 (*Penicillium oxalicum*, CTd-C-EPI-5B) consistently produced the highest and most statistically significant fold-changes, followed by PEN1 (*Penicillium oxalicum*, CTd-L-EPI-1F), which showed moderate but significant induction (up to 3.5–4-fold in β-1,3-glucanase and POX). ASPR (*Aspergillus niger*, RT-L-EPI-9D) generally recorded the lowest fold-changes, typically below 2-fold, with responses that were both delayed in onset and reduced in magnitude relative to the two *P. oxalicum* isolates (**Supplementary table 3.1**).

4. Discussion

The endophytic mycobiome associated with maize in the present study comprised diverse fungal genera such as *Fusarium, Penicillium, Cladosporium, Alternaria, Aspergillus, Sarocladium, Mucor*, and *Kalmusia* from Bihar, and *Fusarium, Penicillium, Aspergillus*, and *Purpureocillium* from Delhi reflecting the compositional richness of the culturable maize mycobiome across the two agro-climatic regions surveyed. This diversity is broadly consistent with earlier reports describing *Fusarium, Trichoderma, Penicillium, Aspergillus, Sarocladium*, and *Colletotrichum* as dominant endophytic colonisers of maize tissues, with their distribution shaped by tissue type, geography, and agronomic practice (Sanglard et al., 2017; Fisher et al., 1992; Awais et al., 2024). Seasonal and cultivar-driven shifts in mycobiome structure have also been documented, with *Aspergillus, Fusarium, Penicillium, Sarocladium, Stenocarpella, Talaromyces*, and *Ustilago* frequently recovered under varying climatic conditions (Katati et al., 2023), and *Penicillium* and *Alternaria* consistently reported in the maize phyllosphere, with certain cultivars favouring higher relative *Penicillium* abundance (Vilanova et al., 2018; Chavan et al., 2018; Kong et al., 2020). Tissue-specific partitioning of the maize mycobiome viz., *Penicillium, Pleosporales, Fusarium, Sarocladium*, and *Rhizomucor* in leaves versus *Penicillium, Gibberella, Fusarium*, and *Aspergillus* in roots (Potshangbam et al., 2017), further supports the ecological plausibility of recovering *Aspergillus*- and *Penicillium*-dominant isolates as candidate biocontrol potential group in the present work.

Screening of these isolates for antagonism against *Rhizoctonia solani* f. sp. *sasakii* revealed marked inter- and intra-generic variation in mycelial inhibition, with *Aspergillus niger* isolates from Bihar inhibiting the pathogen by 14.16–47.81% and *Penicillium oxalicum* isolates from Delhi by 24.60–53.14%, while other genera such as *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Kalmusia*, *Mucor*, and *Sarocladium* showed comparatively weaker inhibition. On the basis of in planta performance, three isolates such as *A. niger* (RT-L-EPI-9D) and *P. oxalicum* (CTd-C-EPI-5B and CTd-L-EPI-1F) were selected for further evaluation, having produced resistant disease reactions with PDI values of 13.98, 18.37, and 18.59, respectively, at 25 days after inoculation. These findings are consistent with the growing body of evidence that endophytic fungi can suppress plant pathogens by limiting colonisation and disease

progression (Narayanan et al., 2022; Shofman et al., 2025; Muhammad et al., 2024). Comparable antagonism has been reported for other endophytic *Aspergillus* and *Penicillium* species: an endophytic *Aspergillus fumigatus* strain from corn inhibited *A. flavus* by approximately 77% while thickening host cell walls and inducing antioxidant enzymes (Aji & Rofiyanti, 2025); *Trichoderma asperellum*, *Chaetomium cochliodes*, and *Penicillium citrinum* reduced *Magnaporthiopsis maydis* inoculum and promoted plant growth when applied as seed or endophyte inoculants (Degani et al., 2021); and *Aspergillus terreus* and *Penicillium citrinum* from sunflower induced resistance and improved biomass against *Sclerotium rolfsii* (Waqas et al., 2015). Similarly, *Aspergillus tubingensis* conferred the strongest protection against Fusarium wilt in pepper among related endophytic inducers (Attia et al., 2022a), *Aspergillus terreus* from legume hosts suppressed *R. solani*-induced damping-off (Abdelaziz et al., 2023), and *Penicillium bilaiae* has been validated as a safe biocontrol agent against *R. solani* in field trials (Nicoletti et al., 2004). Taken together, these parallels reinforce *Aspergillus* and *Penicillium* species as a recurrent and reliable source of biocontrol activity against Rhizoctonia-incited diseases across host systems.

Under field conditions, the selected isolates translated their antagonistic potential into practical disease suppression and yield benefit: RT-L-EPI-9D recorded PDI values of 41.30 and 52.93 at 10 and 20 DAI with a 31.69% yield increment over control; CTd-L-EPI-1F recorded PDI of 23.02 and 61.62 with a 32.11% yield increment; and CTd-C-EPI-5B recorded PDI of 42.22 and 60.73 with the highest yield increment of 33.79%. These results parallel earlier field-scale observations in which endophytic Aspergillus species such as *A. flavus* and *A. tubingensis* were used to control seedling infection and preserve corn yield (Terna et al., 2021), underscoring that the yield gains observed here are consistent with the established capacity of native endophytic biocontrol agents to protect maize productivity under field conditions rather than being an isolated outcome.

Seed bio-priming with the three isolates *A. niger* (RT-L-EPI-9D; ASPR), *P. oxalicum* (CTd-L-EPI-1F; PEN-1), and *P. oxalicum* (CTd-C-EPI-5B; PEN-2) significantly induced defence-related enzymes in maize seedlings in a time- and isolate-dependent manner. β-1,3-glucanase and superoxide dismutase (SOD) peaked early, within 24–48 h, while catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO) showed sustained, late-phase induction extending to 96 h. *Penicillium oxalicum* (CTd-C-EPI-5B) consistently elicited the strongest responses among the three isolates across nearly all genes assayed. This two-phase induction pattern mirrors defence signalling dynamics reported for other endophyte–cereal interactions. In eggplant, endophytic *Aspergillus terreus* applied against *Alternaria solani* triggered expression of peroxidase (POD) and polyphenol oxidase (PPO), implicating these enzymes in endophyte-mediated protection (Attia et al., 2022b), consistent with the sustained POX and PPO induction observed here. In pearl millet, *Penicillium chrysogenum* (PenC-JSB9) elicited rapid and enhanced expression of LOX and POX by 48 h and of chitotriosidase (CHT) as early as 24 h against downy mildew (Murali et al., 2013), a temporal pattern broadly analogous to the early β-1,3-glucanase/SOD burst and the subsequent oxidative-enzyme phase recorded in the present study. More generally, plant–microbe interactions are known to reshape host defence at both the molecular and biochemical levels (Niemeyer & Perez, 1995), and endophytes such as *Purpureocillium lilacinum* and *Piriformospora indica* have been shown to trigger long-lasting, non-specific systemic resistance in cereals through sustained upregulation of defence genes, even in tissues distant from the colonisation site (Kimotho et al., 2024; Waller et al., 2005; Fontana et al., 2021) — a feature consistent with the durability of the antioxidant response seen through 96 h in the present study. Field-level induction of PPO and CAT by fungal endophytes in maize has similarly been reported (Byregowda et al., 2025), supporting the physiological relevance of the enzyme dynamics observed under controlled bio-priming conditions.

Collectively, the present findings indicate that *A. niger* (RT-L-EPI-9D) and *P. oxalicum* (CTd-L-EPI-1F, CTd-C-EPI-5B), isolated as native components of the maize mycobiome, act through a dual mode of action: direct antagonism against *R. solani* f. sp. *sasakii* and priming of host defence enzymes, with *P. oxalicum* (CTd-C-EPI-5B) emerging as the

most effective isolate across biocontrol efficacy, field disease suppression, yield enhancement, and defence-enzyme activation. This dual mechanism is consistent with the broader literature on endophyte-mediated protection, in which biocontrol and induced systemic resistance frequently act in concert rather than in isolation, and it positions these native isolates as promising, environmentally compatible candidates for the sustainable management of banded leaf and sheath blight in maize.

5. Conclusion

This study demonstrates that native endophytic isolates from the maize mycobiome; *Aspergillus niger* (RT-L-EPI-9D) and *Penicillium oxalicum* (CTd-L-EPI-1F and CTd-C-EPI-5B)—offer a dual mode of protection against BLSB disease caused by *Rhizoctonia solani* f. sp. *sasakii*. *In vitro* screening confirmed strong mycelial inhibition, while *in planta* and field trials validated resistant disease reactions (PDI 13.98–18.59 at 25 DAI) alongside substantial yield gains (31.69–33.79% over control). Beyond direct antagonism, seed bio-priming with these isolates activated a time- and isolate-dependent defence-enzyme response in maize seedlings, marked by an early burst of β-1,3-glucanase and SOD (24–48 h) followed by sustained induction of CAT, POX, and PPO through 96 h patterns consistent with a primed innate immune state. *Penicillium oxalicum* (CTd-C-EPI-5B) consistently outperformed the other isolates across all parameters, emerging as the most promising candidate. Together, these findings position *A. niger* and *P. oxalicum* as effective, environmentally sustainable bio-priming agents that combine direct biocontrol with induced systemic resistance, offering a practical strategy for managing BLSB in the field and improving maize productivity.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest associated with the manuscript

Ethical Statement

Our manuscript entitled '**Bio-Priming with Endophytic Mycobiome-Derived Penicillium oxalicum and Aspergillus niger Activates Defence Enzymes in Maize and Suppresses Banded Leaf and Sheath Blight Disease**' complies with the Ethical Rules applicable to the journal.

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Author Contribution

VV: Methodology; Investigation Formal analysis; Data curation; Visualization; Writing – original draft.RG: Conceptualization; Supervision; Project administration; Resources; Validation; Writing – review & editing. Corresponding author.AK: Conceptualization; Supervision; Methodology; Resources; Validation; Writing – review & editing. Corresponding authorVI: Data curation.

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Data Availability

The datasets presented in this study can be found in online repositories (GenBank).

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Figures

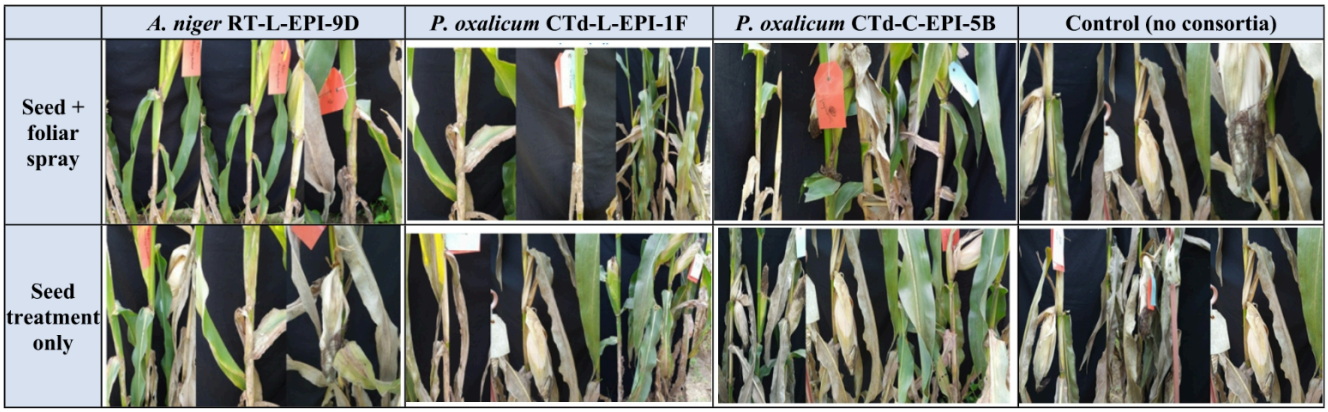


Figure 1

Figure 3.1. Field-level effect of fungal mycobiome consortia on banded leaf and sheath blight (BLSB) severity in the susceptible maize (Zea mays L.) variety Vivek QPM-9, photographed at 45 days after seed germination. Plants were

treated with *Aspergillus niger*RT-L-EPI-9D, *Penicillium oxalicum* CTd-L-EPI-1F, or *Penicillium oxalicum* CTd-C-EPI-5B, each applied either as a combined seed treatment plus foliar spray (top row) or as a seed treatment alone (bottom row), and compared with untreated control plants without consortia inoculation. Differences in leaf and sheath necrosis, senescence, and overall canopy health among treatments relative to the control indicate the protective effect of the applied mycobiome consortia against BLSB under natural field infection.

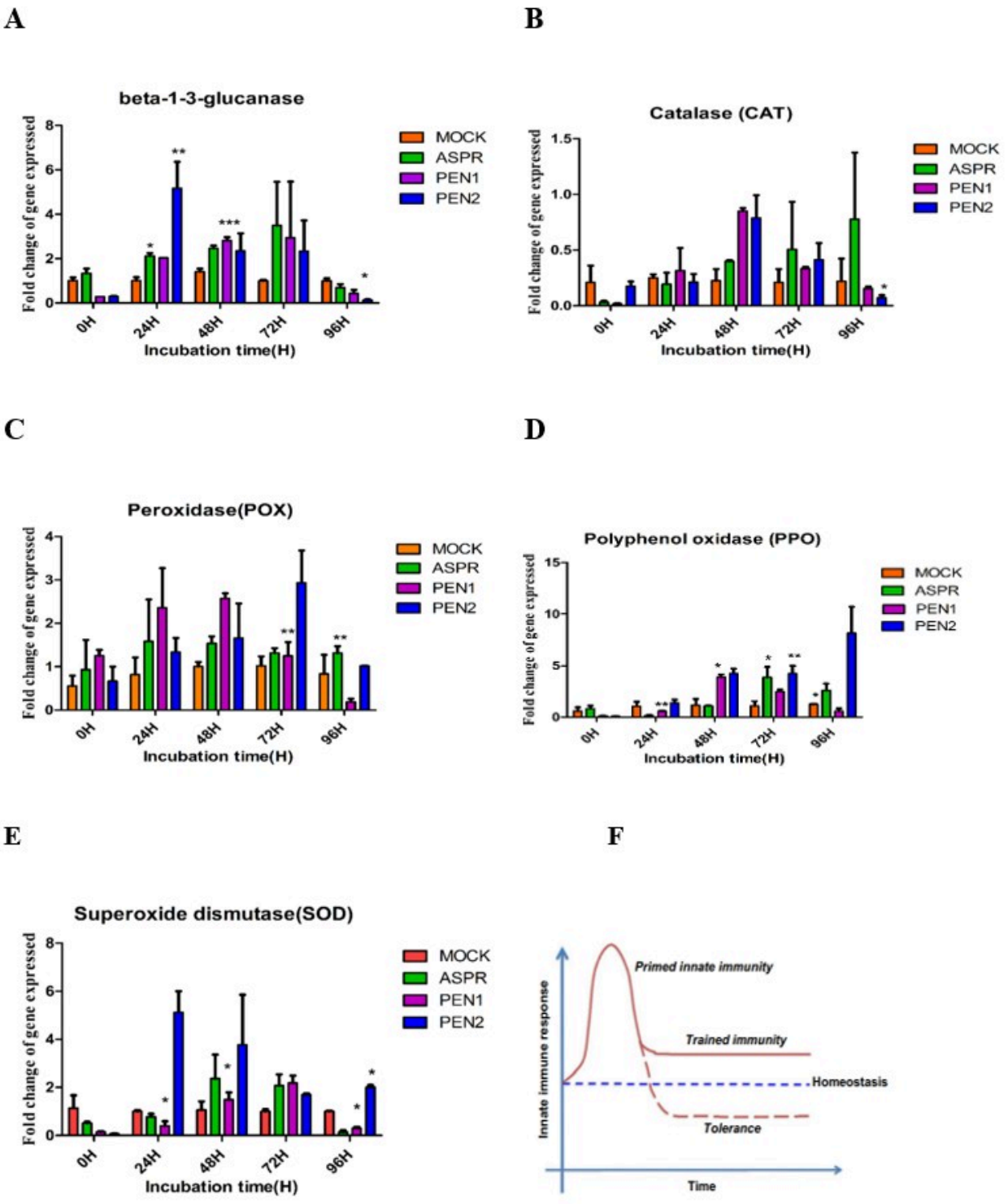


Figure 2

Figure 3.2. Relative expression of defence-related genes (A–E) in maize seedlings following seed bio-priming with selected fungal isolates — RT-L-EPI-9D (*Aspergillus niger*; ASPR), CTd-L-EPI-1F (*Penicillium oxalicum*; PEN1), and CTd-C-EPI-5B (*Penicillium oxalicum*; PEN2). Error bars represent the mean ± SD. Statistical significance was determined by two-way ANOVA with Bonferroni post-test relative to the mock control (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns, not significant). (F) Schematic representation of the generalized primed plant response under different stimulations.

Supplementary Files

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- floatimage1.png