Scientific and Technical Progress Report (STPR)[Final] (R&D projects)

on

DBT-NER Project entitled

'Harnessing Endophytes and Arbuscular Mycorrhizal Fungi from Citrus Microbiome for Plant and Soil Health Management in North East India

PPRN No: BT/PR40047/NER/95/1662/2020

Submitted by

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1. Project Title:	Harnessing Endophytes and Arbuscular Mycorrhizal Fungi from Citrus Microbiome for Plant and Soil Health Management in North East India
2. DBT Sanction Order No. & Date:	NO. BT/PR40047/NER/95/1662/2020 Dtd. February 10, 2021
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4. Date of Sanction:	10th February 2021
5. Date of Completion:	9th August 2025
6. Approved Cost:	Rs 20,82,120
7. Budget Released:	Rs 19,18,645
8. Objectives	1. Isolation, characterization and identification of endophytes (bacterial and fungal), Arbuscular Mycorrhizal Fungi (AMF) and plant growth promoting rhizobacteria (PGPR) from Citrus microbiome of NE region of India including Sikkim. 2. Elucidation of the mechanisms involved in growth promotion and disease suppression by endophytes, AM fungi and PGPR. 3. Development of a cost-effective microbial consortia of selected potential endophytes AM fungi and PGPR. 4. Validation of the potential microbial consortia in plant and soil health management of commercial citrus varieties.

9. METHODOLOGIES

Study area and sampling design

Plant tissue and rhizospheric soil samples were collected from *Citrus reticulata* (mandarin) and *Citrus limon* (lemon) plants grown across different agro-climatic zones of Arunachal Pradesh and Assam, India. Representative sites were selected to capture environmental variation with respect to altitude, rainfall, and soil type. At each location, five healthy, disease-free trees of each *citrus* species were randomly selected, ensuring a minimum spacing of 10 m between trees.

Plant tissue collection (Period 2021-2022)

From each tree, three types of tissues were collected: leaves, stems (young twigs), and fine roots. Fully expanded, asymptomatic leaves were excised from the mid-canopy using sterile scissors, while young twig segments (5–8 cm) were cut from secondary branches. Fine root samples (~2–3 cm segments) were carefully excavated near the dripline to obtain actively growing rootlets. All samples were handled using sterile gloves and instruments, which were surface-sterilized with 70% ethanol between collections to avoid cross-contamination. Plant tissues were placed individually in pre-labelled sterile polyethylene bags and immediately stored in insulated ice boxes (4–8 °C) for transport to the laboratory.

Rhizospheric soil collection (Period 2021-2022)

Rhizospheric soil was obtained by gently shaking root systems to recover soil adhering closely to the root surface. Additionally, soil cores were collected at 0–15 cm depth from three equidistant points around the tree canopy dripline using sterile spatulas. These subsamples were pooled to generate a composite rhizospheric soil sample (~200 g) per tree. The collected soil was placed into sterile bags, sealed, and stored at 4 °C until further processing.

Sample labelling and metadata recording

Each sample was labelled with a unique alphanumeric code indicating state, district, site, tree number, tissue/soil type, and date of collection. Metadata, including GPS coordinates, were recorded systematically for each sampling site.

Isolation of endophytic fungi (Period 2021-2022)

Isolation of endophytic and rhizospheric fungi was carried out within six hours of sample collection. Plant tissues obtained from *Citrus* spp. were first rinsed thoroughly with tap water to remove adhering debris, and subsequently cut into a total of 108 segments using sterile knives while wearing sterile gloves. These segments comprised 36 each of leaves, bark, and fine root branches, with an approximate size of 2 × 0.5 cm.

Surface sterilization of plant tissues was performed following the protocol of (Strobel et al., 2002) with slight modifications. Briefly, tissue segments were sequentially immersed in 70% ethanol for 3 min**,** followed by 4% sodium hypochlorite (NaClO) solution for 5 min, then 70% ethanol for 1

min, and finally 0.1% mercuric chloride ($HgCl_2$) for 3 min. The tissues were subsequently rinsed three times with sterile distilled water. To verify the effectiveness of sterilization, 200 µL of water from the third rinse was plated onto Potato Dextrose Agar (PDA) and incubated at 28 °C for 4 days. The absence of microbial growth confirmed successful surface sterilization (Schulz et al., 1993).

Endophytic fungi were then isolated by placing surface-sterilized tissue segments on three different fungal-specific growth media: Czapek-Dox Agar (CDA), Potato Dextrose Agar (PDA), and Rose Bengal Chloramphenicol Agar (RBCA)**.** All media were procured from HiMedia Laboratories Pvt. Ltd. (India). Each 90 × 15 mm Petri plate contained 25 mL of medium, and four tissue segments were placed diagonally per plate. For each tissue type, a total of 12 segments were inoculated across the three media. The plates were incubated at 28 ± 1 °C until visible mycelial growth emerged from the tissue segments.

Identification

Pure fungal isolates were identified on the basis of their colony morphology and microscopic reproductive features during growth on Potato Dextrose Agar (PDA). Microscopic examinations were conducted using a Leica DM-2400 microscope after staining the fungal structures with lactophenol cotton blue**.** The observed colony characteristics and reproductive structures were compared with descriptions provided in standard fungal identification manuals (Barnett & Hunter, 1998). Isolates that failed to produce spores under the given culture conditions were classified as *Mycelia sterilia.*

Estimation of colonization frequencies

The colonization frequency (CF%) of endophytic fungi was estimated by following the formula of (Hata & Futai, 1995):

$$\mathrm{CF} = \left(\frac{\mathrm{Ncol}}{\mathrm{Nt}}\right) \times 100$$

where,

N_{COL} = number of leaf/bark/root segments colonized by specific fungus

Nt = total number of leaf/bark/root segments plated

Diversity analysis

The composition of fungal species within plant parts was estimated by calculating alpha diversity. For this, the species richness (*S*) was estimated to determine the total number of fungal species from each of the plant parts (Margalef, 1973). The Shannon-Wiener index was used to measure diversity, accounting for richness and evenness using the following formula (Shannon, 1948).

$$H' = -\sum_{\{i=1\}}^{\{S\}} p_{\mathrm{i}} \ln(p_{\mathrm{i}})$$

Where,

H' = Shannon diversity index

S = total number of species (species richness)

p_i = proportion of individuals belonging to the i^{th} species (i.e., n_i/N), where n_i is the number of individuals of fungal species i, and N is the total number of individuals of all fungal species.

ln = natural logarithm

Simpson's index was measured to emphasise the dominance of the fungal species within the plant parts. The following formula was used to measure the Simpson's index (Simpson, 1949).

$$D = \sum_{\{i=1\}}^{\{S\}} p_i^2 = \sum_{\{i=1\}}^{\{S\}} (\frac{n_i}{N})^2$$

Where:

D = Simpson's index (measures dominance, so lower values indicate higher diversity)

n_i = number of individuals of fungal species i

N = total number of individuals of all fungal species

p_i = proportion of species i

S = total number of species

Additionally, Pielou's evenness was assessed to determine evenness of their distribution within the plant parts by using the following formula (Pielou, 1966).

$$J' = \frac{H'}{\ln(S)}$$

Where,

J' = Pielou's evenness

H' = Shannon-Weiner diversity index

S = total number of species (species richness)

ln = natural logarithm

Determination of soil physical properties (Period 2021-2022)

Soil bulk density

Soil bulk density was measured following the core method (Blake & Hartge, 1986). Undisturbed soil cores were collected from the rhizospheric zone (0–15 cm depth) using a stainless-steel cylindrical core sampler (5 cm internal diameter, 5 cm height). The cores were carefully trimmed flush at both ends to maintain a known volume **(**$V = \pi r^2 h$**)**. Fresh weight of the soil cores was recorded immediately after collection. Samples were oven-dried at 105 °C for 24 h, cooled in a desiccator, and reweighed. Bulk density (BD) was calculated using the equation:

$$\text{BD (g cm}^{-3}) = \frac{Wd}{V}$$

where,
Wd = oven-dry weight of soil (g)
V = volume of the core (cm^3)

Soil moisture content

Soil moisture content was determined using the gravimetric method (Shukla et al., 2014). Approximately 100 g of fresh rhizospheric soil was collected in pre-weighed aluminium containers. The initial fresh weight was recorded, after which samples were oven-dried at 105 °C for 24 h and cooled in a desiccator before reweighing. Soil moisture percentage was calculated as:

$$\text{Moisture content (\%)} = \frac{Wf - Wd}{Wd} x100$$

where,
Wf = fresh weight of soil (g)
Wd = oven-dry weight of soil (g)

Determination of soil chemical properties (Period 2021-2022)

Soil pH

Soil pH was measured in a soil–water suspension using a calibrated glass electrode pH meter (Brouder et al., 2005). Briefly, 10 g of air-dried, sieved (<2 mm) soil was mixed with 25 mL of deionized water to maintain a 1:2.5 (w/v) soil: water ratio. The suspension was stirred intermittently for 30 min before recording pH values once stabilized.

Soil organic carbon (SOC)

Soil organic carbon was estimated by the Walkley–Black wet oxidation method (Walkley & Black, 1934). A 0.5 g soil sample was digested with 1 N potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4). The residual dichromate was titrated against 0.5 N ferrous ammonium sulfate (FAS) solution using diphenylamine as an indicator. SOC (%) was calculated

from dichromate consumption and corrected with a factor of 1.3 to account for incomplete oxidation.

Available nitrogen

Available nitrogen was determined following the alkaline $KMnO_4$ method (Bv, 1956). In this procedure, 5 g of soil was digested with alkaline $KMnO_4$ solution, and the released ammonia was distilled and absorbed in boric acid, followed by titration with standard sulfuric acid.

Available phosphorus

Available phosphorus was estimated by the Olsen method (Olsen, 1954), suitable for neutral to alkaline soils. Two and a half grams of soil was extracted with 50 mL of 0.5 M $NaHCO_3$ (pH 8.5) for 30 min and filtered through Whatman No. 42 filter paper. The filtrate was treated with ammonium molybdate and stannous chloride, and the blue color intensity was measured spectrophotometrically at 882 nm. Concentrations were calculated against KH_2PO_4 standards and expressed as mg kg^{-1}.

Available potassium

Available potassium was determined by extraction with 1 N ammonium acetate (NH_4OAc, pH 7.0) (Hanway, 1952). Five grams of soil was shaken with 25 mL of extractant for 30 min and filtered. Potassium concentration in the filtrate was quantified using a flame photometer calibrated with KCl standards, and results were expressed as mg kg^{-1} of soil.

Soil biological properties (Period 2022-2023)

Microbial biomass carbon (MBC)

Soil microbial biomass carbon was determined using the chloroform fumigation–extraction method (Vance et al., 1987). Fresh soil samples (10 g, field-moist) were divided into two sets: fumigated and non-fumigated controls. The fumigated samples were exposed to ethanol-free chloroform vapour in a desiccator for 24 h in the dark at 25 °C, followed by extraction with 40 mL of 0.5 M K_2SO_4. Non-fumigated samples were extracted similarly without fumigation. Extracts were filtered and analyzed for organic C using a TOC analyzer.

Dehydrogenase activity (DHA)

Soil dehydrogenase activity was assayed according to the method of Casida Jr, 1977). One gram of fresh soil was incubated with 2,3,5-triphenyltetrazolium chloride (TTC) solution at 37 °C for 24 h. The reduction of TTC to triphenyl formazan (TPF) was extracted with methanol, and the absorbance was measured at 485 nm using a UV–Vis spectrophotometer.

Urease activity

Urease activity was estimated following the method of Tabatabai, 1994. Five grams of fresh soil was incubated with 2.5 mL of 10% urea solution and 20 mL of phosphate buffer (pH 6.7) at 37 °C for 2 h. The released ammonium was extracted with 2 M KCl and quantified colorimetrically using the indophenol blue method at 578 nm.

β-Glucosidase (BGLU) activity

β-Glucosidase activity was determined according to Tabatabai, 1994. One gram of soil was incubated with 4 mL of modified universal buffer (pH 6.0) and 1 mL of 25 mM p-nitrophenyl-β-D-glucopyranoside (pNPG) at 37 °C for 1 h. The reaction was stopped with 0.1 M $CaCl_2$ and Tris buffer (pH 12).

Phosphomonoesterase (PMA) activity

Acid phosphomonoesterase activity was measured using the method of Tabatabai, 1994. Soil (1 g) was incubated with 4 mL of modified universal buffer (pH 6.5) and 1 mL of 25 mM p-nitrophenyl phosphate (pNPP) solution at 37 °C for 1 h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to stop the reaction. The amount of p-nitrophenol released was measured spectrophotometrically at 400 nm.

Estimation of plant growth-promoting traits of endophytic fungi (Period 2022-2023)

Indole-3-acetic acid (IAA) production

IAA production was quantified following the colorimetric method of (Munasinghe et al., 2017)i with modifications. Fungal isolates were inoculated into 50 mL of Potato Dextrose Broth (PDB) supplemented with 0.5 mg mL^{-1} L-tryptophan and incubated at 28 ± 2 °C on a rotary shaker (120 rpm) for 7 days. After incubation, cultures were centrifuged at 10,000 rpm for 10 min, and 2 mL of the cell-free supernatant was mixed with 4 mL of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid). The mixture was incubated in the dark at room temperature for 30 min, and the development of a pink colouration indicated IAA production. Absorbance was measured at 530 nm using a spectrophotometer (Eppendorf Biospectrometer kinetic 6136FP603197). IAA concentration (μg mL^{-1}) was estimated from a standard curve prepared using pure IAA solutions.

Phosphate solubilization

Fungal isolates were spot-inoculated onto Pikovskaya's agar medium containing insoluble tricalcium phosphate (TCP) as the sole P source and incubated at 28 ± 2 °C for 7 days. Phosphate solubilization was indicated by the appearance of a clear halo zone around the fungal colony (Pandey et al., 2006). The solubilization index (SI) was calculated as:

$$\text{Solubilization index (SI)} = \frac{(\text{colony zone diameter} + \text{clearing zone diameter})}{(\text{colony diameter})}$$

Antagonistic assay of endophytic fungi against *Phytophthora infestans* (Period 2023-2024)

The antagonistic potential of endophytic fungal isolates against *Phytophthora infestans* was evaluated using the dual-culture method on Potato Dextrose Agar (PDA) (Khare et al., 2010). Actively growing cultures of *P. infestans* were maintained on V8 juice agar and subcultured 7 days prior to the assay to obtain fresh mycelium. Endophytic fungal isolates were cultured on PDA for 5–7 days before use.

For the assay, 5 mm agar plugs were excised aseptically from the periphery of actively growing cultures of both the pathogen and the test fungi. A plug of *P. infestans* was placed approximately 10 mm from the edge of a PDA plate (90 mm diameter), and a plug of the test fungal isolate was placed directly opposite at an equal distance from the edge, leaving ~60 mm between the two inocula. Control plates were inoculated with *P. infestans* alone. All plates were incubated at 20 ± 2 °C in the dark until the pathogen in the control plates reached the plate margin (7–10 days). Each treatment was performed in triplicate. The antagonistic activity was determined by the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{\text{R2} - \text{R1}}{\text{R1}} \text{X100}$$

Where,

R1 - Radius of the pathogen's radial growth toward the opposite side on the control plate

R2 - Radius of the pathogen's radial growth toward the opposing antagonist on the test plate

Preparation of fungal consortia in liquid culture (Period 2023-2024)

Fungal consortia were prepared using endophytic isolates that exhibited promising plant growth–promoting (PGP) traits (e.g., IAA production, phosphate solubilization) and antagonistic activity against phytopathogens.

Selection and compatibility testing

Representative isolates were selected based on their performance in preliminary PGP and antagonistic assays. Pairwise compatibility among isolates was evaluated on Potato Dextrose Agar (PDA) plates by dual inoculation at opposite margins and observing interactions after 7 days of incubation at 28 ± 1 °C. Isolates showing no antagonism or overgrowth were considered compatible and selected for consortium preparation (Mili et al., 2023).

Standardization of inoculum

Each selected isolate was cultured on PDA plates at 28 ± 1 °C for 5–7 days. A 5 mm mycelial plug from the actively growing margin was inoculated into 50 mL Potato Dextrose Broth (PDB) in 250 mL Erlenmeyer flasks and incubated at 28 ± 1 °C on a rotary shaker at 120 rpm for 72 h. Fungal

biomass was homogenized and standardized to a spore or propagule concentration of approximately 1×10^7 CFU mL^{-1} using sterile PDB as diluent. Concentrations were determined using a hemocytometer for spore-producing fungi or viable counts (CFU) for mycelial fungi (Talukdar et al., 2020).

Consortium assembly

For consortium preparation, equal volumes of standardized inoculum from each isolate were mixed aseptically into sterile PDB to obtain a final concentration of ~1×10^7 CFU mL^{-1} per isolate. The mixed culture was incubated at 28 ± 1 °C on a rotary shaker at 120 rpm for 24–48 h to allow acclimatization and interaction among isolates while preventing overgrowth by fast-growing species (Talukdar et al., 2020).

Quality control and storage

The viability and relative abundance of each isolate in the consortium were verified by serial dilution plating on PDA and by observing colony morphology. Absence of contamination was confirmed by streaking aliquots on nutrient agar. The freshly prepared liquid consortium was stored at 4 °C for up to 72 h prior to experimental use, with gentle mixing before application (Talukdar et al., 2020).

Greenhouse pot experiment with *Citrus* Seedlings treated with fungal consortium (Period 2024-2025)

Experimental design

A greenhouse pot experiment was conducted to evaluate the effect of a fungal consortium on the growth performance of six-month-old *Citrus limon* seedlings. The experiment followed a completely randomized design with two treatments: (i) seedlings treated with 10% (v/v) fungal consortium, and (ii) seedlings treated with sterile Potato Dextrose Broth (PDB) diluted to 10% (v/v), serving as control. Each treatment was replicated three times (n = 3), with one seedling per pot constituting a replicate.

Plant material and potting substrate

Uniform *Citrus* seedlings (six months old) were selected from nursery stock. Seedlings of similar height and vigour were transplanted into 5 L plastic pots containing sterilized soil–sand–compost mixture (2:1:1, w/w). Pots were arranged randomly on greenhouse benches to minimize positional effects.

Preparation and application of fungal consortium

The fungal consortium was prepared in liquid culture by mixing compatible isolates standardized to ~1×10^7 CFU mL^{-1} per isolate. A 10% (v/v) suspension was prepared by diluting the consortium stock with sterile distilled water. For each seedling, 500 mL of the 10% consortium suspension was applied as a soil drench at the base of the stem. Control seedlings received an equal volume

(500 mL) of 10% sterile PDB suspension. A booster drench of the same volume was administered 30 days after the initial inoculation (Talukdar et al., 2020)**.**

Greenhouse maintenance

Seedlings were maintained under greenhouse conditions at 25 ± 3 °C with a 12–14 h photoperiod and relative humidity of 50–70%. Irrigation was provided with tap water as needed to maintain soil moisture near field capacity. Fertilizer was not applied during the first 30 days; thereafter, a balanced NPK (20:20:20) solution was applied uniformly at 50% recommended dose every 30 days. No fungicides were used during the experiment (Joshi, 2020).

Estimation of physiological and morphological parameters of *Citrus* Seedlings (Period 2024-2025)

The physiological and morphological responses of six-month-old *Citrus* seedlings subjected to fungal consortia treatments after inoculation. Measurements were performed on three biological replicates per after 6 months of transplantation.

Photosynthetic rate

Net photosynthetic rate was measured using a portable photosynthesis system (Licor-6400). Fully expanded leaves (third or fourth from the shoot apex) were selected for measurement between 09:00–11:30 h. Chamber conditions were maintained at 25 ± 1 °C, CO_2 concentration of 400 μmol mol^{-1}, photon flux density of 1200 μmol m^{-2} s^{-1}, and relative humidity of 50–60%. Three leaves per plant were recorded, and mean values were used for plant-level analysis (Jaafar et al., 2012).

Plant height and stem diameter

Plant height was measured from the soil surface to the apical meristem using a measuring tape. The stem diameter was measured 2 cm above the soil surface using digital callipers. Measurements were taken at each sampling interval.

Leaf count and leaf area

The total number of fully expanded leaves per seedling was recorded. Leaf area was estimated from scanned images of representative leaves (three per plant) using ImageJ software (Costa et al., 2014). Images were converted to binary and calibrated with a scale bar prior to analysis. Leaf area was expressed in cm^2 per leaf and extrapolated to total leaf area per seedling.

Root morphology (length and surface area)

At final harvest, roots were carefully washed, spread uniformly in a water-filled tray, and scanned at 600 dpi resolution. Root length and surface area were quantified using ImageJ software following threshold adjustment, skeletonization, and particle analysis. Root length was expressed in cm per plant, and surface area in cm^2 per plant.

Plant biomass

Shoot and root systems were separated at harvest, blotted to remove excess water, and weighed immediately to record fresh biomass. Samples were oven-dried at 65 °C until constant weight and expressed as dry biomass (g plant^{-1}). Root-to-shoot ratios were calculated on a dry weight basis.

Analysis of soil physicochemical and biological properties (Period 2024-2025)

At the termination of the greenhouse experiment (6 months after inoculation), rhizospheric soil samples were collected from each treatment pot for assessment of physicochemical and biological properties. Soil adhered to the roots was carefully brushed off, homogenized, and passed through a 2 mm sieve for further analysis.

Physicochemical properties

Soil pH was determined in a 1:2.5 (w/v) soil–water suspension using a calibrated digital pH meter (Brouder et al., 2005). Soil organic carbon (SOC) content was estimated by the Walkley–Black dichromate oxidation method (Walkley & Black, 1934). Available nitrogen (N) was quantified by the alkaline permanganate method (Subbiah, 1956), available phosphorus (P) by Olsen's method using 0.5 M $NaHCO_3$ extract (Olsen, 1954), and available potassium (K) by flame photometry following extraction with neutral 1N ammonium acetate . Soil bulk density and moisture content were measured using the core method (Blake & Hartge, 1986) and gravimetric method, respectively.

Biological properties

Soil microbial biomass carbon (MBC) was measured by the chloroform fumigation–extraction method (Vance et al., 1987). Dehydrogenase activity (DHA) was assayed using 2,3,5-triphenyltetrazolium chloride (TTC) reduction to triphenyl formazan (Casida Jr, 1977)c. Urease activity was determined colorimetrically by quantifying ammonium released after incubation with urea (Tabatabai, 1994). β-glucosidase activity (BGLU) was measured using p-nitrophenyl-β-D-glucopyranoside substrate hydrolysis (Tabatabai, 1994). Phosphomonoesterase (PMA) activity was determined using p-nitrophenyl phosphate substrate hydrolysis under controlled pH conditions (Tabatabai, 1994).

Statistical analysis

All experimental data, including plant growth, physiological traits, and soil physicochemical and biological parameters, were subjected to one-way analysis of variance (ANOVA) to assess treatment effects. Means were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

10. RESULTS AND DISCUSSION

Collection of plant and soil samples

During the project period, extensive field surveys were conducted across various *Citrus* orchards in the northeastern states of Assam and Arunachal Pradesh for the collection of plant and soil samples. The focus was on both *Citrus reticulata* (Khasi mandarin orange) and *Citrus limon* (Assam lemon), chosen from ecologically diverse locations to ensure a broad representation of environmental and soil conditions influencing the associated microbial communities (Fig 1).

In Assam, samples were collected from Boragaon, Tinsukia (Lat: 28.5453ºN, Long: 95.4835ºE), Motapong, Tinsukia (Lat: 27.5632ºN, Long: 95.4847ºE), and Kakopather, Tinsukia (Lat: 27.5910ºN, Long: 95.8041ºE), all representing *Citrus reticulata*. Additional sampling sites included Boko, Kamrup (Lat: 25.9413ºN, Long: 91.3184ºE), Chakrasila, Kamrup (Lat: 25.9252ºN, Long: 91.2775ºE), Umring Kuna, Kamrup (Lat: 25.8917ºN, Long: 91.2687ºE), and Jimiligaon, Rani, Kamrup (Lat: 25.8897ºN, Long: 91.5233ºE), all of which also featured *Citrus reticulata*. Two locations were used for collecting *Citrus limon* samples: Tezpur University, Tezpur (Lat: 26.7000ºN, Long: 92.8355ºE) and Kanyaka Bahumukhi Pam, Biswanath Chariali (Lat: 26.7411ºN, Long: 92.8874ºE).

In Arunachal Pradesh, collections were made from Old Pukhuri, Wakro (Lat: 27.7828ºN, Long: 96.2430ºE), Changlang near Tezu (Lat: 27.9340ºN, Long: 96.2218ºE), Pasighat (Lat: 28.2553ºN, Long: 95.5806ºE), and Dambuk (Lat: 28.2542ºN, Long: 95.5802ºE). These sites are located in relatively undisturbed, ecologically diverse hilly terrain and all hosted *Citrus reticulata* plants.

The selection of these twelve diverse sites with accurately recorded GPS coordinates facilitated the comprehensive study of fungal diversity and soil properties in *Citrus* orchards. The collected plant and soil samples formed the basis for analyzing endophytic fungi with potential plant growth-promoting traits, and antifungal properties. These representative sites across varied geographic zones were crucial for understanding the ecological dynamics of citrus-associated microbial communities in Northeast India.

Isolation of endophytic fungi from *Citrus reticulata* and *Citrus limon*

A total of twelve distinct sporulated endophytic fungal taxa were isolated from *Citrus reticulata* and *Citrus limon* collected across various locations in Assam and Arunachal Pradesh. The identified fungal genera include *Aspergillus* sp., *Colletotrichum* sp., *Penicillium* sp., *Fusarium* sp., *Bipolaris* sp., *Curvularia* sp., *Alternaria* sp., *Stemphylum* sp., *Mycelia sterilia*, along with three unidentified morphotypes (Morphotypes 1, 2, and 3).

In Citrus reticulata, the most frequently isolated fungi varied by location. At Borgaon, *Colletotrichum* sp. and *Penicillium* sp. were dominant, while *Aspergillus niger* was least encountered. In Motanpong, *Aspergillus* sp. was the most prevalent, and *Curvularia* sp. was the least. Kakopathar showed a dominance of *Colletotrichum* sp., with *Penicillium* sp. and *Aspergillus niger* being least observed. Similarly, *Colletotrichum* sp. dominated the fungal community in Old Pukhuri Wakro, with *Curvularia* sp. occurring infrequently. In Changlang Tezu, *Aspergillus* sp.

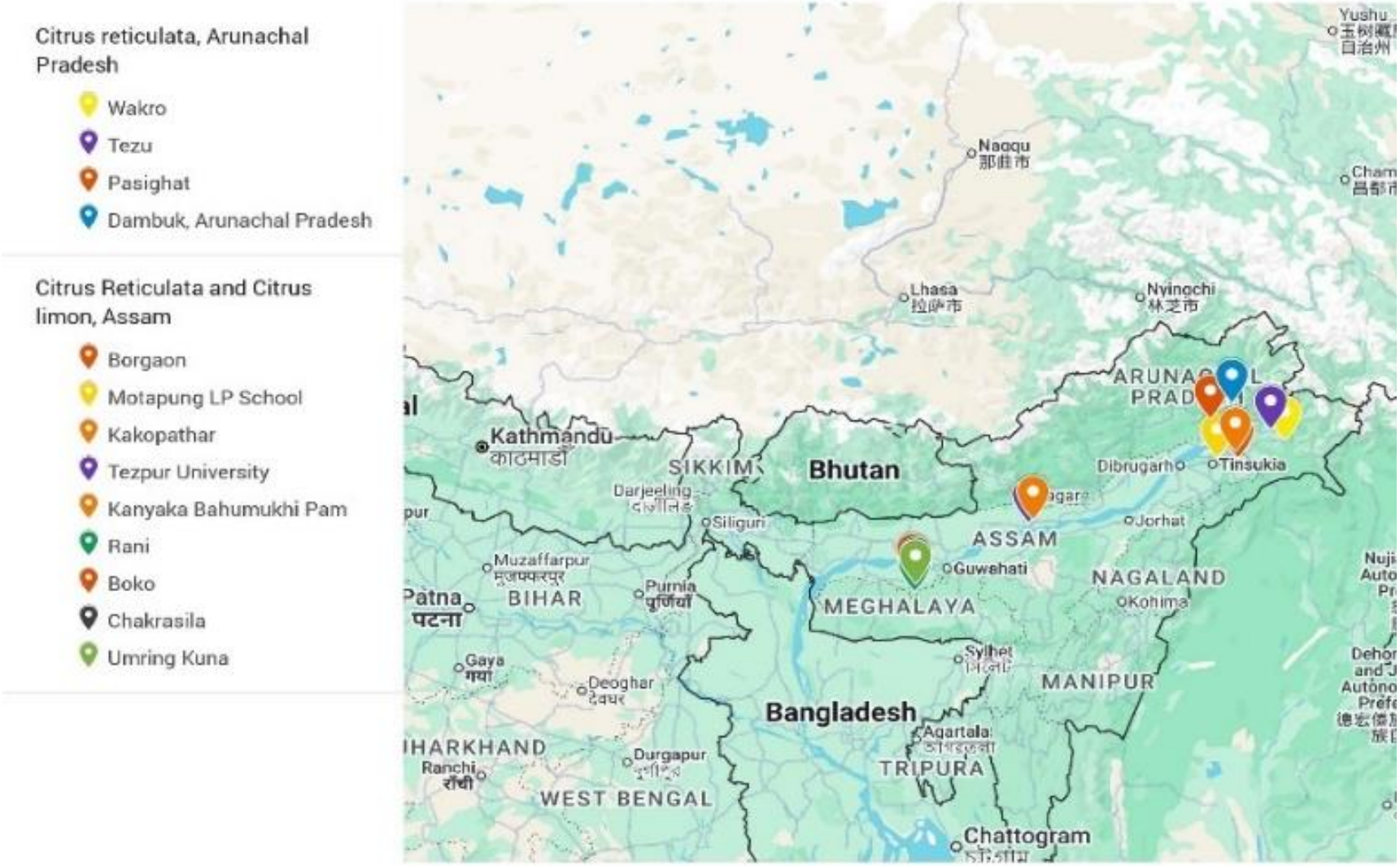


Fig 1: Locations of Collection sites of plant and soil samples from different *Citrus* orchards of Assam and Arunachal Pradesh (Source, Google Maps

Fig 2: Photo documentation during collection of plant and soil samples from different *Citrus* orchards of Assam and Arunachal Pradesh (2021-22).

and *Penicillium* sp. were most common, while *Alternaria* sp. was least represented. Pasighat samples showed a predominance of *Aspergillus* sp., and Morphotype 1 was the least isolated. At Dambuk, *Penicillium* sp. was most frequent, whereas *Fusarium* sp. and *Aspergillus* sp. appeared sparsely. Boko was characterized by a high frequency of *Bipolaris* sp., while *Colletotrichum* sp., *Curvularia* sp., and Morphotype 3 were among the least observed. In Chakrasila, *Aspergillus* sp. was dominant, with *Stemphylum* sp. being the least frequent. At Umring Kuna, a combination of *Aspergillus* sp., *Fusarium* sp., and *Bipolaris* sp. were most frequently isolated, while Morphotype 2 appeared least. Jimiligaon showed a high occurrence of *Aspergillus* sp (Graph 1).

In *Citrus limon*, *Aspergillus* sp. was most frequently isolated from Kanyaka Bahumukhi Paam, with *Colletotrichum* sp. being the least. At Tezpur University, Mycelia sterilia was the most dominant isolate, while Morphotype 2 was least observed (Graph 2).

Endophytic fungi isolated from citrus orchards in both Assam and Arunachal Pradesh revealed considerable overlap in fungal taxa, despite variations in environmental conditions such as soil type, altitude, and agricultural practices. Dominant genera such as *Fusarium*, Colletotrichum, *Penicillium*, and *Aspergillus* were consistently recovered from both regions. This pattern suggests that factors beyond the external environment play a crucial role in shaping the composition of the endophytic fungal community associated with citrus plants. Previous studies have similarly observed the widespread occurrence of these genera in a range of host plants and geographic locations, indicating their broad ecological adaptability and potential endophytic lifestyle (Liao et al., 2025; Mohammad Golam Dastogeer et al., 2020).

One of the most influential factors contributing to the similarity in fungal assemblages across regions is plant host specificity. Host plants are known to exert selective pressure on endophytic communities through physiological and biochemical interactions. Endophytic fungi often display a degree of host specificity or preference, meaning that particular fungal species are more likely to colonize certain plant hosts regardless of varying environmental contexts (Hardoim et al., 2008; Mohammad Golam Dastogeer et al., 2020). In the case of citrus, the host plant appears to favor the recruitment of a core group of fungal endophytes, which could explain the recurrent presence of similar taxa across diverse agroclimatic conditions (Hardoim et al., 2008).

Furthermore, the influence of citrus genotype cannot be overlooked. Many citrus species, including *Citrus reticulata* and *Citrus limon*, possess shared physiological traits and secondary metabolites such as flavonoids, phenolics, and alkaloids, which are known to affect microbial colonization (Chen et al., 2024). These phytochemical characteristics may create a selective microenvironment that supports the colonization of specific fungal genera, contributing to the conservation of core endophytic communities (Gadd & McGregor, 2024). This biochemical compatibility likely promotes the proliferation of *Fusarium*, *Colletotrichum*, and *Penicillium* species, which are frequently reported in citrus-associated microbiomes (Crouch et al., 2014). Fig 3 shows the isolation of endophytic fungi in various culture medium and Fig 4 shows the microscopic photographs.

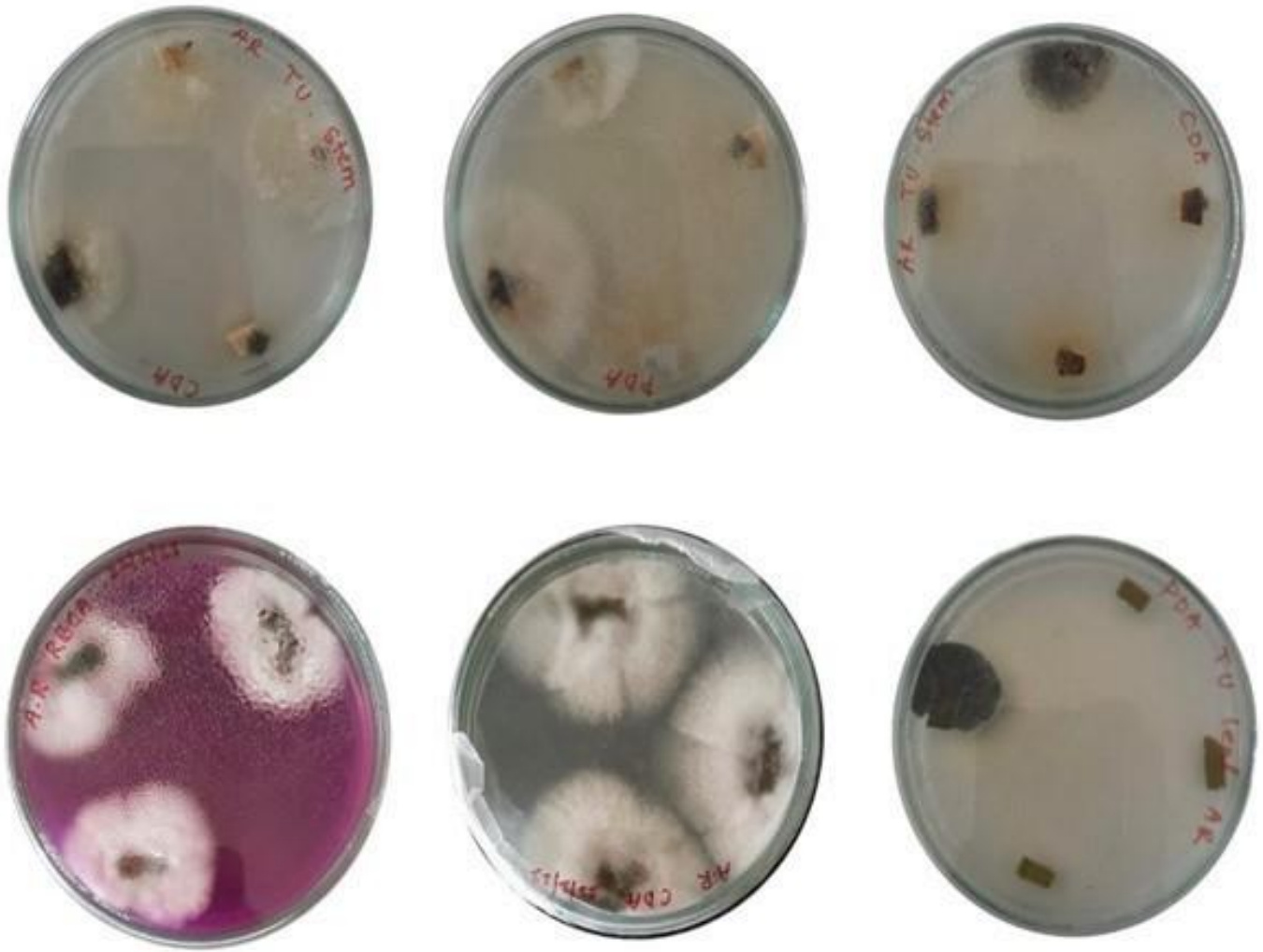


Fig 3: Endophytic fungi growing out from the surface-sterilised *Citrus limon* and *C. reticulata* tissue fragments

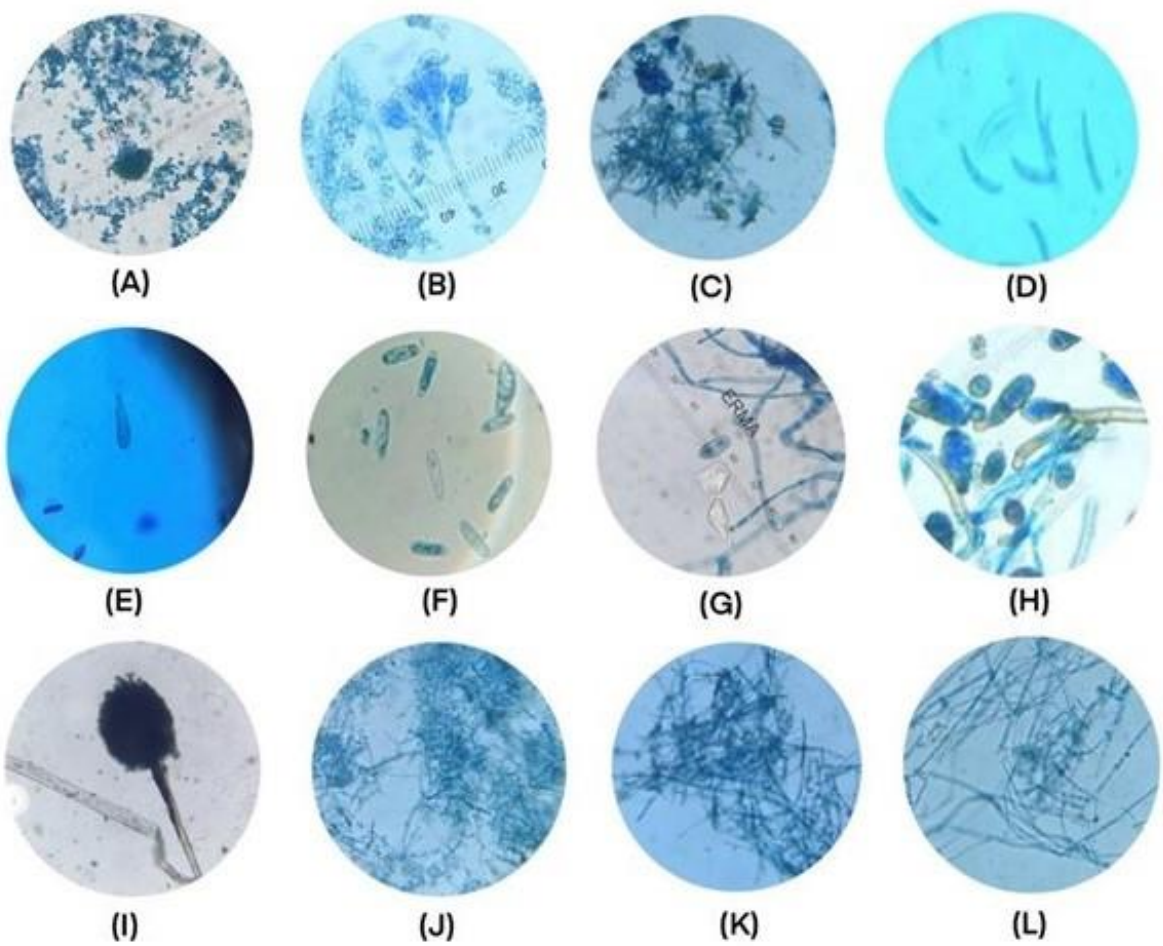
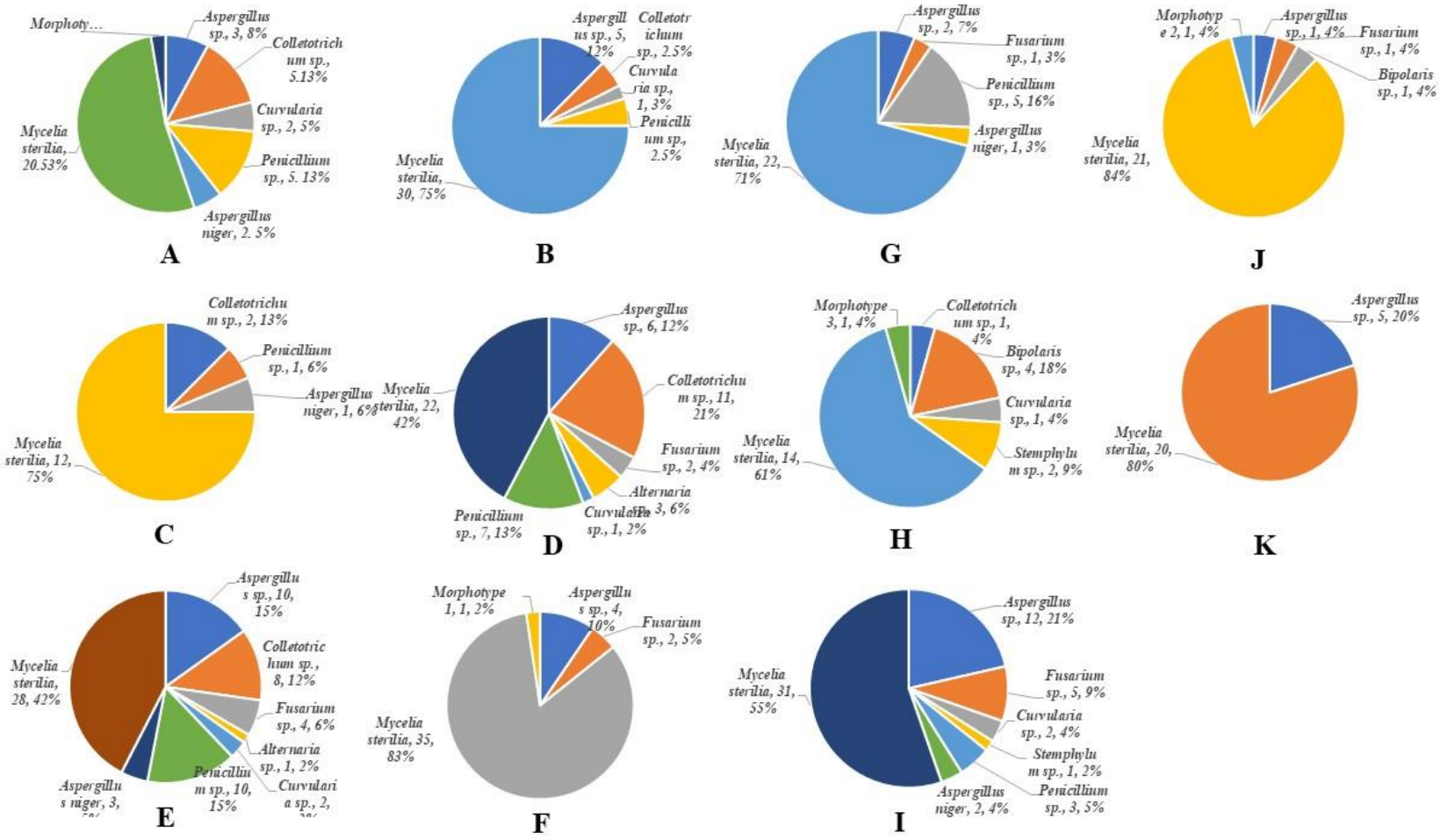
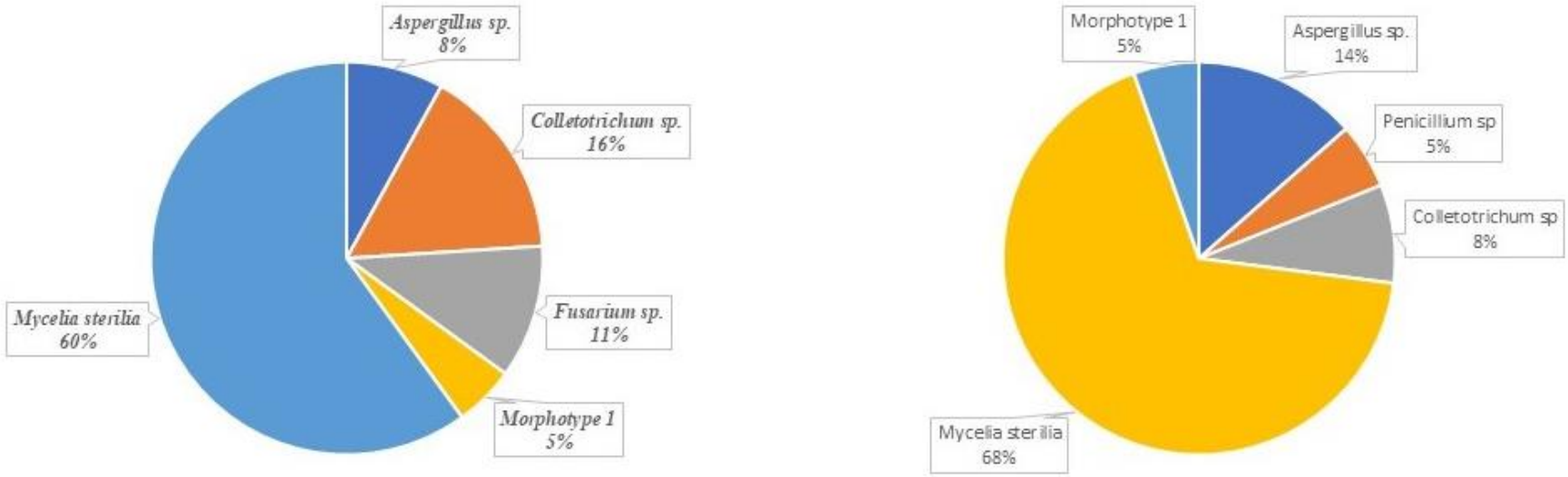


Fig 4: Microscopic photographs of endophytic fungi isolated from *C. reticulata* and *C. limon* (40x magnification), A) *Aspergillus* sp. B) *Penicillium* sp. C) *Curvularia* sp. D) *Fusarium* sp. E) *Alternaria* sp. F) *Colletotrichum* sp. G) *Bipolaris* sp. H) *Stemphylum* sp. I) *Aspergillus niger* J) Morphotype 1 K) Morphotype 2 L) Morphotype 3.



Graph 1: Percentage of occurrence of different species of Endophytic fungi isolated from *Citrus reticulata* collected from different parts of Assam and Arunachal Pradesh A) Borgaon B) Motapong C) Kakopathar D) Wakro E) Tezu F) Pasighat G) Dambuk H) Boko I) Chakrasila J) Umring Kuna K) Rani



Graph 2: Percentage of occurrence of different species of Endophytic fungi isolated from *Citrus limon* collected A) Tezpur University B) Kanyaka Bahumukhi Pam

Diversity analysis

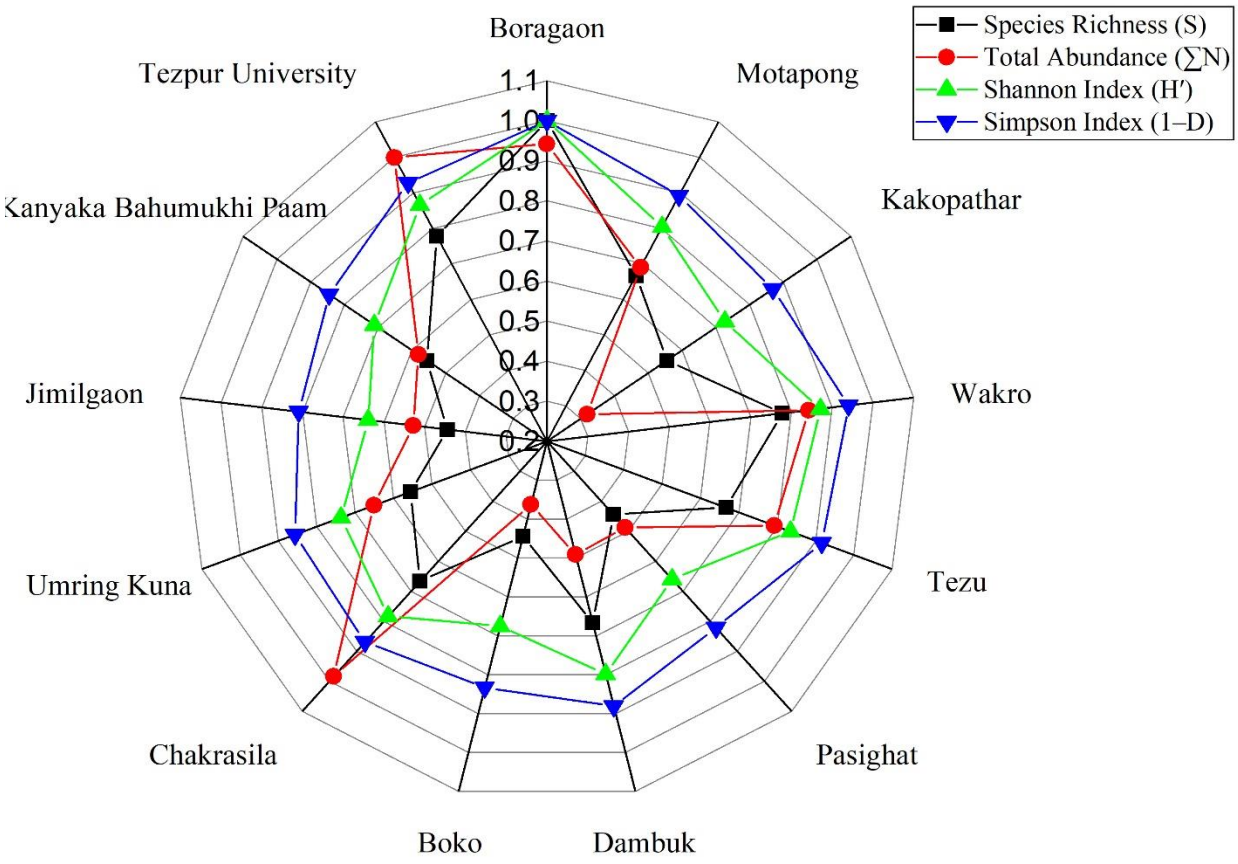
The diversity analysis of endophytic fungi isolated from citrus orchards across multiple regions of Assam and Arunachal Pradesh revealed notable spatial variability in community richness and evenness. Boragaon exhibited the highest species richness (S = 9) and total fungal abundance (∑N = 69.29), alongside a Shannon diversity index (H′) of 1.94 and a Simpson index (1–D) of 0.84, indicating a highly diverse and balanced endophytic community structure. In contrast, sites such as Pasighat and Jimiligaon showed the lowest species richness (S = 4) and reduced diversity values (H′ = 1.28 and 1.24, respectively), with lower Simpson indices (0.69 and 0.68), suggesting dominance by a few fungal taxa and limited species evenness (Graph 3).

Tezpur University and Wakro displayed relatively high diversity levels, with species richness of 7 and Shannon indices of 1.68 and 1.69, respectively. These values suggest that certain regions, despite differences in agroclimatic zones, harbor robust and heterogeneous fungal communities (Hardoim et al., 2015). The variation in diversity indices across locations may be attributed to differences in microclimate, altitude, soil chemistry, and land use practices, all of which are known to influence the structure of endophytic communities (Hardoim et al., 2015). Locations like Motapong, Tezu, Dambuk, and Chakrasila showed moderate diversity values (S = 6), although Chakrasila recorded one of the highest total abundances (∑N = 72.35) with a Shannon index of 1.52, suggesting an uneven yet rich fungal population.

Moderate diversity values observed at sites like Kakopathar (H′ = 1.41) and Umring (H′ = 1.43) also indicate spatial heterogeneity in fungal colonization. Such differences could be linked to local environmental stressors, nutrient availability, and the degree of anthropogenic influence, which are known to modulate endophytic community dynamics (Arnold & Lutzoni, 2007; Kharwar et al., 2011). Interestingly, the lemon-growing site of Kanyaka Bahumukhi Paam showed lower diversity (S = 5, H′ = 1.38), whereas Tezpur University (S = 7, H′ = 1.68) exhibited a rich and even endophytic fungal profile. This may be due to the genetic and phytochemical differences between *Citrus reticulata* and *Citrus limon*, as plant genotype and tissue chemistry can significantly influence endophyte recruitment and colonization (Karliński et al., 2010).

Physicochemical and biological properties of *Citrus* orchards

The soil properties of the citrus orchards investigated across Assam and Arunachal Pradesh showed marked variability in biological, physical, and chemical parameters, each of which plays a critical and interconnected role in maintaining soil health and supporting microbial communities. Biologically, microbial biomass carbon (MBC), dehydrogenase activity (DHA), urease (URA), β-glucosidase (BGLU), and phosphomonoesterase (PMA) were used as indicators of microbial abundance, activity, and functionality. Arunachal Pradesh sites, particularly Changlang (MBC: 2.18% of SOC, DHA: 287.72 mg ml^{-1} INTF g^{-1} soil $2h^{-1}$, URA: 3.62 µg NH_4^+ g^{-1} soil, BGLU: 41.94 µg NH_4^+ g^{-1} soil, PMA: 308.34 µg pNP g^{-1} h^{-1}), exhibited the highest values, suggesting a thriving and functionally diverse microbial population. In contrast, Assam sites like Boragaon and Boko recorded lower values for MBC (0.72–0.99%) and enzyme activities, indicating comparatively weaker microbial activity and organic matter transformation (Table 1). These biological



Graph 3: Radar map depicting normalized alpha diversity indices (Richness, Total Abundance, Shannon Index and Simpson Index) scaled to a 0–1 range. Normalization was performed by dividing the observed value of each index by its respective maximum observed value to enable equitable comparison across inherently disparate scales.

indicators are directly influenced by the availability of carbon substrates, nutrient levels, and soil moisture, which are in turn governed by chemical and physical properties (Kaushal, 2023).

Physically, bulk density and moisture content played a vital role in moderating microbial and enzymatic activities. Arunachal Pradesh soils had lower bulk densities (1.09–1.11 g/cc) and significantly higher moisture content (12.25–12.68%), particularly at Dambuk, Pasighat, and Changlang. These conditions favor microbial growth by enhancing pore space, aeration, and water availability, which are crucial for enzymatic reactions and microbial metabolism (Drew, 1992). Conversely, higher bulk density and lower moisture content in Assam sites like Chakrasila (bulk density: 1.18 g/cc, moisture: 9.36%) likely constrained microbial proliferation and reduced biochemical activity. Soil compaction reduces oxygen diffusion, which can limit aerobic microbial functions and reduce enzymatic turnover (Drew, 1992).

Chemically, soil pH, organic carbon (OC), and available macronutrients (N, P, K) collectively contributed to shaping the microbial environment. Soils across all sites were slightly acidic (pH 5.18–5.66), a range within which many plant-associated fungi and bacteria thrive (Rousk et al., 2009). Organic carbon was markedly higher in Arunachal soils, with Changlang (5.64%), Old Pukhuri (5.50%), and Pasighat (5.09%) registering the top values (Table 1). High OC supports microbial biomass by providing essential carbon sources for growth and enzyme production (Khatoon et al., 2017)k. This directly correlated with higher MBC and DHA observed in these regions. Available nitrogen (up to 153.83 kg/ha at Dambuk) and phosphorus (up to 369.20 kg/ha at Chakrasila and Kanyaka) were also influential, as they support microbial synthesis of functional proteins, nucleic acids, and enzymes, thus enhancing overall soil biological activity (Parthasarathi & Ranganathan, 1999).

Importantly, these soil properties are highly interdependent. Organic carbon improves soil structure, thereby reducing bulk density and enhancing moisture retention two physical properties that directly favour microbial survival and enzymatic action (Khatoon et al., 2017). In turn, active microbial communities mineralize organic matter, releasing plant-available forms of nitrogen and phosphorus that enhance chemical fertility. Moisture acts as a medium for nutrient diffusion and enzyme-substrate interaction, linking physical and biochemical processes (Datta et al., 2017). The high biological activity in moist, carbon-rich, and nutrient-available soils in Arunachal Pradesh underscores these synergistic relationships (Datta et al., 2017). On the other hand, soils with low OC, compact structure, and poor moisture (e.g., Boragaon and Tezpur University) exhibited reduced biological functioning, indicating that integrated management of these properties is essential for maintaining soil health and productivity in citrus ecosystems (Datta et al., 2017).

Indole acetic acid production by the fungal endophytes

The observed variation in IAA production among endophytic fungal isolates indicates functional diversity in their auxin biosynthetic capabilities (Graph 4). The significantly higher IAA concentrations recorded in the presence of tryptophan (TRP) across all isolates support the notion that TRP acts as a critical precursor for IAA biosynthesis via the TRP-dependent pathway. This pathway is well-documented among plant-associated microorganisms, where TRP is converted into IAA through various intermediates such as indole-3-pyruvate and indole-3-acetamide (Lu et al., 2025).

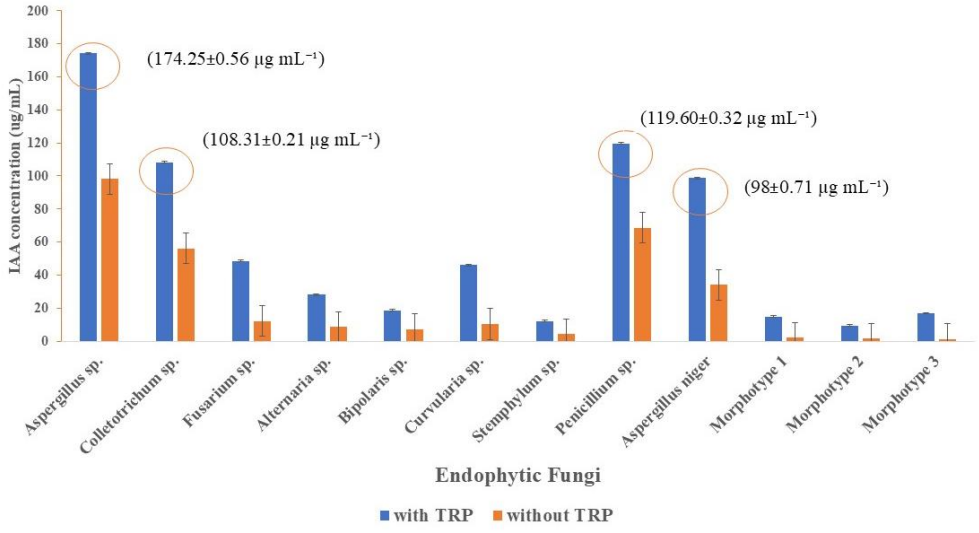
Aspergillus sp., which produced the highest level of IAA ($174.25 \pm 0.56\ \mu g\ mL^{-1}$ with TRP), has been previously reported as an efficient IAA producer in several studies involving rhizospheric and endophytic fungi (Liu et al., 2023). The enhanced production by *Penicillium* sp. ($119.60 \pm 0.32\ \mu g\ mL^{-1}$) and *Aspergillus niger* ($98 \pm 0.71\ \mu g\ mL^{-1}$) further highlights the auxin-producing potential of filamentous ascomycetes, which are known to promote plant growth via multiple mechanisms including phytohormone production, phosphate solubilization, and siderophore release (Moreno-Salazar et al., 2020).

The moderate IAA levels observed in *Colletotrichum* sp. ($108.31 \pm 0.21\ \mu g\ mL^{-1}$) align with previous findings that this genus, though often pathogenic, can also behave as a mutualistic endophyte under specific host or environmental conditions (Jahn et al., 2021). Fungi like Fusarium sp., *Curvularia* sp., and *Alternaria* sp. produced moderate to low amounts of IAA, suggesting that

either they utilize TRP less efficiently or follow alternative, less active biosynthetic pathways (Tang et al., 2023).

The generally low IAA production by unidentified morphotypes and species such as *Stemphylium sp*. suggests either the absence of a functional TRP-dependent pathway or the reliance on TRP-independent routes, which are typically less efficient (Tang et al., 2023). Furthermore, the marked reduction in IAA levels under TRP-free conditions across all isolates confirms the substrate-limited nature of IAA biosynthesis in fungi, consistent with findings from endophytic and rhizospheric studies (Tang et al., 2023).

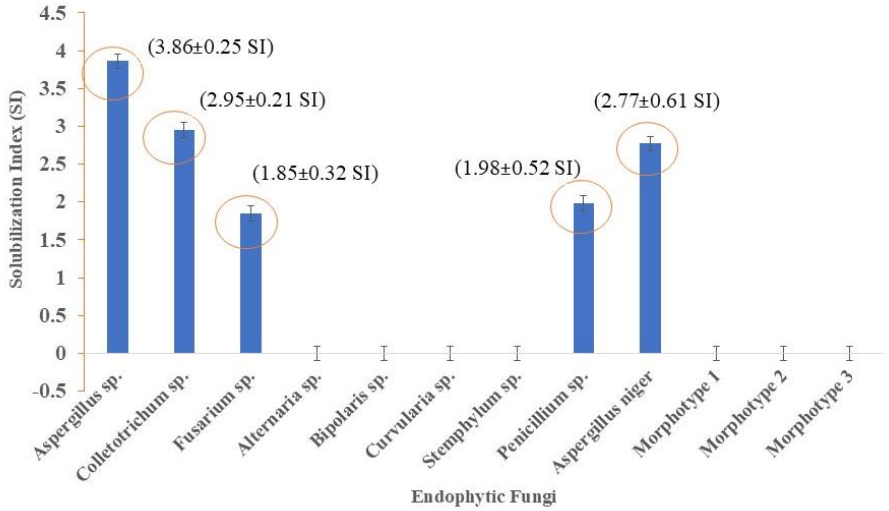
Taken together, these results indicate that endophytic fungi associated with citrus species harbour diverse auxin-producing capabilities, with TRP supplementation playing a central role in amplifying IAA production. Harnessing high IAA-producing strains such as Aspergillus sp. and Penicillium sp. may offer biotechnological potential for developing fungal bioinoculants to promote plant growth under both normal and stress conditions.



Graph 4: Indole-acetic acid production by endophytic fungi isolated from *Citrus* plant tissues.

Phosphate solubilization

Phosphate-solubilising fungi (PSF) play a critical role in enhancing plant phosphorus nutrition by converting insoluble phosphates into bioavailable forms through the production of organic acids and enzymes (García-Latorre et al., 2023). The high solubilization index observed in *Aspergillus* sp. (3.86 ± 0.25) confirms its strong phosphate solubilization potential, a characteristic well-documented in both rhizospheric and endophytic strains of *Aspergillus* (Pandey et al., 2006). The production of organic acids such as gluconic and citric acid by *Aspergillus* sp. contributes to pH reduction in the surrounding medium, aiding phosphate release from mineral complexes (Dolatabad et al., 2017).



Graph 5: Phosphate solubilization by endophytic fungi isolated from *Citrus* plant tissues.

Colletotrichum sp. and *Aspergillus niger* also exhibited considerable phosphate solubilization, with SI values of 2.95 and 2.77 respectively. Although *Colletotrichum* is primarily known as a pathogen, certain endophytic strains have been shown to possess plant growth-promoting traits, including phosphate solubilization (Jamilano-Llames & dela Cruz, 2025). *Aspergillus niger*, a widely studied industrial fungus, has been repeatedly reported as an efficient PSF capable of solubilizing various inorganic phosphates (Nascimento et al., 2021).

Moderate solubilization by *Fusarium* sp. and *Penicillium* sp. (SI values ~1.85–1.98) suggests that while these fungi are not the most efficient PSFs, they still contribute to phosphorus availability in the rhizosphere. Previous studies have identified certain *Fusarium* and *Penicillium* strains that produce organic acids such as lactic and oxalic acid, aiding in phosphate solubilization (Arias et al., 2023).

In contrast, isolates such as *Alternaria* sp., *Bipolaris* sp., *Curvularia* sp., and the three morphotypes exhibited little to no phosphate solubilization. This could be attributed to either the absence of organic acid production or inefficient enzymatic systems required for mobilizing inorganic phosphate (de Oliveira Mendes et al., 2014). These results indicate that not all endophytic fungi possess the same capacity for phosphate solubilization, and highlight the potential of specific genera like *Aspergillus* and *Colletotrichum* for development as biofertilizers (Graph 5).

Table 1: Physicochemical and Biological Properties of rhizospheric soil of *Citrus* orchards of *Citrus reticulata* and *Citrus limon* collected from Assam and Arunachal Pradesh.

Location	**MBC (% of SOC)**	**DHA (mg ml^{-1} INTF g^{-1} soil 2h^{-1})**	**URA (µg NH_4^+ g^{-1} soil 2h^{-1})**	**BGLU (µg NH_4^+ g^{-1} soil 2h^{-1})**	**PMA (µg pNP g^{-1} h^{-1})**	**Bulk Density (g/cc)**	**Moisture (%)**	**pH**	**Organic C (%)**	**Avail. N (kg/ha)**	**Avail. P (kg/ha)**	**Avail. K (kg/ha)**
Boragaon,	0.72 ± 0.12 m	196.25 ± 12.56 i	1.06 ± 0.04 m	25.14 ± 0.38 i	129.88 ± 3.57 l	1.10 ± 0.02 b	4.26 ± 0.15 f	5.42 ± 0.07 c	2.43 ± 0.15 e	68.86 ± 12.55 e	5.38 ± 0.30 i	150.18 ± 14.73 a
Motapong	1.06 ± 0.05 k	248.03 ± 20.28 e	1.20 ± 0.07 k	23.38 ± 0.58 l	152.08 ± 2.29 g	1.09 ± 0.02 bc	5.36 ± 0.23 e	5.66 ± 0.21 a	3.10 ± 0.10 de	65.58 ± 12.63 e	7.54 ± 0.58 h	145.58 ± 10.32 b
Kakopathar	1.29 ± 0.10 h	203.00 ± 19.05 h	1.20 ± 0.04 l	26.56 ± 1.65 h	146.34 ± 3.13 i	1.10 ± 0.01 b	5.18 ± 0.08 e	5.24 ± 0.10 d	3.44 ± 0.13 d	66.25 ± 9.41 e	1.90 ± 0.40 j	106.91 ± 9.38 e
Old Pukhuri	1.86 ± 0.10 d	302.43 ± 46.33 b	3.05 ± 0.12 c	39.42 ± 0.99 c	290.36 ± 4.32 c	1.10 ± 0.01 b	12.25 ± 0.09 b	5.32 ± 0.17 d	5.50 ± 0.08 ab	129.99 ± 5.62 b	24.70 ± 2.90 e	85.52 ± 13.50 e
Changlang	2.18 ± 0.23 a	287.72 ± 41.11 c	3.62 ± 0.19 a	41.94 ± 0.84 a	308.34 ± 5.54 b	1.11 ± 0.01 b	12.59 ± 0.11 a	5.44 ± 0.10 c	5.64 ± 0.08 a	75.42 ± 7.93 d	12.02 ± 1.32 f	78.06 ± 3.65 f
Pasighat	2.15 ± 0.21 b	280.67 ± 35.75 d	3.05 ± 0.09 b	39.92 ± 1.00 b	295.00 ± 7.37 b	1.09 ± 0.01 b	12.45 ± 0.06 a	5.26 ± 0.05 d	5.09 ± 0.04 c	72.71 ± 9.43 d	11.89 ± 1.88 g	88.27 ± 5.20 e
Dambuk	2.02 ± 0.25 c	338.20 ± 15.78 a	2.65 ± 0.07 d	38.62 ± 0.59 d	281.12 ± 9.36 d	1.10 ± 0.02 bc	12.68 ± 0.19 a	5.18 ± 0.17 e	5.32 ± 0.12 b	153.83 ± 8.54 a	11.42 ± 1.79 g	82.78 ± 4.57 e
Boko	0.99 ± 0.04 l	202.90 ± 15.20 h	1.88 ± 0.06 h	28.76 ± 0.73 e	150.50 ± 2.03 h	1.11 ± 0.04 bc	12.26 ± 0.16 b	5.20 ± 0.09 e	2.47 ± 0.08 e	128.60 ± 5.16 b	318.53 ± 10.38 a	137.70 ± 2.12 c
Chakrasila	1.12 ± 0.09 j	206.20 ± 12.72 h	1.89 ± 0.03 f	26.90 ± 1.43 g	134.12 ± 2.70 k	1.18 ± 0.05 a	9.36 ± 0.22 d	5.58 ± 0.10 b	2.57 ± 0.03 e	124.08 ± 8.01 b	369.20 ± 13.25 c	123.92 ± 4.99 d
Umring Kuna,	1.33 ± 0.09 f	212.58 ± 5.57 g	1.88 ± 0.06 g	28.58 ± 1.06 f	153.50 ± 3.80 f	1.13 ± 0.06 b	10.18 ± 0.08 c	5.46 ± 0.14 bc	2.59 ± 0.05 e	128.23 ± 3.82 bc	308.39 ± 24.49 c	128.45 ± 7.73 d
Jimiligaon,	1.32 ± 0.02 g	218.53 ± 4.67 f	1.90 ± 0.07 e	24.78 ± 2.09 k	154.30 ± 3.70 e	1.15 ± 0.02 b	10.25 ± 0.08 c	5.44 ± 0.10 bc	2.53 ± 0.01 e	108.17 ± 5.40 c	309.32 ± 17.15 c	130.48 ± 2.75 d
Tezpur University,	1.18 ± 0.04	218.19 ± 8.49 f	1.81 ± 0.10 j	21.80 ± 0.67 m	127.94 ± 2.08 m	1.16 ± 0.02 b	10.59 ± 0.11 c	5.36 ± 0.05 c	1.88 ± 0.05 f	107.23 ± 1.36 c	218.90 ± 10.48 d	105.45 ± 0.90 e
Kanyaka Bahumukhi Pam	1.52 ± 0.06 i	220.84 ± 3.79 f	1.87 ± 0.02 i	24.79 ± 0.47 j	142.53 ± 2.69 i	1.13 ± 0.03 b	10.25± 0.12 c	5.58 ± 0.12 b	2.57 ± 0.03 e	126.94 ± 8.70 b	369.20 ± 13.25 a	118.09 ± 1.57 m

Distinct lowercase letters in each column denote a significant difference within soil samples at a 5% significance level ($p \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

Antagonistic activity against *Phytophthora infestans*

The results indicate that certain endophytic fungi possess potent antagonistic properties, likely attributable to the production of antifungal metabolites, competition for space and nutrients, and secretion of lytic enzymes. *Penicillium* sp., showing the highest inhibition (97.6%), is well known for producing secondary metabolites such as griseofulvin, penicillin, and various polyketides with broad-spectrum antifungal activity (Elhamouly et al., 2022). Its high inhibitory effect suggests strong potential as a biocontrol agent.

Colletotrichum sp. and *Aspergillus niger* also demonstrated significant antagonistic effects (>94%), which may be due to the synthesis of cell wall-degrading enzymes like chitinases and glucanases or volatile organic compounds (VOCs) that suppress pathogen growth (Hung et al., 2015). Although *Colletotrichum* is commonly associated with pathogenicity, several non-pathogenic strains function as endophytes and exhibit biocontrol potential under specific ecological conditions (Rai et al., 2023).

Fusarium sp. exhibited substantial inhibition (82.35%), likely through competitive exclusion or production of antifungal peptides and fusaric acid, as reported in previous biocontrol studies (Sajeena et al., 2020). In contrast, species like *Alternaria* sp. showed only moderate inhibition, and others including *Bipolaris*, *Curvularia*, and morphotypes showed limited to no antagonism, possibly due to lack of effective antifungal mechanisms or slower growth rates in dual culture assays (Table 2) (Fig 5).

Overall, the findings highlight *Penicillium* sp., *Colletotrichum* sp., *Aspergillus niger*, and *Fusarium* sp. as promising candidates for further evaluation in plant protection strategies, especially against fungal phytopathogens. Their strong inhibitory effects in vitro provide a foundation for potential development into biofungicides or integrated disease management agents.

Preparation of fungal consortium

In a collaborative effort to enhance citrus cultivation through microbial biotechnology, Tezpur University successfully developed four distinct fungal consortia derived from endophytic fungi isolated from the roots and stems of *Citrus limon* and *Citrus reticulata*. These consortia were meticulously formulated based on the isolates demonstrated plant growth-promoting (PGP) traits, such as indole-3-acetic acid (IAA) production, phosphate solubilization, nitrogen fixation, and ammonia production. The selection process for the constituent fungal strains also considered their antagonistic potential against common citrus pathogens, as well as their ability to tolerate environmental stressors (Fig 6).

Table 2: Antagonistic activity against Phytophthora infestans by endophytic fungi associated with Citrus tissues.

Endophytic fungi	Inhibition of Pathogen (%)
Aspergillus niger	94.1±0.54 b
Aspergillus sp.	3.5±0.41 h
Colletotrichum sp.	94.11±0.21 b
Fusarium sp.	82.35±0.25 c
Alternaria sp.	52.4±0.12 d
Bipolaris sp.	15.2±0.24 e
Curvularia sp.	2.3±0.31 i
Stemphylum sp.	8.2±0.21 f
Penicillium sp.	97.6±0.51 a
Mycelia sterilia	8.2±0.47 f
Morphotype 1	4.7±0.33 g
Morphotype 2	16.4±0.41 e
Morphotype 3	8.5±0.35 f

Distinct lowercase letters in each column denote a significant difference within fungal species at a 5% significance level ($p \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

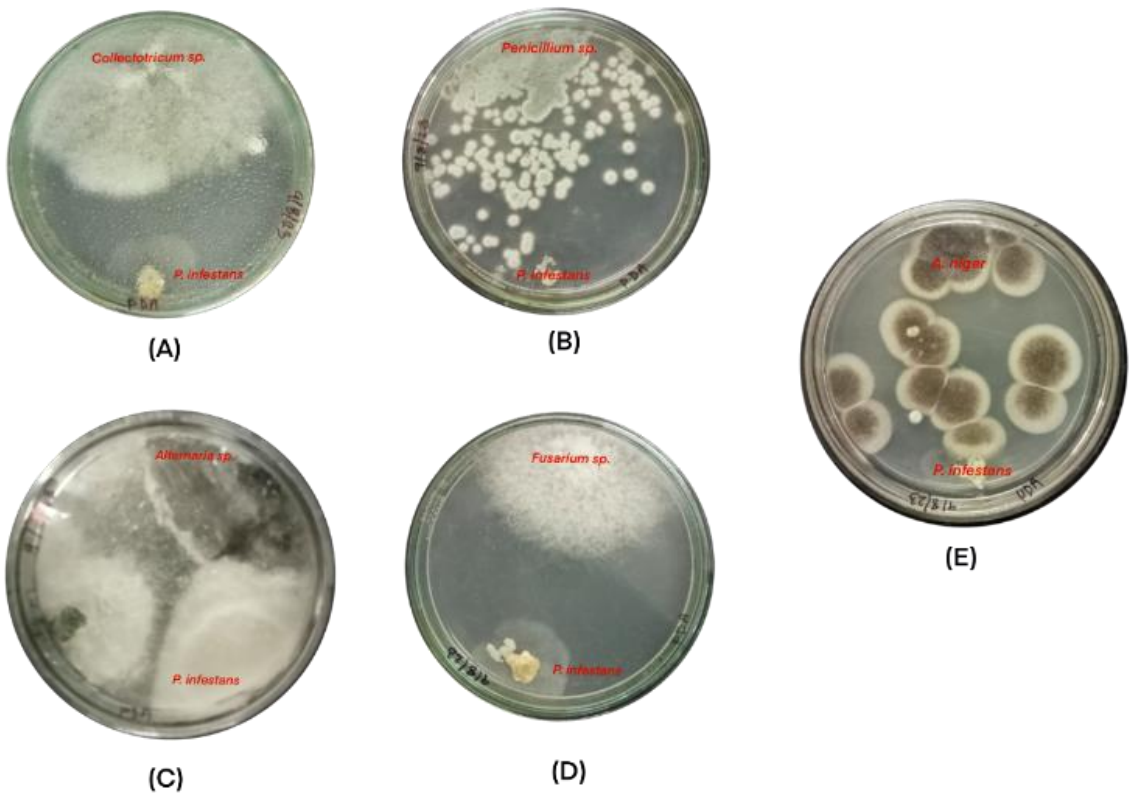


Fig 5: Antagonistic activity by *Citrus* endophytic fungi against *Phytopthora infestans* (Dual culture assay) A: *Colletotrichum* sp. B: *Penicillium* sp. C: *Alternaria* sp. D: *Fusarium* sp. E: *Aspergillus niger*.

To ensure the viability and effectiveness of the formulated fungal consortia, each consortium was maintained under controlled laboratory conditions, with monthly monitoring of colony-forming units (CFU), which ranged between 10^4 and 10^7 CFU/mL. These fungal consortia were subsequently applied as bio-inoculants to two-year-old C. limon seedlings cultivated under controlled greenhouse conditions. The application method involved soil drenching around the rhizosphere zone to promote colonization and symbiotic interaction with the host plant.

To assess the physiological impact of the fungal inoculation, photosynthetic activity in the treated *C. limon* seedlings was monitored on a weekly basis from July to December 2024. Key physiological parameters, including net photosynthetic rate, was measured using a portable photosynthesis system These measurements aimed to evaluate the influence of fungal colonization on the plant's photosynthetic efficiency and overall health.

Additionally, monthly assessments of rhizospheric microbial population were conducted to evaluate the stability and persistence of the inoculated fungal consortia over the six-month period.

For comparative analysis, a parallel experimental setup was conducted using seven bacterial consortia developed by Assam Agricultural University (AAU), Jorhat. These bacterial consortia were derived from rhizospheric bacteria isolated from healthy citrus orchards and were similarly characterized for PGP traits such as IAA production, phosphate solubilization, siderophore production, and antagonism against citrus pathogens. The bacterial consortia were applied to a separate batch of *C. limon* seedlings using similar inoculation and monitoring protocols.

This comparative study was designed to evaluate and contrast the relative effectiveness of endophytic fungal consortia and bacterial consortia in enhancing *Citrus* seedling growth, improving photosynthetic performance, and sustaining beneficial microbial populations in the rhizosphere. The outcome of this investigation is expected to provide valuable insights into the development of effective microbial bioformulations for sustainable *Citrus* cultivation in the northeastern region of India.

Photosynthetic activity

The photosynthetic performance of *Citrus limon* seedlings treated with microbial consortia was assessed to evaluate the physiological benefits conferred by different microbial bio-inoculants. Plants treated with the bacterial consortia developed by Assam Agricultural University (AAU), Jorhat, recorded net photosynthetic rates ranging from 12 to 18 μmol CO_2 m^{-2} s^{-1} over the monitoring period from July to December 2024. These values indicate a moderate but steady photosynthetic response, suggesting that the bacterial consortia were effective in maintaining baseline photosynthetic efficiency under greenhouse conditions. This performance could be attributed to the rhizospheric activity of the bacterial strains, which promote plant growth primarily through mechanisms such as phosphate solubilization, nitrogen fixation, and hormone production (Santoyo et al., 2021), thereby indirectly supporting photosynthetic metabolism.

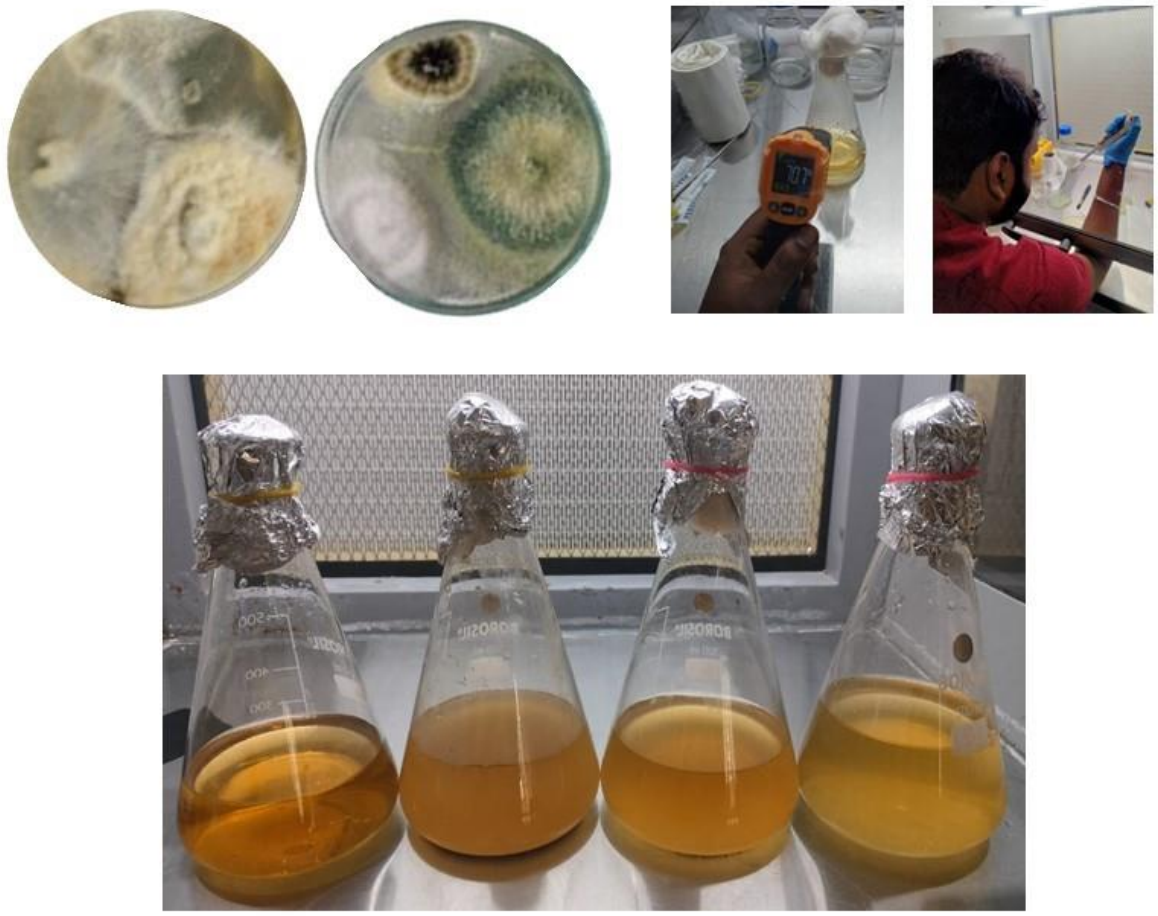


Fig 6: Preparation of fungal consortia

In contrast, seedlings inoculated with fungal consortia developed by Tezpur University (TU) showed significantly elevated photosynthetic rates, ranging from 20 to 28 μmol CO_2 m^{-2} s^{-1}. This represents a 30–50% increase in photosynthetic efficiency compared to the AAU bacterial consortia (Graph 6). The marked enhancement highlights the greater bioefficacy of endophytic fungi, which, unlike bacteria, colonize internal plant tissues and directly influence host metabolic processes (García-Latorre et al., 2023). Endophytic fungi are known to produce a wide range of plant growth-promoting compounds, including auxins, cytokinins, and gibberellins, as well as to improve nutrient availability (Tsavkelova et al., 2012). The significant improvement in photosynthetic performance in TU-treated seedlings may be attributed to the enhanced carbon assimilation capacity facilitated by endophytic colonization. These fungi likely improved stomatal conductance, chlorophyll content, and enzyme activity related to photosynthesis (Santoyo et al., 2021). In contrast, the relatively modest effect of the AAU bacterial consortia on photosynthetic parameters suggests that their influence may be more localized to the rhizosphere and restricted in their capacity to modulate systemic physiological responses (Santoyo et al., 2021).

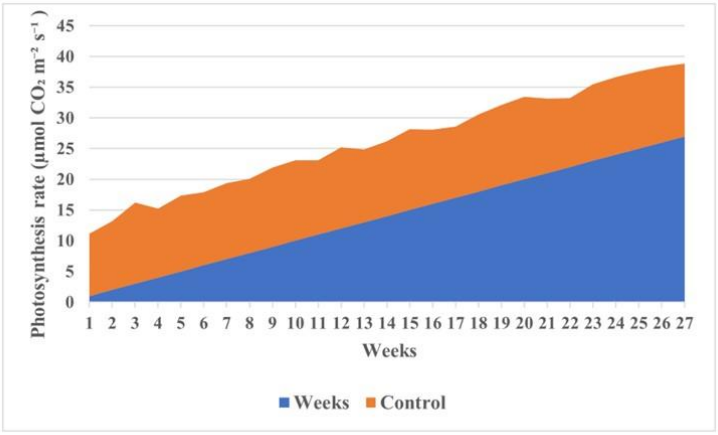
Impact of fungal consortium in growth of *Citrus* seedlings

The growth performance of 6-month-old *Citrus* seedlings varied significantly among the tested consortia (Table 3). All four fungal consortia (TU1–TU4) exhibited a substantial increase in plant height, leaf count, root length, leaf area, and root area compared to the seven bacterial consortia (AAU1–AAU7).

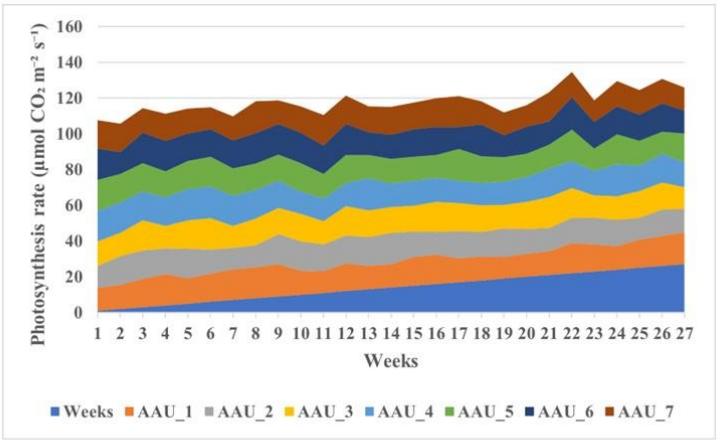
Among the fungal treatments, TU2 recorded the highest plant height (74.5 ± 3.5 cm), which was significantly greater ($p \leq 0.05$) than TU1, TU3, and all bacterial consortia. TU2 was grouped in the "a" category, while TU4 (71.2 ± 3.4 cm) shared a statistically similar group **(**ab**)**, and TU1 (69.3 ± 3.2 cm) and TU3 (66.8 ± 3.1 cm) followed in groups b and c, respectively. In contrast, bacterial consortia showed significantly lower plant height values, ranging from 27.3 ± 1.4 cm (AAU3) to 30.9 ± 1.8 cm (AAU7), all grouped in e or f categories.

Root length also increased significantly in seedlings treated with fungal consortia. TU2 again recorded the highest root length (31.2 ± 1.8 cm), statistically greater than all other treatments (a), followed by TU4 (30.1 ± 1.7 cm, ab), TU1 (28.9 ± 1.6 cm, b), and TU3 (27.6 ± 1.5 cm, **c**). Bacterial treatments showed root lengths between 11.0 ± 0.6 cm (AAU3) and 13.0 ± 0.9 cm (AAU7), all significantly lower.

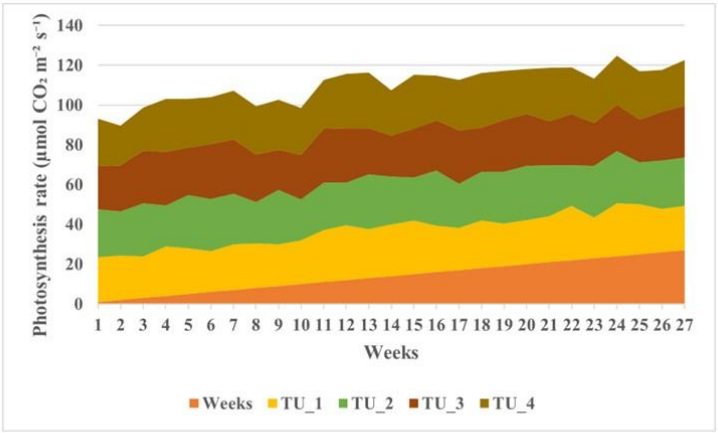
Leaf area and root area followed the same pattern. TU2 showed the maximum values (39.0 ± 2.2 cm^2 leaf area and 25.8 ± 1.4 cm^2 root area), placing it in group a, significantly higher than all bacterial treatments and even other TU groups. TU1, TU3, and TU4 also showed marked improvements in these parameters, forming distinct statistical groups (b**,** c**,** ab, respectively), whereas all bacterial consortia occupied the lowest groups (e and f).



A



B



C

Graph 6: Photosynthesis rates of the citrus seedlings under the treatment A) Control (without consortia) B) 7 bacterial consortia developed by AAU Jorhat, C) Four fungal consortia developed by Tezpur University during the period of July-December 2024 (assessed with Licor-6400).

Fig 7: Growth of *Citrus limon* seedlings under the application of A) Eight bacterial consortia developed by AAU, Jorhat, B) Four fungal consortia developed by Tezpur University.

The significantly enhanced growth parameters observed in fungal consortia-treated seedlings can be attributed to multiple plant growth-promoting mechanisms exhibited by fungi, such as auxin (IAA) production, phosphate solubilization, siderophore production, and improved nutrient uptake (Vyas & Bansal, 2018). Fungi like *Penicillium*, *Aspergillus*, and *Fusarium* sp. have been shown to promote vigorous root and shoot development through phytohormonal signaling and efficient colonization of the rhizosphere (García-Latorre et al., 2023; Watts et al., 2023). These capabilities enhance not only primary growth metrics but also root surface area and nutrient translocation efficiency. In contrast, bacterial consortia, though beneficial, often exhibit slower colonization and limited persistence under field conditions compared to fungi (Potshangbam et al., 2017). Furthermore, fungal hyphae enable better soil exploration, enhancing water and nutrient absorption, which is crucial during the early establishment of seedlings (Hardoim et al., 2015).

The performance of TU2 across all measured parameters suggests it contains a highly synergistic fungal combination with potent PGP (plant growth-promoting) and possible biocontrol properties, likely contributing to better root architecture, greater photosynthetic surface area, and increased biomass accumulation. This aligns with previous reports where fungal consortia significantly outperformed individual strains and bacterial inoculants in various horticultural crops (Santoyo et al., 2021). The growth comparison of the *Citrus limon* seedlings are shown in Fig 7.

Table 3: *Citrus* seedling growth under the application of fungal and bacterial consortia developed by Tezpur University and Assam Agricultural University.

Treatments	Plant Height (cm)	Root Length (cm)	Leaf Area (cm^2)	Root Area (cm^2)
Control	22.5 ± 1.2 g	8.6 ± 0.5 g	10.8 ± 0.7 g	6.7 ± 0.4 g
AAU1	28.4 ± 1.5 f	11.6 ± 0.7 f	14.5 ± 0.9 f	9.6 ± 0.5 f
AAU2	29.7 ± 1.6 ef	12.4 ± 0.8 ef	15.8 ± 1.0 ef	10.2 ± 0.6 ef
AAU3	27.3 ± 1.4 f	11.0 ± 0.6 f	13.9 ± 0.8 f	9.1 ± 0.5 f
AAU4	28.9 ± 1.5 f	11.8 ± 0.7 f	14.9 ± 0.9 f	9.8 ± 0.5 f
AAU5	30.2 ± 1.7 e	12.7 ± 0.8 e	16.3 ± 1.1 e	10.5 ± 0.6 e
AAU6	28.2 ± 1.5 f	11.5 ± 0.7 f	14.3 ± 0.9 f	9.4 ± 0.5 f
AAU7	30.9 ± 1.8 e	13.0 ± 0.9 e	16.8 ± 1.1 e	10.9 ± 0.6 e
TU1	69.3 ± 3.2 b	28.9 ± 1.6 b	36.2 ± 2.0 b	23.7 ± 1.3 b
TU2	74.5 ± 3.5 a	31.2 ± 1.8 a	39.0 ± 2.2 a	25.8 ± 1.4 a
TU3	66.8 ± 3.1 c	27.6 ± 1.5 c	34.5 ± 1.9 c	22.9 ± 1.2 c
TU4	71.2 ± 3.4 ab	30.1 ± 1.7 ab	37.4 ± 2.1 ab	24.9 ± 1.3 ab

Distinct lowercase letters in each column denote a significant difference within plant growth under application of consortia at a 5% significance level ($p \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

Impact of fungal consortium on soil chemical properties

The application of both bacterial and fungal plant growth-promoting (PGP) consortia significantly enhanced soil fertility parameters of the potted soil of *Citrus* seedlings (Available N, P, K, and Soil organic carbon) compared to uninoculated soils, with fungal consortia outperforming bacterial consortia in all measured traits (Table 4).

In terms of soil available nitrogen (N), fungal consortia treatments exhibited markedly higher values, with TU2 recording the maximum (90.1 ± 0.25 mg/kg**)**, followed closely by TU4 (89.7 ± 0.25 mg/kg) and TU1 (88.5 ± 0.25 mg/kg), all of which were statistically grouped in the top Duncan classes (a–c). In contrast, bacterial consortia such as AAU3 and AAU7 showed significantly lower nitrogen enhancement, falling into the lower statistical groupings (k–l). The elevated nitrogen levels under fungal consortia can be attributed to enhanced biological nitrogen fixation, stimulation of root exudation, and increased microbial biomass turnover (Santoyo et al., 2021; Tian et al., 2023).

Soil available phosphorus (P) availability also increased significantly, with TU2 again showing the highest phosphorus content (27.5 ± 0.41 mg/kg), followed by TU4 (26.9 ± SD) and TU1 (26.2 ± 0.54 mg/kg). These values were significantly higher than those recorded under all bacterial treatments. This improvement is largely due to the solubilization of inorganic phosphate through the secretion of organic acids (such as gluconic, oxalic, and citric acids) and phosphatases by phosphate-solubilizing fungi like *Penicillium* and *Aspergillus* (Kumar & Prasher, 2023). The enhanced colonization capacity of fungal hyphae also improves P acquisition by extending the nutrient absorption zones beyond the rhizosphere (Luo et al., 2024).

With respect to available potassium (K) levels, the fungal consortia again recorded superior outcomes, where TU2 showed the highest value (168.9 ± 0.25 mg/kg), and other fungal groups like TU4 and TU1 also showed significantly enhanced potassium content. Fungal strains are known for releasing chelating agents and organic acids which help in solubilizing bound forms of potassium, thus making it bioavailable (Das & Pradhan, 2016). In contrast, bacterial consortia such as AAU3 and AAU7 demonstrated lower potassium mobilization efficiency.

The soil organic carbon (SOC) content was also significantly higher in soils treated with fungal consortia. TU2 again ranked highest (0.91 ± 0.31%), followed by TU4 (0.90 ± 0.54%), while bacterial consortia such as AAU3 and AAU7 had the lowest SOC values (0.70 and 0.71%, respectively). The higher SOC under fungal treatments can be explained by increased microbial biomass contribution, root biomass, and exudates as well as more effective decomposition and humification of organic matter by saprophytic fungal components (Grinhut et al., 2007). These fungi stimulate the buildup of stable carbon pools in soil, enhancing soil health and fertility in the long term (Grinhut et al., 2007).

Table 4: Effect of bacterial (AAU1–AAU7) and fungal (TU1–TU4) consortia on soil chemical properties of the potted soil.

Consortia	Available Nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	SOC (%)
AAU1	78.3 ± 0.02 i	21.5 ± 0.25 i	145.4 ± 0.58 j	0.72 ± 0.41 j
AAU2	80.1 ± 0.05 g	22.3 ± 0.23 g	150.2 ± 0.51 g	0.76 ± 0.36 g
AAU3	75.4 ± 0.12 l	19.8 ± 0.25 l	142.5 ± 0.23 l	0.70 ± 0.45 l
AAU4	82.0 ± 0.24 e	23.1 ± 0.21 e	155.1 ± 0.35 e	0.79 ± 0.41 e
AAU5	77.8 ± 0.32 j	20.7 ± 0.31 j	149.0 ± 0.52 h	0.74 ± 0.56 i
AAU6	79.3 ± 0.45 h	21.9 ± 0.38 h	147.6 ± 0.34 i	0.75 ± 0.37 h
AAU7	76.9 ± 0.48 k	20.1 ± 0.47 k	144.3 ± 0.52 k	0.71 ± 0.54 k
TU1	88.5 ± 0.25 c	26.2 ± 0.14 c	165.4 ± 0.45 c	0.88 ± 0.25 c
TU2	90.1 ± 0.25 a	27.5 ± 0.41 a	168.9 ± 0.25 a	0.91 ± 0.31 a
TU3	87.2 ± 0.21 d	25.7 ± 0.12 d	163.1 ± 0.14 d	0.86 ± 0.15 d
TU4	89.7 ± 0.25 b	26.9 ± 0.54 b	167.3 ± 0.41 b	0.90 ± 0.54 b

Distinct lowercase letters in each column denote a significant difference within soil properties under application of consortia at a 5% significance level ($p \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

Impact of fungal consortium on soil biological properties

The application of microbial consortia significantly influenced the soil biochemical parameters, including dehydrogenase activity (DHA), urease (URA), β-glucosidase (BGLU), phosphomonoesterase activity (PMA), and microbial biomass carbon (MBC) (Table 5).

Among the fungal consortia, TU2 demonstrated the highest values across all parameters: DHA (3.4 ± 0.09 ml^{-1} INTF g^{-1} soil 2 h^{-1}), URA (24.1 ± 0.7 µg NH_4^+ g^{-1} soil 2 h^{-1}), BGLU (2.6 ± 0.07 µg PNP g^{-1} h^{-1}), PMA (4.4 ± 0.09 µg pNP g^{-1} h^{-1}), and MBC (228 ± 5.9 mg kg^{-1}), which were significantly superior to all bacterial consortia ($p < 0.05$). This was closely followed by TU4 and TU1, which also showed significantly higher enzyme activities and MBC compared to the bacterial treatments.

Among bacterial consortia, AAU4 and AAU6 were the top performers, with AAU4 recording DHA (2.6 ± 0.06), URA (19.8 ± 0.5), BGLU (2.0 ± 0.05), PMA (3.7 ± 0.08), and MBC (195 ± 4.5). However, even the best-performing bacterial consortia were significantly lower than the fungal consortia TU2 and TU4 (DMRT: e–j vs. a–d groupings). The lowest activity was observed in AAU3, with DHA (2.1 ± 0.09), URA (17.6 ± 0.7), BGLU (1.6 ± 0.07), PMA (3.2 ± 0.10), and MBC (178 ± 5.5), all of which were statistically grouped in the lower ranks (DMRT: l).

Overall, the results suggest that fungal consortia were more effective in enhancing soil enzymatic activities and microbial biomass compared to bacterial consortia. The elevated DHA and PMA indicate increased microbial metabolic activity and phosphorus mobilization potential, while higher MBC reflects an enriched microbial biomass pool. These enhancements could be linked to

greater fungal mycelial density and functional diversity in the rhizosphere, contributing to improved soil health and nutrient cycling, as supported by previous findings (Zhang et al., 2021).

Table 5: Effect of bacterial (AAU1–AAU7) and fungal (TU1–TU4) consortia on soil biological properties**.**

Consortia	DHA (ml^{-1} INTF g^{-1} soil $2h^{-1}$)	URA (µg NH_4^+ g^{-1} soil $2h^{-1}$)	BGLU (µg PNP/g/hr)	PMA (µg pNP g^{-1} h^{-1}))	MBC (mg/kg)
AAU1	2.3 ± 0.08 j	18.5 ± 0.6 i	1.8 ± 0.06 i	3.4 ± 0.09 j	186 ± 5.2 i
AAU2	2.5 ± 0.07 g	19.2 ± 0.5 g	1.9 ± 0.05 g	3.6 ± 0.08 g	192 ± 4.8 g
AAU3	2.1 ± 0.09 l	17.6 ± 0.7 l	1.6 ± 0.07 l	3.2 ± 0.10 l	178 ± 5.5 l
AAU4	2.6 ± 0.06 e	19.8 ± 0.5 e	2.0 ± 0.05 e	3.7 ± 0.08 e	195 ± 4.5 e
AAU5	2.4 ± 0.07 i	18.3 ± 0.6 j	1.7 ± 0.06 j	3.5 ± 0.09 i	184 ± 5.0 j
AAU6	2.5 ± 0.07 h	19.0 ± 0.5 h	1.9 ± 0.05 h	3.6 ± 0.08 h	190 ± 4.7 h
AAU7	2.2 ± 0.08 k	17.9 ± 0.6 k	1.7 ± 0.06 k	3.3 ± 0.09 k	180 ± 5.3 k
TU1	3.1 ± 0.10 c	22.5 ± 0.8 c	2.4 ± 0.08 c	4.1 ± 0.10 c	215 ± 6.1 c
TU2	3.4 ± 0.09 a	24.1 ± 0.7 a	2.6 ± 0.07 a	4.4 ± 0.09 a	228 ± 5.9 a
TU3	3.0 ± 0.08 d	21.8 ± 0.6 d	2.3 ± 0.07 d	4.0 ± 0.08 d	210 ± 5.7 d
TU4	3.3 ± 0.09 b	23.6 ± 0.7 b	2.5 ± 0.07 b	4.3 ± 0.09 b	225 ± 6.0 b

Distinct lowercase letters in each column denote a significant difference within soil properties under application of consortia at a 5% significance level ($p \leq 0.05$) based on Duncan's Multiple Range Test (DMRT).

11. Conclusion and achievements

The project successfully demonstrated that harnessing endophytes and arbuscular mycorrhizal fungi from the citrus microbiome in Northeast India leads to significant improvements in plant growth and soil health management in commercial citrus crops, notably outperforming bacterial consortia in nearly all assessed parameters. This research confirmed that the citrus microbiome in Assam and Arunachal Pradesh contains a high diversity of endophytic fungi, with dominant genera such as *Aspergillus*, *Penicillium*, *Colletotrichum*, and *Fusarium* consistently isolated from multiple agroclimatic zones, regardless of environmental variation. The study established that host plant specificity and genotype are major drivers of the recurrent core fungal community in citrus, while local soil properties and management practices modulate richness and evenness. Developing cost-effective fungal consortia by combining endophytic strains with strong plant growth-promoting (PGP) traits such as high IAA production, phosphate solubilization, and antagonistic activity against pathogens resulted in a marked enhancement across citrus seedling growth metrics, including plant height, root length, leaf area, and photosynthetic rates. Fungal consortia notably surpassed the bacterial consortia from AAU, Jorhat, with TU2 (Tezpur University) delivering the most significant improvements: up to a 30–50% increase in photosynthetic activity and substantial gains in biomass, leaf count, and nutrient uptake. Furthermore, fungal consortium-treated soils exhibited superior physicochemical and biological parameters such as higher available nitrogen, phosphorus, potassium, and soil organic carbon, along with elevated microbial enzyme activities

(DHA, urease, β-glucosidase, phosphomonoesterase) and microbial biomass carbon. These changes indicate that endophytic fungi not only promote rapid plant growth but also enhance soil fertility, nutrient cycling, and overall ecosystem resilience.

Major achievements

- **Comprehensive isolation:** Twelve distinct endophytic fungal taxa were isolated from citrus species across 12 sites in Assam and Arunachal Pradesh, confirming widespread distribution and host specificity despite variable environmental conditions.
- **Diversity assessment:** Detailed diversity, richness, and evenness analysis revealed robust fungal communities, with Boragaon, Tezpur University, and Wakro showing the highest diversity indices. Fungal consortia maintain ecosystem-wide benefits across locations.
- **PGP trait characterization:** *Aspergillus, Penicillium, Colletotrichum,* and *Fusarium* sp. were identified as the most potent PGP agents, with high IAA production (up to 174.25 µg/mL), strong phosphate solubilization (SI > 3.0), and exceptional pathogen inhibition (up to 97.6%).
- **Consortium development & compatibility:** Four fungal consortia were developed and standardized for consistent application, showing robust compatibility and no cross-antagonism among chosen strains.
- **Greenhouse validation:** Application of fungal consortia resulted in a statistically significant improvement in citrus seedling growth and physiological performance compared to both control and bacterial treatments. TU2 achieved the best results in plant height (74.5 cm), root length (31.2 cm), leaf area (39 cm^2), and root area (25.8 cm^2).
- **Soil health improvement:** Fungal consortia markedly increased available N, P, K, organic carbon, and key biological activity metrics in soil, substantiating their role as superior biofertilizers for sustainable citrus production.

Summary (200 words)

Tezpur University targeted the harnessing of endophytic fungi from the citrus microbiome in Northeast India for plant and soil health management. Extensive field surveys across Assam and Arunachal Pradesh enabled the isolation of twelve endophytic fungal taxa (including dominant genera such as *Aspergillus, Penicillium, Colletotrichum*, and *Fusarium*) from *Citrus reticulata* and *Citrus limon*, revealing considerable diversity and host specificity despite environmental variation. Diversity indices varied across sites, but the presence of core endophytes was consistent. A series of fungal consortia were developed based on their superior plant growth-promoting traits: high IAA production (up to 174.25 µg/mL), phosphate solubilization, and strong pathogen antagonism (up to 97.6% inhibition of *Phytophthora infestans*). In greenhouse trials, citrus seedlings treated with fungal consortia significantly outperformed both uninoculated controls and bacterial consortia in growth, root development, leaf area, and photosynthesis (up to 30–50% improvement). Fungal consortia also enhanced soil fertility (available N, P, K, SOC) and biological activity. These findings demonstrate that endophytic fungal consortia offer a potent, sustainable bioinoculant strategy for improving citrus productivity and soil health in the region.

12. New Lead

1. Precision microbial consortia formulation

The consistent superiority of certain fungal consortia, particularly TU2, for citrus growth and soil health points to the need for further exploration of synergistic strain combinations and the potential for tailoring bio-inoculant formulations to specific citrus varieties or local soil conditions. Systematic screening of endophytic fungi combinations may yield optimized consortia for different agro-climatic zones.

2. Mechanistic understanding of host–endophyte interactions

The strong impact of host genotype and tissue phytochemistry on endophyte recruitment suggests a lead for targeted studies on the molecular mechanisms underlying host-fungus specificity. Advances in transcriptomics and metabolomics could help elucidate how citrus plants select beneficial microbes and how these microbes modulate plant responses for disease resistance and growth promotion.

3. Biocontrol agents

The discovery of endophytic strains with high antagonistic activity against *Phytophthora infestans* (e.g., *Penicillium sp*., *Colletotrichum* sp., *Aspergillus niger*) underscores the bio-prospecting potential for developing new biofungicides or integrated disease management strategies for citrus and other crops. Further research may aim at isolating and characterizing antifungal metabolites and validating field-level biocontrol efficacy.

4. Enhancement of soil health and carbon sequestration

The observed increase in soil organic carbon and microbial biomass with fungal consortia hints at a dual role in fertility improvement and long-term soil carbon sequestration. This aligns with climate-smart agriculture practices and invites research into the role of citrus endophytes in sustainable carbon cycling and mitigation of soil degradation.

5. Crop diversification and wider application

With the functional versatility of the identified endophytes, there is scope to test these fungal consortia in other horticultural and plantation crops in the Northeast and similar agro-ecological regions. Such cross-crop trials could validate their broad-spectrum plant growth-promotion and stress-resilience effects.

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Consolidated Statement of Accounts (*Towards final Settlement of Accounts*)

(For the period from Official Sanctioned Date to Official Completion Date...10/02/2021 to 09/08/2025)

1. Title of the Project: Harnessing Endophytes and Arbuscular Mycorrhizal Fungi from Citrus Microbiome for Plant and Soil Health Management in North East India
2. Name of the Grantee Institution: Tezpur University
3. Principal Investigator: Prof. Nirmali Gogoi

Sanction Order No. & Date of Sanctioning of the Project: BT/PR40047/NER/95/1662/2020 Dtd. February 10, 2021

4. Actual Duration of the Project: 10th February 2021 to 9th August 2025

A. Consolidated Budget Statement

(Financial figures given in this CBS are shown in lakhs)

Budgetary Object Heads	Total Sanctioned Grant within each Budget Head *(as per the Sanctioned Order)*	Grants Release Made by DBT							Expenditure Incurred *(This must align with UCs and SoEs submitted yearly)* **							Amount Refunded through BharatKosh	Unspent Balance left with the Institution	Remark *(if any)*
		Details to be furnished Financial Year wise							Details to be furnished Financial Year wise									
		10th Feb 2021 to 31st march 2021	2021 to 2022	2022 to 2023	2023 to 2024	2024 To 2025	1st April 2025 to 9th August 2021	Total	10th Feb 2021 to 31st march 2021	2021 to 2022	2022 to 2023	2023 to 2024	2024 To 2025	1st April 2025 to 9th August 2025	Total			
Grants for Creation of Capital Assets		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Grants-in-aid General		**6.76760**	0.00	**5.96885**	0.00	0.00	**6.45000**	**19.18645**	0.00	**5.46885**	**1.24000**	**5.41947**	0.00	**5.79798**	**17.9263**	**0.04908**	***0.65202**	*Amount of Rs **0.60813** lakh was lapsed on 20th December 2024 and was unable to be utilized till 31st March 2024
Interest Earned/ any other Receipt(s)	--	0.00	**0.04908**	0.00	0.00	0.00	0.00	**0.04908**	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Total		**6.76760**	**0.04908**	**5.96885**	0.00	0.00	**6.45000**	**19.23553**	0.00	**5.46885**	**1.24000**	**5.41947**	0.00	**5.79798**	**17.92630**			

**Indicate if there was any deviation/revision as against the sanctioned order, and reason thereof in remark section

B. Details of Equipment Procured During the Project Period

(Financial figures given in this equipment procured details are shown in lakhs)

S. No.	Name of Equipment(s)/ Asset(s) Acquired	Sanctioned Cost	Quantity Procured	Actual Cost *(Figures not to roundoff)*	Balance to be reimbursed
	1	2	3	4	5
a.	Nil	0.00	0.00	0.00	0.00
b.	Nil	0.00	0.00	0.00	0.00
c.	Nil	0.00	0.00	0.00	0.00
d....	Nil	0.00	0.00	0.00	0.00
Total		0.00	0.00	0.00	0.00

- *The signatories hereby certify that the expenditure incurred from the released grant was utilized solely for the purpose of implementation of the project under consideration, and in compliance with the sanction order. We also certify that monthly emoluments of engaged human resource in this project were disbursed in accordance with the duly notified norms/guidelines of the Government Department/Ministry/Autonomous Bodies. Detailed expenditure, if asked for, will be submitted for the purpose of Audit.*

- *It is also certified that the Institute has not utilized more than the amount sanctioned under the 'Overhead' component.*

27/10/25

PROJECT INVESTIGATOR

(Name, Signature and Stamp)

Prof. Nirmali Gogoi
Department of Environmental Science
Tezpur University

FINANCE OFFICER OF THE INSTITUTION

(Name, Signature and Stamp)
Finance Officer
Tezpur University

HEAD OF THE INSTITUTION

(Name, Signature and Stamp)
Registrar i/c
Tezpur University

Please also note the following:

1. *Grant utilization details for this UC and all other pending UCs of this project need to be compulsorily updated in PFMS website. After that please generate GFR- 19 from PFMS website. Get signed GFR-19 and upload the signed GFR-19 in PFMS website and enclose original copy of the same with financial documents to be submitted to DBT.*

2. *Please cross check and ensure that all financial documents are complete in all respect and all are in proper order and in proper DBT formats.*

3. *Please ensure that all financial documents should tally with each other:*

    a. *Details in Capital Assets Acquired Certificate are matching with equipment expenditure details given in UC &SoE and are in consonance with the DBT approvals/ sanction equipment /Capital assets item list.*

    b. *Expenditure details in Utilization Certificates are matching with the GFR-19 generated from PFMS website.*

4. *All financial documents duly signed by PI, Finance & Accounts Officer, and Executive Authority of Institution. In case of NGO/ Private Institutions UC &SE also needs to be Audited by Charted Accountant.*

5. *All financial documents are required to be submitted by email to the program officer.*

Capital Assets Acquired (*wholly or substantially*) Out of Government Grants
(*Separate Register to be maintained by Grantee Institution*)

(For the financial year 2021 to 2025, for the period from 10/02/2021 to 9/08/2025)

(Financial figures given in this CAAC are shown in lakhs)

1.	Name of the Sanctioning Authority	:	Department of Biotechnology, Govt. of India
2.	S. No. of Assets Register	:	N/A
3.	Name of the Grantee Institution	:	Tezpur University
4.	Project No. & Date of sanction order	:	BT/PR40047/NER/95/1662/2020 Dtd. February 10, 2021
5.	Amount of the sanctioned grant for creation of capital assets	:	Nil
6.	Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order	:	N/A
7.	Particulars of assets actually created or acquired *(Details as per enclosed format)*	:	N/A
8.	Value of the assets as on date	:	N/A
9.	Purpose for which assets utilized at present	:	N/A
10.	Encumbered or not	:	N/A
11.	Reasons, if encumbered		N/A
12.	Disposed of or not	:	N/A
13.	Reasons and authority, if any, for disposal	:	N/A
14.	Amount realized on disposal *(before initiating such disposal process prior approval of DBT shall be obtained)*	:	Nil
15.	Remarks	:	Nil

Details of Capital Assets Acquired During the Period

(Financial figures given in this CAAC are shown in lakhs)

S. No.	Name of Equipment/ Asset	Sanctioned Cost	Quantity Procured	Actual Cost *(Actual figure, not to roundoff)*	Date of Placing order	Date of Receiving Equipment	Payment Voucher No. with Date *(Copy of payment voucher(s) may please be enclosed)*	Balance to be reimbursed *[Provide reason(s) for fluctuation in price (if any)]*
	1	2	3	4	5	6	7	8
Sanctioned Equipment/Asset								
a.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
b.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
c.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
d.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
e.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
f.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
g....	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
Equipment/Asset acquired without sanction but for which the prior approval of the DBT was obtained								
a.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
b.	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
c....	Nil	0.00	Nil	0.00	Nil	Nil	Nil	Nil
Total		0.00	Nil	0.00	Nil	Nil	Nil	Nil

27/10/25

PROJECT INVESTIGATOR
(Name, Signature and Stamp)
Prof. Nirmali Gogoi
Department of Environmental Science
Tezpur University

FINANCE OFFICER OF THE INSTITUTION
(Name, Signature and Stamp)
Finance Officer
Tezpur University

HEAD OF THE INSTITUTION
(Name, Signature and Stamp)
Registrar i/c
Tezpur University