BIODIVERSITAS
Volume 26, Number 12, December 2025
Pages: 5977-5988

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d261202

Diversity and biofertilizer potential of root endophytic fungi in Arabica coffee

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Manuscript received: 16 July 2025. Revision accepted: 23 November 2025.

Abstract. *Sofyan, Rosmana A, Nasaruddin, Bahrun H, Kurniawan, Syakur A, Risal D. 2025. Diversity and biofertilizer potential of root endophytic fungi in Arabica coffee. Biodiversitas 26: 5977-5988.* Sustainable Arabica coffee cultivation requires eco-friendly biological inputs to improve plant growth and soil quality. This study explored the diversity and physiological potential of root endophytic fungi isolated from Arabica coffee plants at two age-structured plots within one village in Maros, South Sulawesi, Indonesia, and evaluated their capacity to quantify phytohormone-related signals and assess compost enrichment. Endophytic fungi were isolated from coffee roots and characterized morphologically to the putative genus level, while biochemical assays estimated IAA-equivalents and GA-like (GA_3) compounds. A randomized block design was used to assess compost decomposition and biofertilizer responses of Arabica seedlings in non-sterile soil. Results showed that a total of fourteen isolates were obtained, with *Trichoderma* spp. dominating young roots (<3 years) and *Aspergillus*, *Penicillium*, and *Paecilomyces* more common in older roots (>10 years). Isolate 5C10BM produced the highest IAA-equivalent signal (3.02 ppm), while P11 yielded the highest GA-like signal (8.14 ppm) and showed a trend toward higher compost nitrogen content relative to the uninoculated control. Compost analysis showed that isolate 6C10BM increased C-organic (20.07%), isolate 11C3BM showed nitrogen levels of 1.19% and isolate 14C3BM elevated phosphorus (0.63%) and potassium (0.42%). Plant assays revealed that isolate 2C10BM increased seedling height (45.4 cm), whereas 9C10BM enhanced leaf number and area. The study demonstrates functional diversity among endophytic fungi, with strain-specific capabilities, particularly in isolates 5C10BM, 11C3BM, and 14C3BM, which exhibited indicative traits relevant to biofertilizer and compost-activating potential. These findings provide insights into endophyte-associated functional traits; future work will include molecular identification and verification of root colonization.

Keywords: Biofertilizer, compost enrichment, endophytic fungi, IAA production, sustainable coffee

INTRODUCTION

As one of the world's leading export commodities, coffee ranks as Indonesia's fourth largest global exporter and second in the ASEAN region (Hamzah et al. 2020). Indonesia's competitive advantage stems from its variety and quality; however, this potential remains underutilized in enhancing export competitiveness (Zacharie and Denny 2024). Over the past five years, Indonesian coffee exports have declined by 5%, accompanied by a 5.6% decline in production capacity (Widyantini 2019). This decline was caused by factors, such as limited production capacity, inconsistent quality standards, and intense competition from other coffee-producing countries (Syahputri et al. 2023). Furthermore, environmental influences, such as climate change, pest and disease outbreaks, and reduced soil fertility due to the long-term excessive use of chemical fertilizers also impact coffee production (Essibu 2024). Regionally, Maros District has significant potential for Arabica coffee development, but productivity is hampered by declining soil fertility due to heavy dependence on chemical fertilizers. Uncontrolled use of chemical fertilizers can damage soil structure, reduce water retention, and increase soil acidity (Gao et al. 2023), ultimately reducing coffee plant productivity. Therefore, a comprehensive land management strategy oriented toward environmental sustainability principles is needed to support the continued increase in Arabica coffee productivity.

The use of local biological agents, such as endophytic fungi, has significant potential to sustainably increase coffee plant productivity by improving soil fertility, increasing resistance to disease and environmental stress, and reducing the dependence on chemical fertilizers (García-Latorre et al. 2021). Recent studies have demonstrated that root endophytic fungi act as effective biostimulants by promoting plant growth through phytohormone production and improving soil nutrient cycling. Additionally, these fungi serve as multifunctional compost bioinoculants, enhancing organic matter decomposition and supporting sustainable agricultural systems (Yasmeen et al. 2024). Endophytic fungi are microorganisms that live within plant tissues without causing disease (Pandao et al. 2024), function as biostimulants, biodecomposers, and biofertilizers; improve soil structure and quality; and strengthen plant defence systems (Wemheuer

et al. 2019; Rhouma et al. 2024). These fungi produce bioactive compounds such as Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3), which play a crucial role in the physiological regulation of plant growth (Biswas and Sarojini 2023; Pratiwi et al. 2024). Furthermore, their presence in coffee plant roots functions as decomposers, breaks down organic matter, increases nutrient availability, and aids natural waste management. Endophytic fungi offer dual benefits for coffee cultivation by increasing crop yields and conserving the soil ecosystem.

The potential of endophytic fungi as biological agents in Arabica coffee generally focuses on their role in pathogen biocontrol (Bekele 2022). Practical applications of endophytic fungi in improving soil fertility and managing coffee waste have been sparsely explored. Existing research has focused primarily on their application to crops with rapid rotations, with little attention paid to coffee plants with longer growth cycles (Mantovani et al. 2018; Del Carmen H Rodríguez et al. 2021). Research on root-endophytic fungi is also relatively limited, particularly that linking species diversity to functional potential (Asad et al. 2023). Furthermore, recent research indicates that endophytic microbiota have the potential to improve soil fertility through nitrogen fixation and phosphate solubilization, which can support sustainable and environmentally friendly coffee production (Poveda et al. 2021).

This study to emphasize of these fungi by exploring their role not only in pathogen biocontrol but also as compost activators and biofertilizers in Arabica coffee cultivation. Framed as a site-specific, age-structured case study, we compared two Arabica plots within a single village (Bentenge, Maros) and do not generalize beyond this local context. The objectives of this research were to: (i) characterize the diversity of root fungal endophytes isolated from Arabica coffee, (ii) evaluate their capacity to produce phytohormones, particularly Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3), and (iii) assess their functional effects as compost bioinoculants and biofertilizers on seedling growth performance and compost nutrient parameters. Utilizing these local microbiota can be expected to provide new opportunities for the development of endophytic fungus-based biofertilizers to support efficient and environmentally friendly tropical coffee farming.

MATERIALS AND METHODS

Study area

This study was conducted from May to August 2024 in smallholder coffee plantation in Bentenge Village, Mallawa Sub-district, Maros District, South Sulawesi, Indonesia (Figure 1). Sampling locations were differentiated based on plant age: Location A (coffee plants aged >10 years), located at coordinates 4°50'23.5"S; 119°49'57.2"E; Location B (coffee plants aged <3 years), at coordinates 4°50'27.51"S; 119°49'50.56"E. Endophytic fungi were isolated and morphologically identified at the Plant Pest and Disease Laboratory, Faculty of Agriculture, Universitas Hasanuddin. For site context, both plots were classified as Ultisols (USDA Soil Taxonomy) with fine silty-clay textures. Topsoil (0-20 cm) from each plot was composited from several sampling points for analysis. The soils were acidic (mean pH 5.45) and relatively low in nutrients, containing total N 0.08 %, available P 8.24 mg kg^{-1}, and exchangeable K 0.49 cmol(+) kg^{-1}. Based on (BMKG 2024) climate data (2019-2024), Bentenge Village has a seasonally humid tropical climate (Köppen-Geiger Aw, transitional to Am), characterised by a distinct wet-dry alternation, with wetter and cooler months from January to June and hotter, drier months from August to November. Although both plots are located within the same village, they differ in plant age, canopy structure, and management history, providing contrasting microhabitats for root-associated fungal communities.

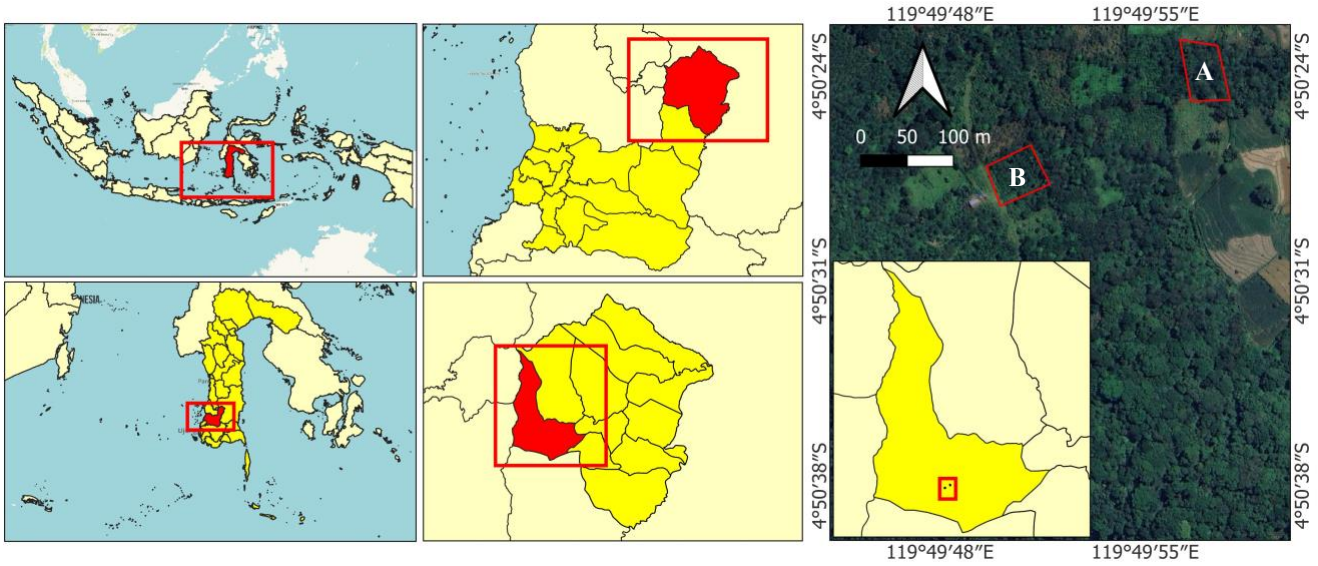

Figure 1. Map of coffee plant root sampling locations in Bentenge Village, Maros District, South Sulawesi, Indonesia. A. Location A (coffee plants aged >10 years). B. Location B (coffee plants aged <3 years)

Root sampling

Root samples from Arabica coffee plants were collected using a systematic random sampling method from two smallholder coffee plantations in Bentenge Village, Maros District, Indonesia. Sampling was stratified by plant age into two groups: mature plants (>10 years, Location A) and young plants (<3 years, Location B), with five plants sampled per group (n = 5). Plants were selected using a predefined grid with a random starting point to minimize selection bias, and the five plants per age class represent the biological replicates. From each plant, three lateral root segments were collected from distinct positions around the crown at approximately 20 cm depth using a hand trowel. This yielded a total of 30 root segments (15 per age class). The root segments were gently cleaned to remove residual soil and placed into labelled brown paper envelopes (A5 size), indicating the sampling point and plant age group. All samples were stored in a cooler box maintained at 4°C to preserve the stability of the endophytic microbial community during transport to the laboratory.

Isolation of endophytic fungi

Isolation of endophytic fungi was performed following a protocol modified from Giba et al. (2020). After transport, root segments were carefully washed under running tap water to remove adhering soil particles. Surface sterilization was performed using a sequential immersion method: 70% ethanol for 60 seconds, 3% sodium hypochlorite (NaOCl) for 60 seconds, followed by 70% ethanol for 30 seconds. The root segments were then rinsed four times with sterile distilled water to remove residual sterilizing agents. The final rinse water was spread onto Potato Dextrose Agar (PDA) to validate the sterilization process; plates without microbial growth after 5 days confirmed successful surface sterilization. To verify the endophytic origin of isolates, surface sterilization efficacy was further tested by imprinting the final rinse and sterilized root fragments on PDA. Plates were monitored for 7 days, and no fungal growth was observed, confirming that sterilization was effective. Additionally, PDA media without tissue inoculum were used as negative (blank) controls, and no contamination was detected. Sterilized root segments (1-2 cm) were aseptically placed on PDA medium (pH 4.5-5.6) supplemented with chloramphenicol (50 mg/L) to inhibit bacterial contamination. Plates were incubated at 28±2°C for 7 days under laboratory conditions. The emerging fungal colonies were monitored daily and purified by successive subcultures on PDA to obtain pure isolates. Each isolate was labeled based on the sampling location and plant age group.

Morphological identification

Endophytic fungal morphology was identified through macroscopic and microscopic observations based on these methods (Widayanti et al. 2024). Macroscopic observations included the characteristics of colonies growing on Potato Dextrose Agar (PDA), such as color of surface and bottom of the colony, shape of the edge, texture, growth pattern, and elevation. For microscopic observations, a small portion of the colony was taken and dripped with lactophenol cotton blue solution on a glass slide, and then observed under a light microscope at 40× and 100× magnifications. The observed characteristics included hyphal structure, conidiophore shape, and type and arrangement of conidia, which were then compared with the identification key described (Samson et al. 2014; Maharachchikumbura et al. 2022; Tuerdibieke et al. 2024). Fungal isolates were identified only to the genus level, as morphological identification represents a preliminary taxonomic assessment. We acknowledge that some genera may exhibit overlapping morphological traits, and species-level confirmation will require future ITS-rDNA sequencing. Isolates that showed significant differences in macroscopic morphology were treated as different entities and analyzed further. All isolates used in this study have been preserved in the Fungal Culture Collection of the Plant Pest and Disease Laboratory, Faculty of Agriculture, Universitas Hasanuddin. Each isolate has been assigned an accession code corresponding to its isolate ID (1C10BM-14C3BM), ensuring traceability for future verification and research.

Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) production test

The production of Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) by fungal endophytic isolates was measured using a colorimetric method. This assay was used as a preliminary estimation approach that detects indolic compounds rather than pure IAA or GA_3 specifically, and therefore the obtained values represent approximate concentrations. For IAA production, each fungal isolate was inoculated into 100 mL Potato Dextrose Broth (PDB) supplemented with 0.1% L-tryptophan, followed by incubation at 28°C for 48 hours with shaking at 120 rpm. After incubation, cultures were centrifuged at 8000 rpm for 15 minutes, and 1 mL of the supernatant was mixed with 2 mL Salkowski reagent (1 mL 0.5 M $FeCl_3$ in 50 mL 35% $HClO_4$). The reaction mixture was incubated in the dark for 30 minutes, and the development of a pink color indicated the presence of IAA. Quantification was performed using a UV-Vis spectrophotometer at 530 nm, and concentrations were calculated based on a standard calibration curve of pure IAA (0-10 ppm). For GA_3 production, isolates were incubated in PDB without L-tryptophan for 7 days under similar conditions. The culture supernatant was reacted with concentrated H_2SO_4, and absorbance was read at 254 nm. GA_3 concentrations were determined using a standard curve of GA_3 (0-10 ppm) prepared from Sigma-Aldrich standard solutions. All calibration curves exhibited good linearity within this range ($R^2 \geq 0.99$), ensuring reliable conversion of optical density to concentration values. Each treatment, including a non-inoculated control, was assessed in triplicate (three independent biological replicates, n = 3).

Testing the ability of isolates as biodecomposers of organic waste

The ability of 14 fungal isolates to biodecompose solid organic wastes was tested. The waste used was a mixture of coffee peels, banana stems, and rice straw in a 2:1:1 ratio chopped to a size of approximately 2 cm. Fungal isolates were prepared by initial dilution according to a previously

described method (Demeni et al. 2021). The first inoculum was added to 5 mL of the liquid medium and incubated for 24 h at room temperature. The mixture was then transferred to 10 mL of liquid medium and incubated for 72 h on a shaker at 120 rpm to obtain a starter culture. A non-inoculated control compost was included to distinguish native microbial activity from the inoculated treatments. Each treatment, including the control, was prepared in three independent biological replicates to ensure biological reproducibility. The position of compost sacks was randomized weekly to minimize positional or environmental bias during the fermentation process. A total of 10 mL of the starter inoculum was mixed with 100 mL of distilled water and evenly applied to a sealed sack containing 5 kg of organic waste, and this procedure was repeated four times for each treatment. Fermentation lasted for nine weeks at a controlled room temperature (approximately 28°C), with weekly manual aeration and moisture maintained near optimal levels (~55-60%). Decomposition evaluation was conducted periodically every week by observing changes in temperature, humidity, and weight loss of the material (Shanthipriya et al. 2020). The temperature change was calculated using the following equation:

$$\Delta T = T_t - T_0$$

Where :

T_t : Average temperature of waste pile at a specific time (weekly)

T_0 : Initial temperature on day 0 of decomposition

The weight loss of the organic material was measured using a precision analytical balance (accuracy of 0.001 g) and the decomposition rate was calculated using the following formula:

$$R = \frac{W_0 - W_t}{t}$$

Where :

R : Decomposition rate (g/day)

W_0 : Initial weight of waste (g)

W_t : Weight of the waste on day t

t : Decomposition time (days)

The quality of compost after biodecomposition was evaluated based on physicochemical parameters following the Indonesian National Standard (SNI) 19-7030-2004 for compost quality. The parameters tested included pH (using a pH meter in a 1:2.5 water suspension), water content (oven drying at 105°C), organic carbon (Walley and Black method), total nitrogen (Kjeldahl method), C/N ratio (C and N ratio), available phosphorus (Bray I method), and potassium (NH_4OAc extraction and AAS analysis). Heavy metals and Electrical Conductivity (EC) were not included as they were beyond the present study's scope. The study focused on physicochemical indicators of compost maturity rather than microbial or enzymatic assays.

Testing the ability of isolates as biofertilizers for coffee seedlings

In this study, 14 endophytic fungal isolates were evaluated for their potential as biofertilizers for Arabica coffee seedlings. The seedlings used were two months old and had undergone a two-week initial nursery period. They were then transferred to 25 × 25 cm polybags containing non-sterilized field soil from the research site to reflect realistic microbial interactions typical of smallholder systems. The greenhouse environmental conditions were monitored throughout the experiment, with temperature maintained between 26-30°C, relative humidity at 70-80%, and natural daylight supplemented by ambient light. These conditions reflect typical smallholder nursery environments in the study region. The study employed a Randomized Block Design (RBD) with 15 treatments (14 fungal isolate treatments plus one uninoculated control), each replicated four times (n = 60 independent biological experimental units). Inocula from the isolates were applied to Arabica coffee seedlings using diluted suspensions (20 mL/L), as detailed in Supplementary Method S1 (Sudewi et al. 2020). Each inoculum corresponded to an estimated spore density of approximately 1×10^6 spores mL^{-1}, standardized by culture age and optical density to ensure dose uniformity. The treatment codes used were P1 (1C10BM), P2 (2C10BM), P3 (3C10BM), P4 (4C10BM), P5 (5C10BM), P6 (6C10BM), P7 (7C10BM), P8 (8C10BM), P9 (9C10BM), P10 (10C3BM), P11 (11C3BM), P12 (12C3BM), P13 (13C3BM), and P14 (14C3BM). Isolate application was carried out in the third week after the seedlings were transferred to polybags with an application volume of 20 mL of the isolate solution for each plant. The 8-week observation period was designed to capture early vegetative responses, including seedling establishment and initial root-shoot development, rather than long-term nutrient accumulation. Observations of vegetative growth were carried out from the fourth week to the eighth week after transfer by measuring the plant height, number of leaves, stem diameter, and leaf area. All parameters were observed weekly to assess the effectiveness of each isolate in increasing the growth of Arabica coffee seedlings during the early growth phase. Root colonization by inoculated fungi was not microscopically verified and is acknowledged as a limitation in interpreting inoculant persistence.

Data analysis

Data were analyzed using Analysis of Variance (ANOVA) to test for significant differences between the treatments. Prior to ANOVA, the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. When significant treatment effects were detected ($p<0.05$), mean separation was conducted using Tukey's Honest Significant Difference (HSD) test at $\alpha = 0.05$. Results are reported as mean±Standard Deviation (SD), and the sample size (n) for each experiment is provided in the corresponding figure or table caption. All graphical error bars represent SD based on independent biological replicates. Statistical analyses were performed using IBM SPSS Statistics. Complete raw datasets, SD values, and ANOVA statistical outputs (including F-values and degrees of freedom) are available and can be provided as supplementary material upon request.

RESULT AND DISCUSSION

Morphological identification of endophytic fungi

A total of fourteen endophytic fungal isolates were successfully obtained from the roots of Arabica coffee plants and grouped by plant age: >10 years (1C10BM-9C10BM) and <3 years (10C3BM-14C3BM). Based on macroscopic and microscopic observations, the isolates were assigned to four fungal genera: *Aspergillus*, *Trichoderma*, *Paecilomyces*, and *Penicillium* (Table 1 and Figures 2-3). In the mature plant group (>10 years), *Aspergillus* sp. species were dominant, accounting for 66.7% (six out of nine isolates). The remaining isolates in this group included one representative each of *Trichoderma* sp., *Paecilomyces* sp., and *Penicillium* sp. Conversely, in younger plants (<3 years old), *Trichoderma* sp. was more prevalent (3 of 5 isolates, 60%), followed by *Aspergillus* sp. (40%) (Figure 4). Morphologically, *Aspergillus* sp. isolates were characterized by globular conidial heads and terminal vesicles, whereas *Trichoderma* sp. showed typical brush-like conidiophores with clustered conidia. *Paecilomyces* sp. exhibited sickle-shaped conidia, and *Penicillium* sp. was identified by elongated chains of cylindrical conidia. These observations suggest a potential shift in the root endophytic fungal community structure with plant age, notably the predominance of *Trichoderma* sp. in younger roots.

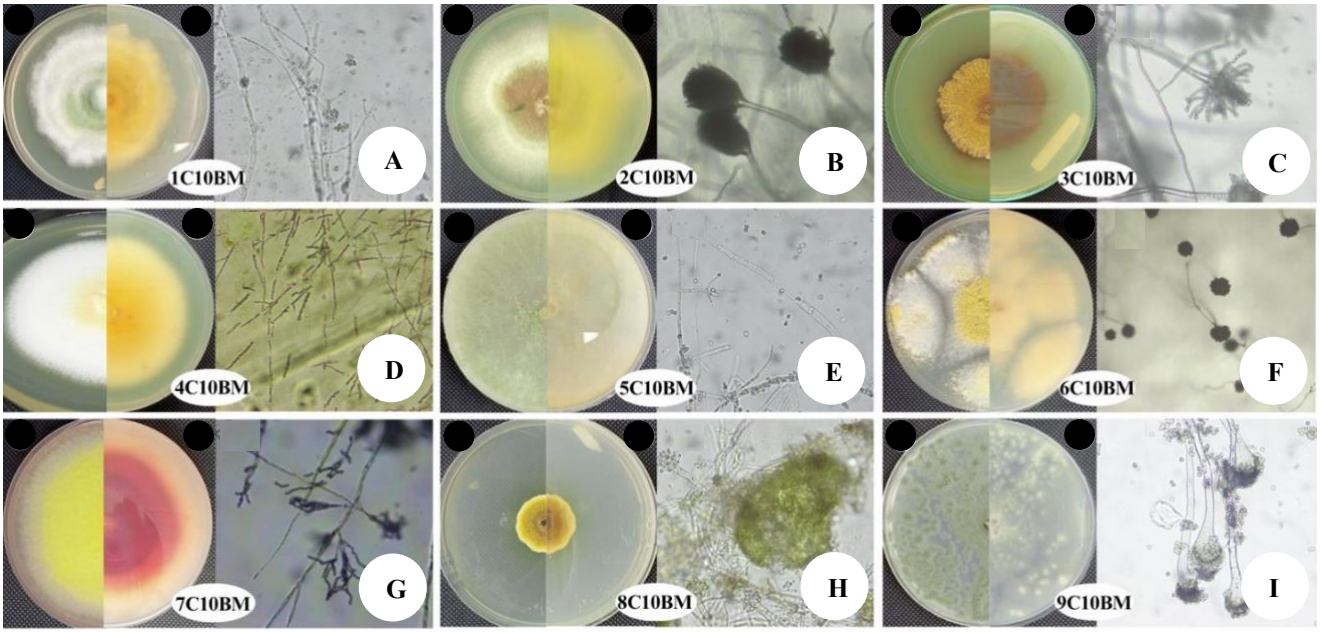

Figure 2. Characteristics of macroscopic view from above and bottom of the colony, and microscopic view of the colony nine endophytic fungal isolates from the roots of Arabica coffee plants aged >10 years. A. 1C10BM, B. 2C10BM, C. 3C10BM, D. 4C10BM, E. 5C10BM, F. 6C10BM, G. 7C10BM, H. 8C10BM, I. 9C10BM

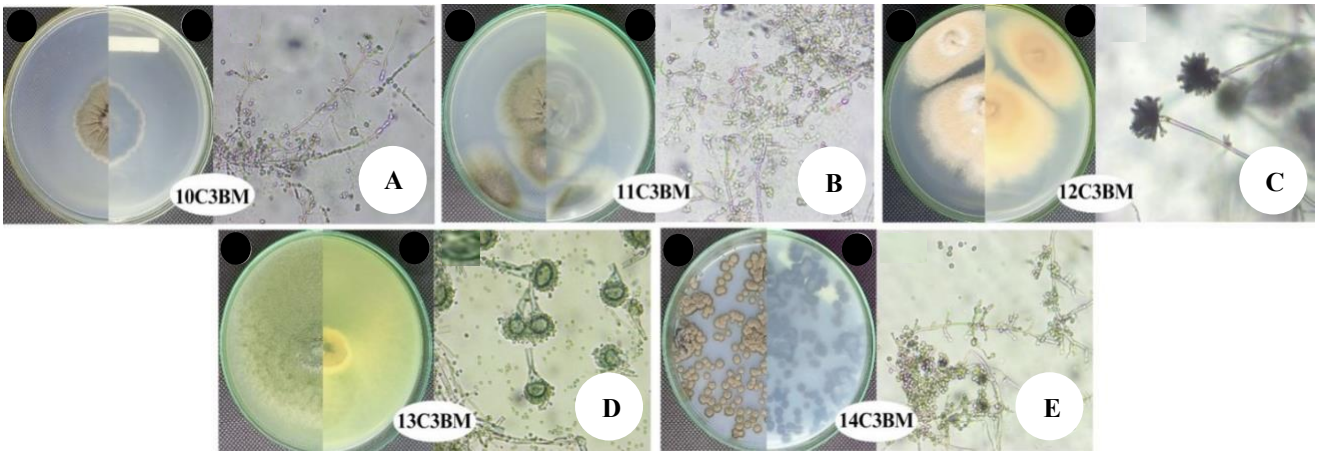

Figure 3. Characteristics of macroscopic view from above and bottom of the colony, and microscopic view of five endophytic fungal isolates from the roots of Arabica coffee plants aged <3 years. A. 10C3BM, B. 11C3BM, C. 12C3BM, D. 13C3BM, E. 14C3BM

Table 1. Macroscopic and microscopic morphological characteristics of 14 endophytic fungal isolates from Arabica coffee roots from two plant age groups (>10 years and <3 years)

Isolates code	Colony (Surface)	Colony (Reverse)	Texture	Hyphae	Conidia	Genus
1C10BM	White at the edge, greyish-green in center	Dark yellow	Cottony	Hyaline, septate, branched	Round-oval, clustered, short chains	*Trichoderma* sp.
2C10BM	White edge, light brown center	Bright yellow	Velvety-powdery	Hyaline, septate, branched	Globular conidiophore, radial conidia	*Aspergillus wentii*
3C10BM	Golden brown to yellow, cream edge	Reddish brown	Granular-wrinkled	Hyaline, septate	Branched conidiophore, small chained conidia	*Aspergillus* sp.
4C10BM	Pale yellowish white	Yellowish orange	Granular-powdery	cylinder, septate	Elongated-cylindrical, chained	*Penicillium* sp.
5C10BM	Pale green to whitish	Yellowish	Cottony	Clear, septate	Small oval, scattered	*Aspergillus* sp.
6C10BM	Bright yellow center, white hairy edge	Pale yellow to cream	Cottony-powdery	Hyaline, septate	Globular conidial head, dense radial conidia	*Aspergillus* sp.
7C10BM	Bright yellow center, light purple edge	Pink	Velvety-powdery	Hyaline, septate	Sickle-shaped, conidial cluster	*Paecilomyces* sp.
8C10BM	Dark brown center, bright yellow ring	Dark brown	Granular-powdery	Hyaline, septate	Small oval conidia on brush-like branch	*Aspergillus* sp.
9C10BM	Uneven greyish-green	Greyish white	Velvety-powdery	Hyaline, septate	Round-oval, short chains	*Aspergillus fumigatus*
10C3BM	Dark brown center, cream edge	Greenish	Granular-floccose	Hyaline, septate	Brush-like conidiophore, round conidia	*Trichoderma* sp.
11C3BM	Dark green, dark center	Brownish grey	Velvety-powdery	Hyaline, septate	Branched conidiophore, chained conidia	*Trichoderma* sp.
12C3BM	Yellowish white, dense center	Yellow-brown	Floccose-cottony	Hyaline, septate	Round vesicle, dense conidia	*Aspergillus* sp.
13C3BM	Olive green to greyish	Light yellow	Velvety-powdery	Hyaline, septate	Terminal vesicle, radial conidia	*Aspergillus fumigatus*
14C3BM	Light brown with black spots	Grey	Granular-wrinkled	Hyaline, septate	Branched conidiophore like brush	*Trichoderma* sp.

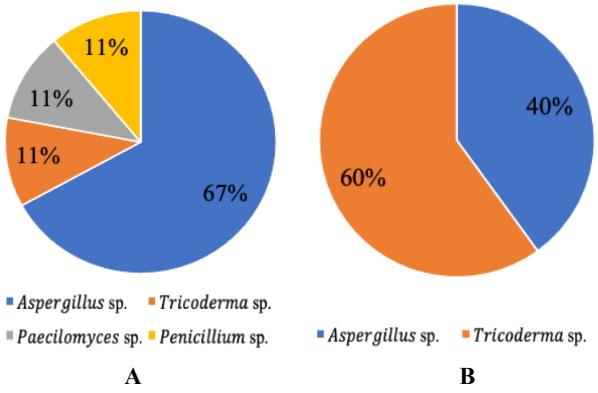

Figure 4. Composition of endophytic fungal genera isolated from *Coffea arabica* roots based on plant age group. A. Fungal genera composition in the roots of plants aged >10 years, B. Fungal genera composition in the roots of plants aged <3 years

Phytohormone production (IAA and GA_3)

Significant variations were observed in the production of Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) among the 14 endophytic fungal isolates from Arabica coffee roots (Figure 5). The highest IAA concentration was recorded in isolate 5C10BM (*Aspergillus* sp.) at 2.92 ppm, which was significantly higher than that in all other treatments ($p<0.05$). Isolates 7C10BM (*Paecilomyces* sp.) and 2C10BM showed moderate IAA production, whereas 11C3BM (*Trichoderma* sp.) exhibited the lowest value (0.12 ppm). GA_3 production follows a different pattern. The isolate 11C3BM (*Trichoderma* sp.) produced the highest concentration (6.41 ppm), followed by 2C10BM and 1C10BM. The lowest GA_3 levels were observed in 10C3BM and 8C10BM. These results suggest that IAA and GA_3 biosynthesis may be isolate-dependent and genus-specific. Overall, the isolate 5C10BM was the most potent auxin (IAA) producer, whereas 11C3BM excelled in gibberellin (GA_3) synthesis. These phytohormonal traits highlight the potential application of specific isolates as functional biofertilizers tailored to promote root growth (IAA) or stem elongation (GA_3). Although isolates with higher IAA or GA_3 levels tended to show positive growth responses, these relationships were not evaluated using correlation analysis; therefore, they should be considered associative rather than causal.

Temperature and moisture profiles during decomposition

Substrate temperature and moisture content during decomposition varied among the fungal treatments, indicating differences in microbial activity (Figure 6). The control group showed the highest average temperature (35.96°C), whereas fungal treatments generally led to slightly lower values, with isolate 14C3BM (*Trichoderma* sp.) exhibiting the lowest temperature (33.89°C). The lower temperature in inoculated treatments likely reflects a faster

transition toward the stabilization phase, following an earlier thermophilic peak, suggesting that certain fungal isolates accelerated organic matter breakdown. Moisture content also differed across the treatments. The control group consistently showed the highest value (96.81%), whereas fungal treatments, particularly 14C3BM, 10C3BM, and 12C3BM, showed reduced moisture levels (<94%), indicating more advanced organic matter degradation. Isolates with higher moisture retention, such as 1C10BM and 3C10BM, likely promote slower breakdown. These findings highlight the role of specific endophytic fungal isolates, especially *Trichoderma* sp., in accelerating composting activity. Because this composting system did not include thermophilic phase monitoring, the temperature decrease observed likely reflects a transition toward stabilization rather than increased microbial activity, consistent with standard composting kinetics.

Waste decomposition efficiency

The fungal treatments resulted in a significant reduction in residual waste weight compared to the control, indicating enhanced composting activity (Figure 7). Isolates 1C10BM, 2C10BM, and 5C10BM (*Aspergillus* sp.) demonstrated the highest efficacy, as reflected by the lowest final waste weight. These isolates also exhibited high IAA production, particularly 5C10BM, which additionally showed a moderate GA_3 output (Figure 5). This suggests a dual function for both organic matter decomposition and plant growth stimulation. The bio-decomposer potential of these isolates was further supported by elevated composting temperatures ranging from 33.9 to 36.0°C (Figure 6), indicative of active microbial metabolism during the mesophilic-thermophilic transition phase. High moisture retention (94.5-95.7%) was maintained during the process, creating favorable conditions for enzymatic activity. Taken together, these results highlight *Aspergillus* sp., particularly isolate 5C10BM, as a strong candidate for integrated biofertilizer-biodecomposer applications.

In addition, Figure 8 provides visual documentation of the composting setup, inoculation procedure, replicate arrangement, and the appearance of decomposed material, supporting the methodological workflow described above.

Compost nutrient composition

The compost treated with endophytic fungal isolates showed significant differences in nutrient composition across most parameters, including water content, C-organic, total nitrogen, C/N ratio, phosphorus, and potassium ($p<0.05$), while pH remained unaffected and near neutral (7.1-7.6) across all treatments (Table 2). Isolate 6C10BM yielded the highest C-organic content (20.07%), suggesting strong carbon-retaining capacity. Isolate 11C3BM exhibited the highest nitrogen level (1.19%), whereas 3C10BM recorded the lowest C/N ratio (14.00), indicative of advanced compost maturity. Notably, isolate 14C3BM produced compost with the highest phosphorus (0.63%) and potassium (0.42%) contents, highlighting its potential for enhancing soil fertility. Several other isolates, including 10C3BM and 13C3BM, also showed elevated K levels (≥ 0.40%). These findings suggest that specific fungal isolates contribute differentially to nutrient enrichment, with 14C3BM standing out as a strong candidate for nutrient-rich compost production.

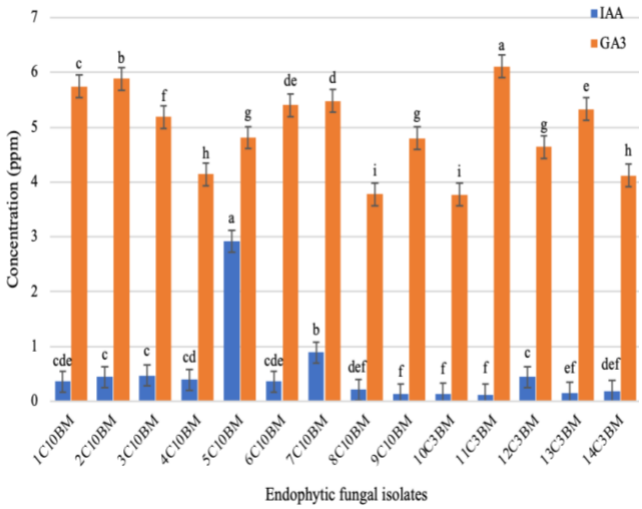

Figure 5. Concentrations of Indole-3-Acetic Acid (IAA) and Gibberellic Acid (GA_3) produced by 14 endophytic fungal isolates from *Coffea arabica* roots. Values represent mean±Standard Deviation (SD) from triplicate biological replicates (n = 3). Bars with different letters (a-i) indicate significant differences (Tukey's HSD test, $p<0.05$)

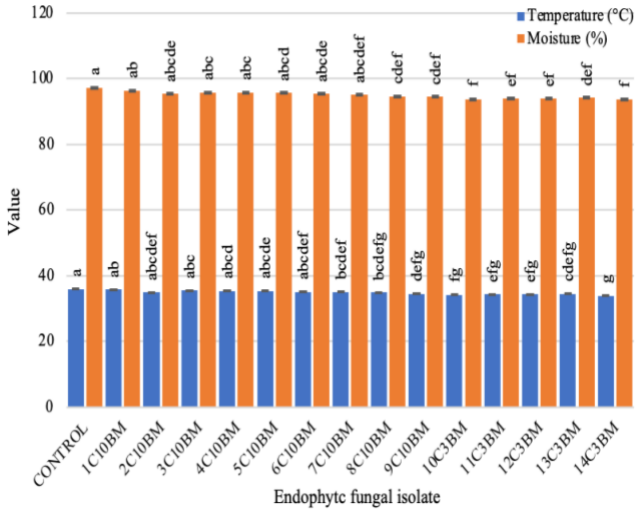

Figure 6. Average substrate temperature and moisture during the decomposition of organic waste treated with 14 endophytic fungal isolates. Values represent mean±SD (n = 4 biological replicates per treatment, including the control). Bars with different letters (a-g) indicate significant differences (Tukey's HSD test, $p<0.05$)

Figure 8. Compost treatment. A. Fungal isolates >10 years, B. Fungal isolates <3 years, C. Results of dilution of 14 fungal isolates in 20 mL/L water, D. Organic waste as compost constituents, E. Repetition and treatment of fungal isolates as biodecomposers, F. Compost decomposed by fungi

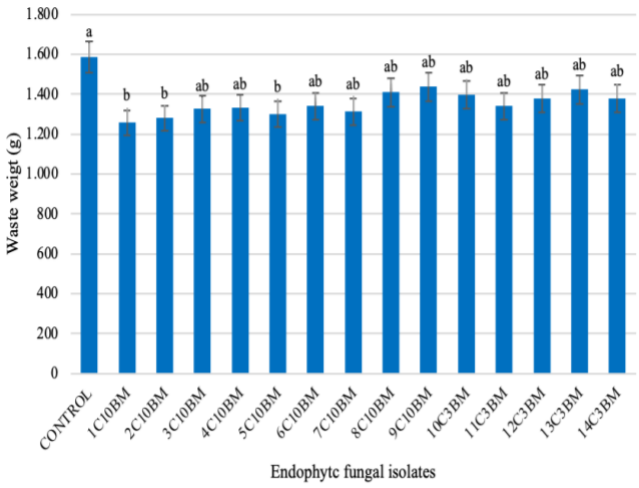

Figure 7. Average waste weight (g) during the decomposition of organic waste by 14 endophytic fungal isolates from *Coffea arabica* roots. Values represent mean±SD (n = 4). Bars with different letters (a-b) indicate significant differences (Tukey's HSD test, $p<0.05$)

Growth promotion in coffee seedlings

The application of endophytic fungal isolates significantly influenced Arabica coffee seedling growth, particularly plant height, stem diameter, leaf number, and leaf area ($p<0.05$) (Table 3). Isolate 2C10BM (*Aspergillus* sp.) induced the greatest plant height (45.4 cm), whereas 4C10BM (*Penicillium* sp.) produced the thickest stem (7.70 mm). These traits were critical for early vigour and structural development. In terms of foliage traits, isolate 13C3BM (*Trichoderma* sp.) recorded the highest leaf number (47.8), whereas isolate 9C10BM resulted in the largest leaf area (143.6 cm²), both key indicators of photosynthetic potential. The results suggest that *Aspergillus* sp. and *Penicillium* sp. isolates enhance shoot elongation and stem robustness, whereas *Trichoderma* sp. isolates, commonly dominant in young plants, were more effective in increasing foliage growth. These patterns indicate that different fungal genera confer distinct growth advantages and can be selectively applied as targeted biofertilizers in nursery management.

Discussion

Age-driven shifts and functional specialization of endophytic fungi

This study isolated a total of 14 root endophytic fungal strains from Arabica coffee plants of different ages. These isolates were distributed across four genera: *Trichoderma*, *Aspergillus*, *Penicillium*, and *Paecilomyces*. The community composition exhibited an age-associated pattern. Younger roots (<3 years) were primarily colonized by *Trichoderma* spp. and *Aspergillus* spp., whereas older roots (>10 years) harbored a more diverse community dominated by *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces* spp. Similar results were also observed in age-related shifts in endophytic fungal communities (Kumar et al. 2020; Pacheco et al. 2022). These findings suggest a possible age-associated shift in endophyte composition; however, because molecular or enzymatic markers of functional specialization were not assessed, this pattern should be interpreted cautiously and viewed as a preliminary ecological tendency rather than confirmed functional differentiation (Richardson et al. 2021). Among the isolates, *Paecilomyces lilacinus* (7C10BM) was exclusively found in mature roots,

consistent with its reported role as a nematode biocontrol agent (Youssef et al. 2020; Pokhare et al. 2024). In contrast, *Trichoderma* isolates (10C3BM, 11C3BM, and 14C3BM) dominated young roots, reflecting their well-documented role in promoting early root development and enhancing plant defense (Asis et al. 2021; Ynfante-Martínez et al. 2023; Vashisht et al. 2024).

With respect to phytohormone biosynthesis, the isolates exhibited functional specialization. *Aspergillus* sp., particularly isolate 5C10BM, produced the highest IAA concentration (2.92 ppm), supporting its involvement in auxin-mediated root development and nutrient uptake. *Trichoderma* isolate 11C3BM exhibited the highest GA_3 production (>6.4 ppm), which is consistent with previous reports of gibberellin synthesis in *Trichoderma* (Dubey et al. 2003; Pollmann et al. 2024). Overall, IAA production was more pronounced in isolates from older roots, while GA_3 production was higher in isolates from younger roots. Santos et al. (2023) reported similar observations, emphasizing that phytohormone production can vary depending on the developmental stage of the host plant.

This functional divergence highlights the potential of combining isolates with complementary traits, such as high-IAA producers (*Aspergillus* 5C10BM) and high-GA_3 producers (*Trichoderma* 11C3BM), for tailored biofertilizer applications across different growth stages of coffee plants.

Table 2. Average nutrient content of compost resulting from waste decomposition by 14 Arabica coffee root endophytic fungal isolates

Treatment isolates	pH	Water content (%)	C-Organic (%)	N (%)	C/N	P (%)	K (%)
1C10BM	7.1	29.00 bcde	16.03 i	0.75 e	21.00 bc	0.35 ef	0.28 cde
2C10BM	7.3	30.00 abcde	17.49 g	0.86 cde	20.00 cd	0.29 f	0.19 f
3C10BM	7.4	26.60 f	16.14 i	1.16 ab	14.00 f	0.37 e	0.31 c
4C10BM	7.1	31.00 ab	19.44 bc	0.92 abcde	21.00 bc	0.55 bcd	0.25 e
5C10BM	7.1	27.83 ef	19.20 c	1.01 abcde	19.00 d	0.49 d	0.27 de
6C10BM	7.3	28.60 cdef	20.07 a	1.04 abcd	19.00 d	0.40 e	0.25 e
7C10BM	7.2	31.20 ab	19.44 bc	0.94 abcde	21.00 bc	0.37 e	0.34 bc
8C10BM	7.1	28.00 def	16.94 h	0.92 abcde	19.00 d	0.52 cd	0.31 c
9C10BM	7.1	29.60 abcde	18.45 de	0.77 de	24.00 a	0.58 abc	0.29 cd
10C3BM	7.1	31.80 a	19.51 bc	0.89 bcde	22.00 b	0.49 d	0.41 a
11C3BM	7.2	30.80 abc	18.21 ef	1.19 a	15.00 f	0.60 ab	0.35 b
12C3BM	7.5	28.40 def	18.02 f	1.05 abcd	17.00 e	0.58 abc	0.33 bc
13C3BM	7.3	30.20 ab	19.62 b	1.00 abcde	20.00 cd	0.62 ab	0.40 a
14C3BM	7.6	30.00 abcde	18.72 d	1.11 abc	17.00 e	0.63 a	0.42 a
ns/SD	Ns	2.28	0.38	0.27	1.61	0.07	0.03

Note: Numbers followed by the same letter (a, b, c, d, e, f, g, h, i) did not significantly different according to Tukey's Advanced Test (HSD) at a significance level of 0.05

Table 3. Average growth of Arabica coffee seedlings after treatment with 14 endophytic fungal isolates as biofertilizers

Treatment isolates	Plant height (cm)	Number of leaves (blades)	Leaf area (cm^2)	Stem diameter (cm)
1C10BM	38.9 bcd	38.8 def	124.9 bcd	6.53 bcde
2C10BM	45.4 a	41.8 abcd	124.8 bcd	6.58 bcde
3C10BM	38.7 bcd	30.0 b	123.7 bcd	5.90 e
4C10BM	41.0 abc	45.3 ab	128.3 abc	7.70 a
5C10BM	38.1 bcde	37.3 cde	118.7 cd	6.18 de
6C10BM	40.2 bc	37.0 cde	136.0 ab	7.10 ab
7C10BM	37.5 cde	35.8 def	131.2 abc	6.98 bc
8C10BM	38.0 bcde	28.3 f	142.8 a	5.90 e
9C10BM	41.9 ab	37.5 bcde	143.6 a	5.95 e
10C3BM	35.0 de	35.8 def	141.0 a	6.45 bcde
11C3BM	38.2 bcd	37.0 cde	125.5 bcd	6.80 bcde
12C3BM	34.0 e	43.8 abc	112.9 d	6.58 bcde
13C3BM	39.0 bcd	47.8 a	121.8 cd	6.58 bcde
14C3BM	35.5 de	41.3 abcd	118.9 cd	6.53 bcde
HSD α 0.05	4.14	7.88	13.92	0.72

Note: Numbers followed by the same letter (a-f) did not significantly different according to Tukey's Advanced Test (HSD) at a significance level of 0.05. Data are presented as mean±SD (n = 4). Values in the same column followed by the same letter are not significantly different according to Tukey's HSD test ($p<0.05$)

Multifunctionality of key isolates: Decomposition, hormone production, and growth promotion

This study highlights the multifunctional potential of several root endophytic fungal isolates from *Coffea arabica*, particularly their combined roles as decomposers, phytohormone producers, and plant growth promoters. Among these, isolate 5C10BM (*Aspergillus* sp.) emerged as a key multifunctional strain. It produced the highest IAA concentration (2.92 ppm), significantly reduced compost weight (1.300 g), and enhanced both plant height and leaf area. These combined traits demonstrate its dual role in auxin-mediated growth stimulation and enzymatic organic matter degradation. Similar multifunctional attributes of *Aspergillus* isolates have also been reported by Abd Ellatif et al. (2022) and Sharma et al. (2023), supporting their value in integrated biofertilizer-biodecomposer applications. However, nutrient differences observed in compost (e.g., N, P, K) fall within the range commonly reported for small-scale composting systems, and without enzymatic or microbial activity measurements, these differences cannot be interpreted as direct causal effects of specific isolates.

In contrast, isolate 11C3BM (*Trichoderma* sp.) displayed a different functional profile. Although its IAA production was relatively low (0.12 ppm), it exhibited the highest GA_3 production (6.41 ppm), moderate waste degradation capacity, and a strong effect on leaf number. This specialization suggests that *Trichoderma* favors GA_3-mediated cell expansion and foliar development rather than auxin-related rooting. Similar genus-level differentiation in hormone biosynthesis has been described previously, with *Aspergillus* and *Paecilomyces* being primarily auxinogenic, while *Trichoderma* excels in gibberellin production via the terpenoid pathway (Dubey et al. 2003; Pollmann et al. 2024). Another important contributor was isolate 14C3BM (*Trichoderma* sp.), which demonstrated efficient phosphate and potassium mobilization, enriching compost with P (0.63%) and K (0.42%). Although its decomposition and growth promotion capacity were moderate, its nutrient-solubilizing ability is particularly valuable for compost enrichment. Similar roles of *Trichoderma* in nutrient mobilization have been reported by Hasanuzzaman et al. (2018) and Müller et al. (2019). When paired with robust decomposers such as *Aspergillus* isolates, this complementary function can improve both compost quality and plant growth.

A notable finding of this study was that no single isolate dominated all functional parameters. Instead, synergistic combinations of isolates with complementary traits—such as thermogenic capacity, hormone production profile, enzymatic efficiency, and nutrient solubilization—should be prioritized in the development of bioformulations. This finding underscores the importance of designing biofertilizer-biodecomposer consortia based on functional specialization rather than single-strain dominance. Functional variation was also influenced by the plant age group from which the isolates were derived. Isolates from mature roots of 5C10BM and 6C10BM generally showed higher decomposition and nutrient enrichment capacities, whereas isolates from younger roots i.e., 14C3BM and 11C3BM were more active in hormone production, which was critical for early plant development. Similar observations of age-driven ecological adaptation have been noted by Richardson et al. (2021) and Miao et al. (2022). This reinforces the idea that host-associated selection plays a key role in identifying effective fungal bioinoculants. However, root colonization or persistence of the inoculated fungi was not microscopically verified in this study. Therefore, the observed growth-promoting effects should be interpreted as indirect indicators of beneficial plant-fungus interactions rather than confirmed endophytic establishment.

While several isolates demonstrated promising multifunctional traits under controlled conditions, the present findings represent short-term and small-scale assessments. Therefore, their broader agronomic implications—such as applications in sustainable coffee farming—remain preliminary and should be validated through future studies that include root colonization assays, enzymatic and microbial activity measurements, greenhouse trials, and field-scale evaluations. Such follow-up work will be essential to confirm functional mechanisms and to determine whether the observed effects can be consistently reproduced under practical farming conditions.

In conclusion, this study demonstrated that Arabica coffee root-endophytic fungi exhibit distinct functional diversity based on plant age, with important implications for agricultural applications. Isolates from mature plants (>10 years old), particularly *Aspergillus* sp. (isolate 5C10BM) and *Penicillium* sp. (isolate 4C10BM), were highly effective in promoting plant height, stem diameter, and compost organic carbon content, indicating their potential as compost bioinoculants and growth stimulants for mature cropping systems. Conversely, *Trichoderma* sp. isolates from younger plants (<3 years old), such as 11C3BM and 14C3BM, demonstrated better GA_3 production and contributed significantly to compost nutrient enrichment (N, P, and K), making them suitable for seedling or nursery-stage applications. The clear functional differentiation among isolates highlights the value of age-targeted endophyte selection strategies tailored to specific plant development phases. Furthermore, several isolates exhibited multifunctionality, combining phytohormone production, composting efficiency, and growth promotion, thus offering strong candidates for integrated biofertilizer-biodecomposer formulations. Future studies should prioritize field-based trials and bioformulation development using locally adapted fungal consortia optimized for sustainable coffee farming systems.

ACKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to the Center for Higher Education Funding and Assessment (PPAPT), Indonesia Endowment Fund for Education Agency (LPDP), Indonesian Education Scholarship (BPI), and the Ministry of Higher Education, Science, and the Technology of Republic Indonesia for doctoral students receiving scholarships with contract number 010612/ PPAPT.1.2/BPI.06/02/2025.

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