International Journal of Agricultural Technology 2026 Vol. 22(6):2637-2658
DOI: https://doi.org/10.63369/ijat.2026.22.6.2637-2658
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Identification and evaluation of selected cellulose-degrading fungi for leaf litter degradation

Ingusan, C. J. D., Parayao, A. M. P. and Waing, K. G. D.*

Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines 3120.

Ingusan, C. J. D., Parayao, A. M. and Waing, K. G. D. (2026). Identification and evaluation of selected cellulose-degrading fungi for leaf litter degradation. International Journal of Agricultural Technology 22(6):2637-2658.

Abstract Cellulase activity resulted to *P. citrinum* obtaining the highest enzymatic index (EI) of 1.33, followed by *A. caelatus* LFJ3 and *A. caelatus* LFJ4 with 1.12 and 1.08, respectively. All these fungi exhibited a moderate positive reaction for cellulase acitivity. The three cellulose-degrading fungi positive for cellulase activity isolated from *Samanea* leaf litter were molecularly identified as *Penicillium citrinum* (KX958025.1)*, Aspergillus caelatus* LFJ3 (KM613140.1), and *A. caelatus* LFJ4 (MH862672.1). Moreover, *P. citrinum* having the highest EI was utilized for *in vitro* degradation of *Samanea* leaves for 60 days. After incubation, leaves were observed to be soft and brown with fungal mycelia all over the surface. Upon incubation, Scanning Electron Microscopy (SEM) micrographs at day 60 revealed the adherence of the fungal hyphae to the surface of the leaf as well as the loosened leaf tissues due to the colonization of the fungi on the leaf mesophyll. In addition, some parts of the leaf surface showed absence of trichomes compared to its abundance on the uninoculated leaf. Small lesion-like texture of the leaf and abundance of spores were also observed. Therefore, *P. citrinum* can possibly be used in the bioremediation field, especially for biodegradation of leaf litter.

Keywords: Carboxymethycellulose, Cellulose, *In vitro* degradation, *Samanea* leaf

Introduction

Waste management involves the collection, transport, treatment, and disposal of waste materials, as well as their management and monitoring, in order to limit their impact on humans and the environment. Leaf litter, fruit and vegetable peel, office paper, printed newspaper, yard rubbish, paper products, and food waste make up the majority of the organic part of Municipal Solid Waste (Ghosh *et al.,* 2020).

Thermal treatment (using heat for waste treatment) such as gasification, incineration, pyrolysis, and burning in open area are some of the most common waste management methods. In addition, landfills like bioreactor landfills, controlled dumps, and sanitary landfills; and biodegradation methods such as

* **Corresponding Author:** Waing, K. G. D. **Email:** kgdwaing@clsu.edu.ph

composting and anaerobic digestion are widely used techniques (Adebayo and Obiekezie, 2018). All of the aforementioned methods in managing waste aims to decompose to prevent accumulation. In developing country like the Philippines, open dump sites are the usual initial and final destination of biodegradable wastes since machineries like composting machines are not thoroughly considered. Through the use of microorganisms, inorganic compounds such as carbon and nitrogen would be able to return to the soil in a more environment-friendly manner (Bani *et al.,* 2018).

Microorganisms serve vital purposes in the preservation and conservation of environment from numerous natural and man-made phenomena, specifically pollution. They provide beneficial tasks that make man's existence simpler and better. Waste management is one of the many uses of microorganisms. Microorganisms are an essential tool for successfully addressing this threat when they are applied appropriately (Adebayo and Obiekezie, 2018).

Fungi are an integral component of the ecosystem since they are regarded as the main agents in the degradation of litter as they are excellent providers of a wide range of enzymes that may break down even cellulose, a naturally occurring polymer arranged in a crystal-like shape, making it difficult to decompose (Krishna and Mohan, 2017). One of the primary elements of forest litter is cellulose, which also plays a significant role in controlling the breakdown of the litter (Sun *et al.,* 2020). Moreover, degradation is a form of biogeochemical activity that is essential to the cycling of nutrients, carbon, and energy within and across ecosystems, as well as between the biosphere and the atmosphere (Bradford *et al.,* 2016).

Several researchers have identified numerous fungal species that were able to degrade cellulose. In the study of Waing *et al.* (2015), *Aspergillus eucalypticola*, *Aspergillus fumigatus*, *Colletorichum gloeosporioides*, *Fusarium oxysporum* and *Penicillum echinulatum* have produced clear zones, thus, they are cellulolytic fungi. Moreover, in the study of Sarsaiya *et al.* (2017), *Aspergillus niger*, *A. fumigatus* and *F. oxysporum* formed large zones, which indicated that they had a lot of potential for cellulase activity. In addition, *Trichoderma reesei* is among the most extensively researched cellulolytic microorganism (Gupta *et al.,* 2016; Dey *et al.,* 2018).

Cellulose is extensively used in a range of industries, such as textile and pulp and paper, contributing to the nation's vast supply of untapped bio-waste resources. Agricultural production and processing, and waste from forestry biomass comprises the cellulosic waste (Baconguis and Pasagdan, 2013). Leaf litters are also rich in cellulose and degradation of litter is crucial to a forest ecosystem's nutritional budget, where nutrient recycling from plant litter has the most significant impact on vegetation. Cellulases breakdown cellulose, which is

important in a variety of agricultural and waste treatment activities since cellulase targets the polysaccharide that is difficult to breakdown due to the presence of cellulose. Therefore, identification of cellulose-degrading fungi has the potential to lead us a step closer in the degradation of waste containing cellulose, according to the studies of Kjoller and Struwe (1982), and Djarwanto and Tachibana (2009) as cited by Sun *et al.* (2020). The cellulose-degrading ability of identified endophytic fungal species have been confirmed to cause mass loss in leaf-litter (Yuan and Chen, 2014). However, there is a lack of quantitative analysis addressing the capacity of various fungi for decomposing leaf litter (Osono, 2019).

Thus, the study aimed to isolate cellulose-degrading fungi from leaf litter of *Samanea* trees and characterize and identified on their morphological characteristics and molecular phylogenetic analysis and evaluate their potential through leaf litter degradation.

Materials and methods

Collection of leaf litters

Leaf litters under degradation stage found on top of the soil were collected from three randomly selected sampling areas within Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The collected leaf litters were air dried and were mixed thoroughly. One hundred (100) grams of leaf litters served as the sample. Furthermore, the sample was pulverized using a blender.

S*erial dilution and plating*

From the pulverized leaf samples, 10 g was transferred to an Erlenmeyer flask with 90 ml of sterile water to achieve 10^{-1} dilution. Then, the flask was sealed tightly sealed and vigorously shaken for the next 5 minutes. One ml was taken from 10^{-1} dilution and added to sterile test tube containing 9 ml of sterile water in order to obtain 10^{-2} up to 10^{-7} dilutions. Moreover, 1 ml of the aliquot from each dilution was transferred to sterilized petri plates with carboxymethylcellulose (CMC) agar and supplemented with streptomycin sulfate with a concentration of 100ug/ml. CMC agar medium (Hankin and Anagnostakis 1975) was prepared, and consisted 0.5 g of peptone, 0.1 g of yeast extract, and 16 g agar. Altogether, these were mixed in 1000 mL distilled water, and 0.5% carboxymethylcellulose was added. The mixture was sterilized using an autoclave at 121 °C and 15 psi, for 15 minutes. After inoculating the plates

with the serially diluted samples through pour-plating method, the plates were incubated for 5 days.

Screening for cellulose-degrading ability

After incubation, the plates were flooded with 0.2% congo red solution for 15 minutes and washed subsequently using 1 M NaCl for 15 minutes (Hankin and Anagnostakis, 1975). Fungal colonies which had clear zones were subjected for purification.

Purification of fungal culture

After incubation, all of the fungi grown with clear zones from all dilutions was inoculated to Potato Dextrose Agar (PDA) plates through triple-point inoculation method. Then, these were incubated for another 7 days at room temperature. It is considered pure if only one appearance of colony is growing on a plate.

Cultural and morphological characterization of fungal isolates

After 7 days of incubation, the characteristics of the fungal culture was observed, specifically the pigmentation and appearance of the colony on obverse and reverse side, and the texture of mycelia.

For morphological characterization, slide culture method of Prakash and Bhargava (2016) was followed. Upon incubation, a sterile syringe containing1 ml of 70% ethyl alcohol was ejected to fix the specimen. After a minute, the cover glass was retrieved and mounted on a new glass slide with a drop of lactophenol cotton blue stain.

Molecular identification

The three fungal isolates with the highest enzymatic index were subjected to molecular identification. Fungal cultures incubated for 7 days were sent to Macrogen (Macrogen Inc., South Korea) for sequencing. The ITS region of the fungal isolates was amplified using the primers ITS4 and ITS5. After receiving the sequence, it was verified through BLAST analysis.

In vitro degradation

In *in vitro* degradation, the method of Yuan and Chen (2014) was adapted and modified. The fungal isolate with the highest enzymatic index was evaluated for its ability to cause degradation in collected leaf litter. Each fermentation jar contained 4 g of freshly fallen leaves cut into 1 cm x 1cm and were sterilized using an autoclave. Using a sterile 6 mm cork borer, a total of 10 mycelial plugs were excavated from the mycelial mat of the selected fungal isolate grown on PDA for 7 days. Then, 10 mycelial plugs were added aseptically to the fermentation jar. The positive control set up contained 4 g of leaf litter sample and it was inoculated with *Trichoderma harzianum* as reference fungi. Then, the jars were stored at room temperature with no light for 30 and 60 days.

Scanning electron microscopy of leaf litter

Dry and uninoculated leaf litter, as well as fungus-inoculated leaf litter collected after 30 and 60 days of incubation were subjected to Scanning Electron Microscope (SEM) (Hitachi SU3800) at CLSU Nanotechnology R&D Facility to achieve an image of 1 mm to 20 µm scale bar. A dissecting blade was used to exposed the longitudinal section of the leaf for viewing.

Statistical analysis

The experiment was done in Completely Randomized Design. Moreover, the cellulase activity was analyzed using the analysis - one-way Analysis of Variance (ANOVA). The Tukey's Honestly Significant Difference was used to compare means with a 5% level of significance.

Results

Enzymatic activity

A total of four fungal isolates were obtained from *Samanea* leaf litter and evaluated for cellulase activity based on clear zone formation on CMC agar (Figure 1). Among the isolates, three tested positive for cellulase activity, while one isolate failed to produce a clear zone, indicating a lack of cellulase production.

Ranking the isolates in ascending order of their enzymatic index (EI) revealed the following: the unidentified species exhibited the lowest EI of 1.00±0.00 (low/non-producer), followed by *Aspergillus caelatus* LFJ4 with an EI of 1.08±0.00 (moderate strength), *A. caelatus* LFJ3 with an EI of 1.12±0.02

(moderate strength), and *Penicillium citrinum*, which exhibited the highest EI of 1.33±0.07 (moderate strength) (Table 1). Although *A. caelatus* LFJ3 displayed the largest mean colony diameter (55.7±3.69mm) and clear zone diameter (62.48 ±3.19mm), *P. citrinum* produced the most significant enzymatic ratio relative to its colony growth. Statistical analysis indicated that the EI of *P. citrinum* was significantly higher than those of all other tested fungi ($p < 0.05$). The EI results of *A. caelatus* LFJ3 and *A. caelatus* LFJ4 were not significantly different from each other, nor was there a significant difference between *A. caelatus* LFJ4 and the unidentified isolate.

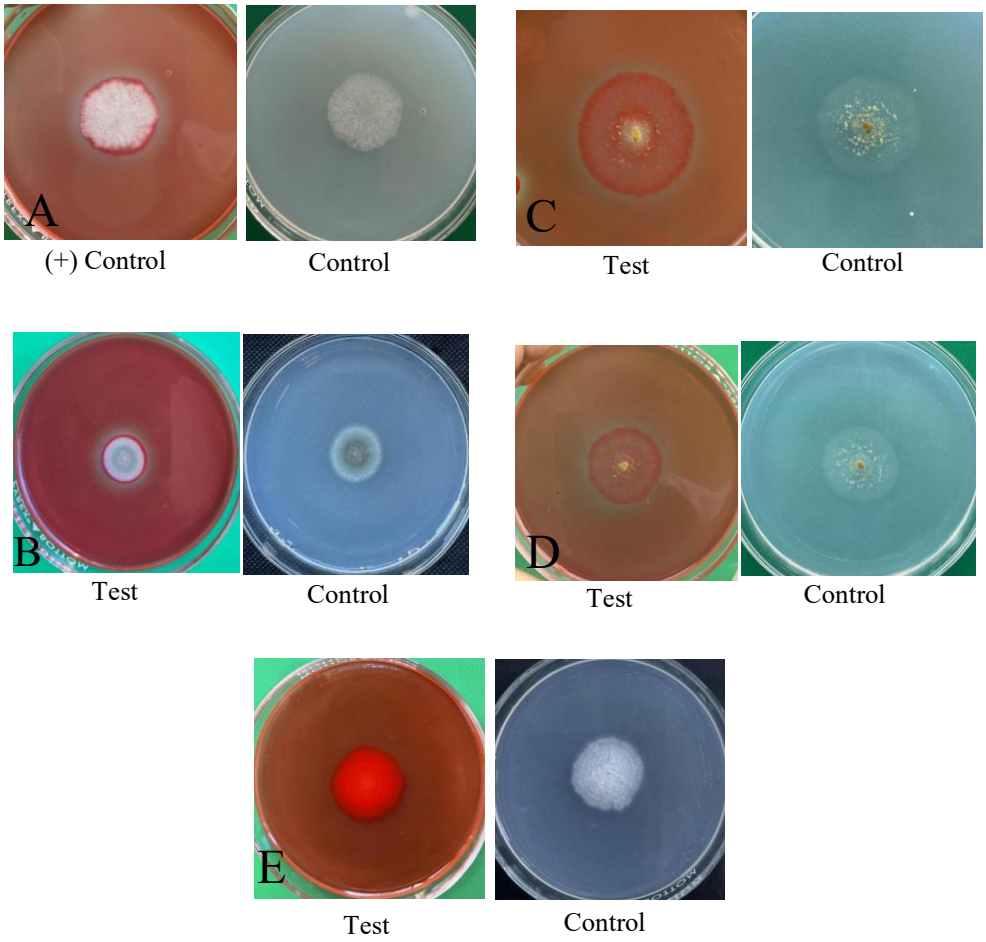


Figure 1. Clear zone produced by different fungi isolated from leaf litter of *Samanea*: (A) *Trichoderma harzianum* as the positive control species, (B) *Penicillium citrinum*, (C) *Aspergillus caelatus* LFJ3, (D) *Aspergillus caelatus* LFJ4, and (E) unidentified species

Table 1. Cellulase activity of different fungal isolates after five days of incubation

Species	Colony Diameter (mm)	Diameter of Clear Zone (mm)	Enzymatic Index (EI)	Reaction Strength
Penicillium citrinum	24.0 ± 0.62^{c}	31.87 ± 0.97^{b}	1.33 ± 0.07^{a}	Moderate
Aspergillus caelatus LFJ3	55.7 ± 3.69^{a}	62.48 ± 3.19^{a}	1.12 ± 0.02^{b}	Moderate
Aspergillus caelatus LFJ4	32.6 ± 1.78^{b}	35.13 ± 1.94^{b}	1.08 ± 0.00^{bc}	Moderate
Unidentified	32.1 ± 1.68^{b}	32.10 ± 1.68^{b}	1.00 ± 0.00^{C}	Low

*Means with the same letter superscript are not significantly different at 5% level of significance

Cultural and morphological characterization of fungal isolates

Penicillium citrinum

After 7 days of incubation on PDA, the culture of the fungi *P. citrinum* appeared to have a flat, velvet-like surface texture, and irregular round shape. The colony has greyish-turquoise color with white outer edge or periphery (Figure 2A) while reverse has pale yellow color (Figure 2B). After 72 hours of incubation of fungal isolates through slide culture method, the glass slides were viewed under microscope. The fungal isolate *P. citrinum* exhibited long conidiophore with phialides and metulae in a finger-like shape indicating biseriated vesicle (Figure 2C). The conidia varied from dark green to dark green-brown color and hyphae was septated and hyaline. Moreover, straight chains of conidiospores are attached to the phialides in columnar form.

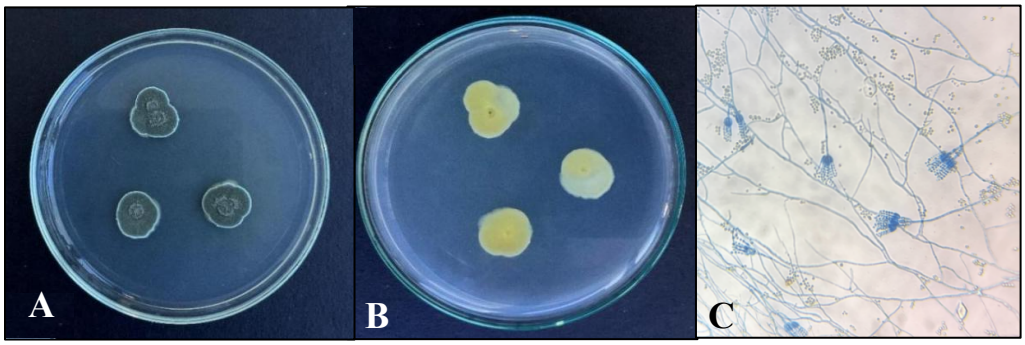


Figure 2. Cultural and morphological characteristics of the isolated fungi *Penicillium citrinum* on PDA; (A) obverse, (B) reverse sides, and (C) biseriated vesicles with distinct columns viewed under HPO (400x)

***Aspergillus caelatus* LFJ3**

Aspergillus caelatus LFJ3 on PDA appeared to have a cottony surface texture in round shape. The colony has light green color in the middle with dense white mycelia to its periphery (Figure 3A) and pale green on its reverse side (Figure 3B). This fungal isolate viewed under microscope revealed long conidiophore, and phialides indicating a uniseriated vesicle (Figure 3C). Moreover, the conidia are yellow-green in color and some are smooth and rough-walled attached to phialides covering only two-thirds of the vesicle in radiate arrangement.

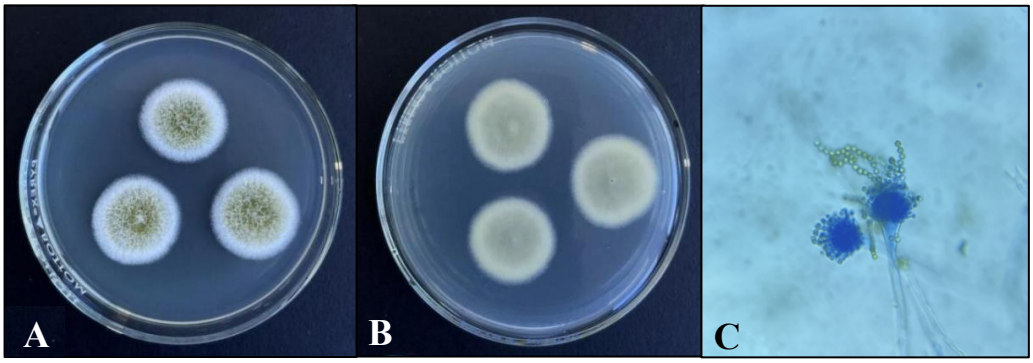


Figure 3. Cultural and morphological characteristics of the isolated fungi *A. caelatus* LFJ3 on PDA; (A) obverse, (B) reverse sides, and (C) uniseriated vesicle in semi-radial arrangement viewed under HPO (400x)

***Aspergillus caelatus* LFJ4**

The surface texture of the third isolate, *A. caelatus* LFJ4, has rough cottony texture. The mature mycelia in the point of inoculation have a darker green-brownish pigment compared to the light green color of the younger mycelia. Moreover, the margin has thin white mycelia (Figure 4A) and pale green-grayish on the reverse (Figure 4B). When viewed under microscope, *A. caelatus* LFJ4, has radiate conidial heads with smooth and rough-walled characteristic. The conidia are attached directly to phialides and vesicle indicating a uniseriated vesicle (Figure 4C).

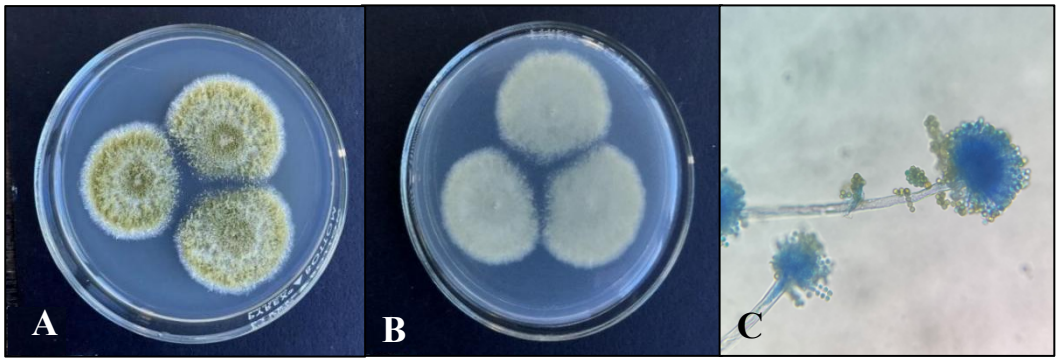


Figure 4. Cultural and morphological characteristics of the isolated fungi *A. caelatus* LFJ4 on PDA; (A) obverse, (B) reverse sides, and (C) uniseriated vesicle in radial arrangement viewed under HPO (400x)

Molecular identification of fungal isolates

Screening for cellulase-degrading fungi resulted to three fungal isolates positive for cellulase activity. To confirm the identity of the fungi isolated from the leaf litter of *Samanea,* the cultural and morphological characterization were complemented with molecular identification wherein the pure culture of fungal isolates was sent to Macrogen (Macrogen Inc., South Korea) for sequencing of the Internal Transcribed Spacer (ITS) region of the fungi. Moreover, the primers used were the forward primer ITS4 and reverse primer ITS5. BLAST analysis revealed that the identities of the fungal species were *P. citrinum* (100%)*, A. caelatus* LFJ3 (98.81%)*,* and *A. caelatus* LFJ4 (100%) shown in Table 2.

Phylogenetic analysis based on Macrogen ITS-5.8S rDNA sequencing and NCBI BLAST searches confirmed the identity of *P. citrinum* (Figure 5), supported by cultural and microscopic traits (Figure 2). Similarly, *A. caelatus* LFJ3 was molecularly identified, with phylogenetic positioning placing it closest to *A. caelatus* reference strains (Figures. 6-7).

Table 2. Identities of the isolated fungi through BLAST analysis with NCBI Genbank Accession Number

Gene Bank Accession Number	Description	Length	E-value	Match	% Identity
MT529486.1	*Penicillium citrinum*	566	0.0	550/550	100.00%
KM613140.1	*Aspergillus caelatus* LFJ3	588	0.0	580/587	98.81%
MH862672.1	*Aspergillus caelatus* LFJ4	610	0.0	594/594	100.00%

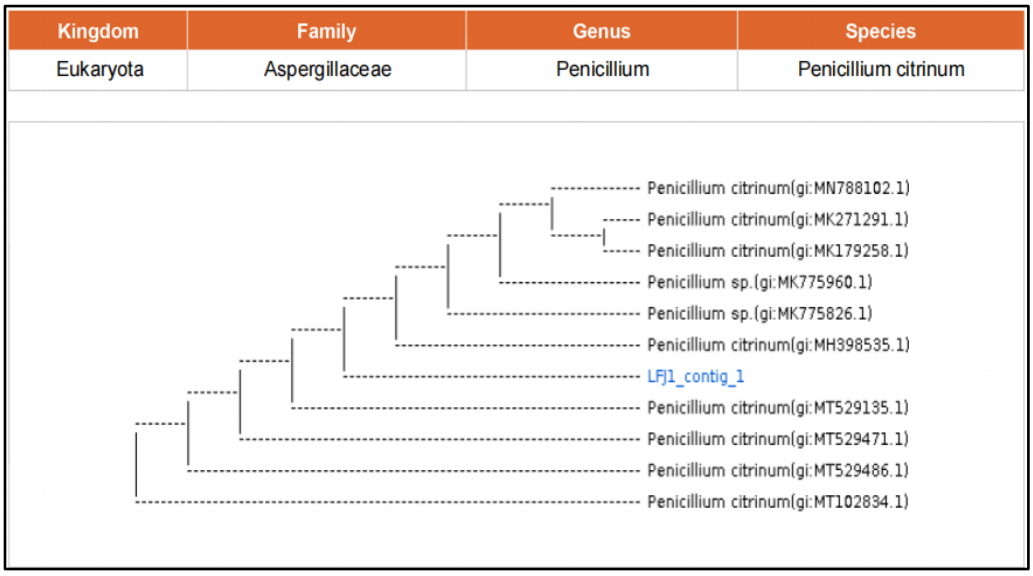


Figure 5. Phylogenetic tree of the fungal isolate *Penicillium citrinum* and their close relatives

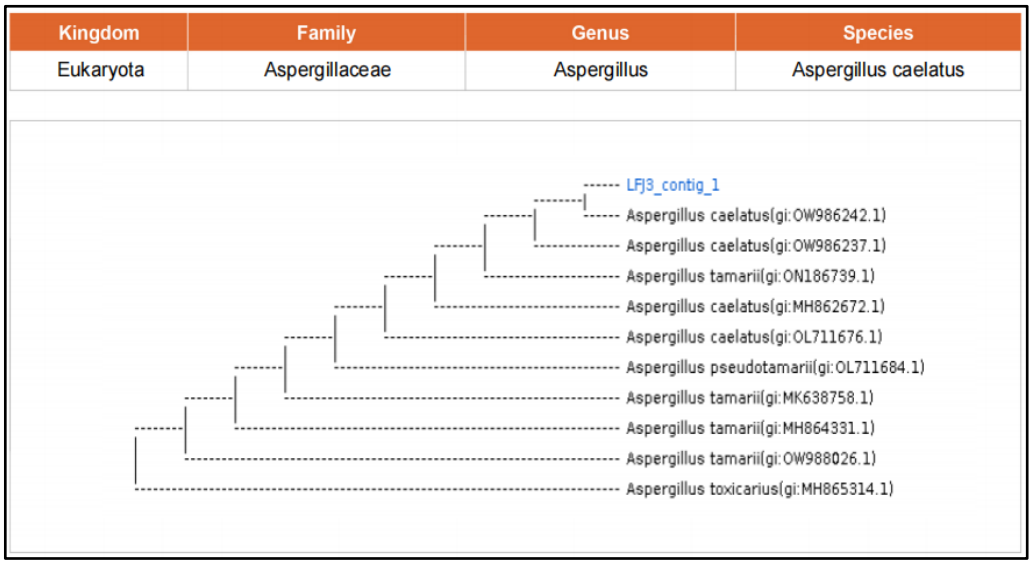


Figure 6. Phylogenetic tree of the fungal isolate *Aspergillus caelatus* LFJ3 and their close relatives

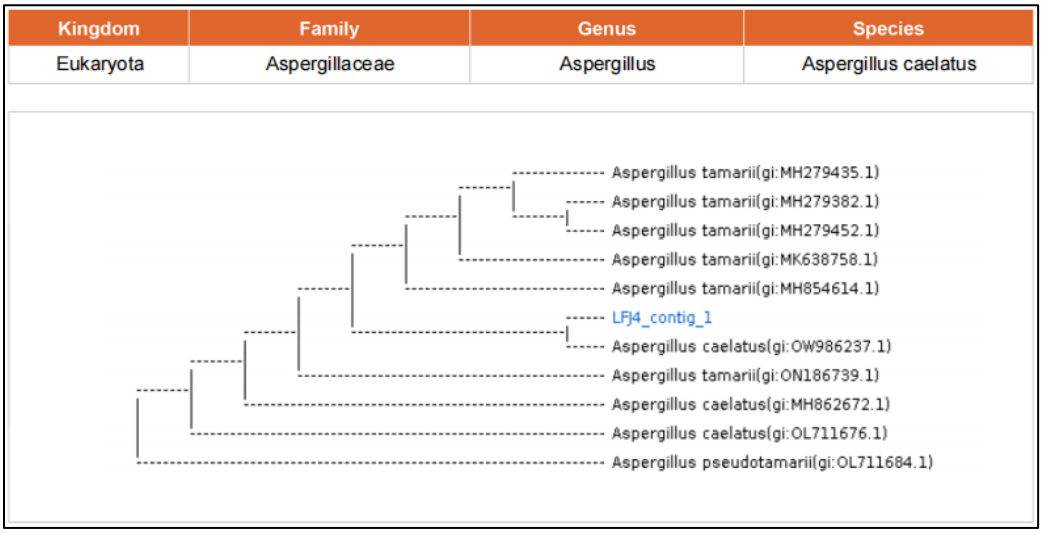


Figure 7. Phylogenetic tree of the fungal isolate *Aspergillus caelatus* LFJ4 and their close relatives

Scanning electron microscopy analysis of leaf litters

On the 30th day of incubation, the leaf litter inoculated with *P. citrinum* in glass jars was observed to be extensively colonized by fungal mycelia (Figure 8A) as viewed under dissecting microscope (Figure 8B). Moreover, on the 60th day of incubation, the leaf litter became soft, darker brown in color and with denser growth of fungal mycelia observed on the leaf surface (Figure 8 C and D).

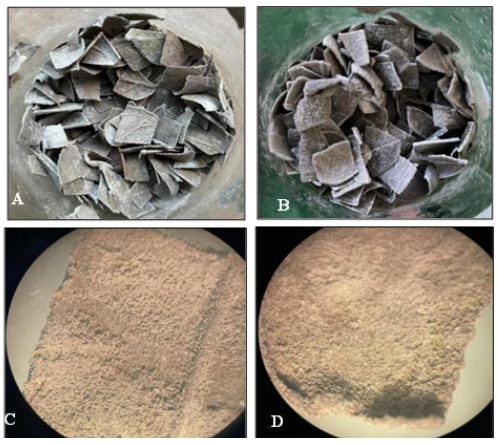


Figure 8. Leaf litter under *in vitro* degradation; (A) leaf litter in a glass jar at 30th day and (B) 60th day of incubation; (C) leaf litter at 30th day and (D) 60th day of incubation viewed under dissecting microscope

The *P. citrinum*'s activity on the leaf litter was further observed through SEM as presented in Figure 9. Before inoculating with the fungi *P. citrinum,* numerous non-glandular and simple trichomes were visible on the longitudinal and surface section of the leaf. Therefore, uninoculated and sterilized leaf showed no growth of fungal hyphae on both leaf surface (Figure 9A) and on its longitudinal section (Figure 9B).

SEM image analysis of the fungus-inoculated leaf litters at day 30 showed extensive growth and proliferation of *P. citrinum* mycelia on the leaf surface. Mycelial colonization was also observed on the longitudinal section of the leaf (Figure 9C), wherein the mycelia adhered and covered most of the leaf surface although the trichomes remained visible (Figure 9D). Moreover, on the 60th day of incubation, further development and increased density of fungal mycelia were observed in the longitudinal section of the leaf (Figure 9E).

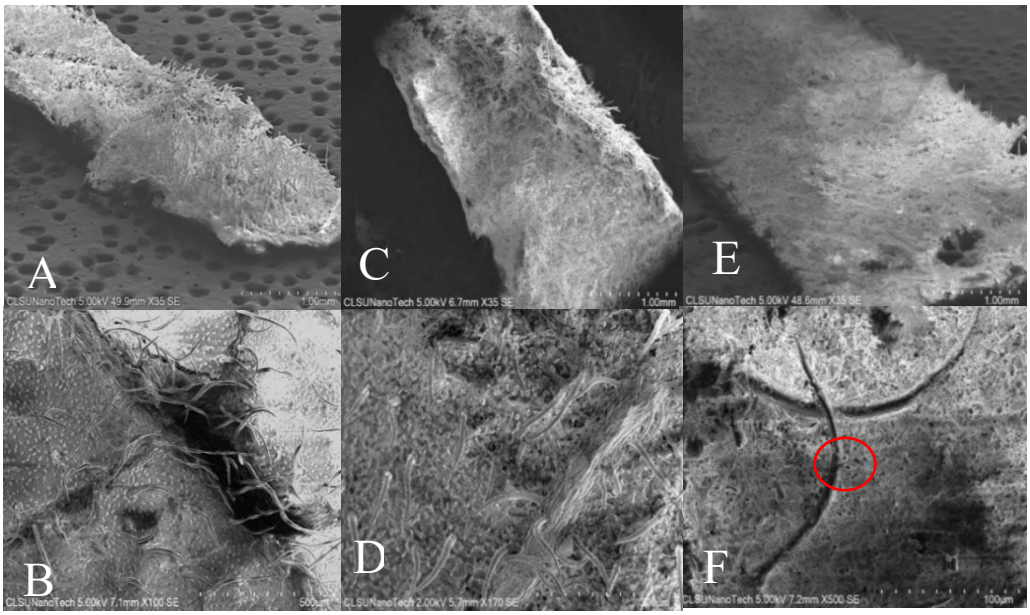


Figure 9. Scanning electron micrographs of *Samanea* leaf sections before and after fungal inoculation at 30 days and 60 days of incubation with *Penicillium citrinum*: (A) uninoculated leaf litter's longitudinal section and (B) leaf litter surface; (C) fungal-inoculated leaf litter's longitudinal section and (D) leaf litter surface after 30 days of incubation; (E) fungal-inoculated leaf litter's longitudinal section; (F) leaf litter surface after 60 days of incubation; and encircled is the ovoid phialide

In a more detailed view of fungus-inoculated leaf litter incubated at 60 days, an interconnected dense network of hyphae is visible surrounding the trichomes at 100 µm scale bar (Figure 10A). At a higher magnification (20 µm scale bar), numerous conidiophore (long stalk-like) and conidiospores (fungal spore) were observed on the longitudinal section of the leaf which may possibly indicates extensive colonization of the leaf mesophyll tissues (Figure 10B). Meanwhile, Figure 11 shows a leaf litter sample with no visible hair-like appendages on the surface (Figure 11A). At 300 µm scale bar, clusters of conidia were observed with numerous small lesion-like structures appearing on the leaf surface (Figure 11B).

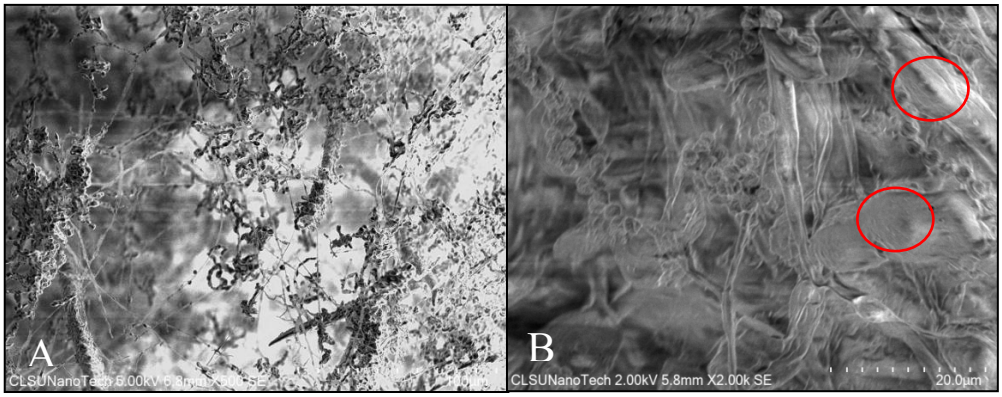


Figure 10. Scanning electron micrograph of leaf litter at day 60: (A) fungal hyphae wrapped around leaf trichomes at 100 µm scale bar, and (B) conidiophore and fungal spores around mesophyll at 20 µm scale bar

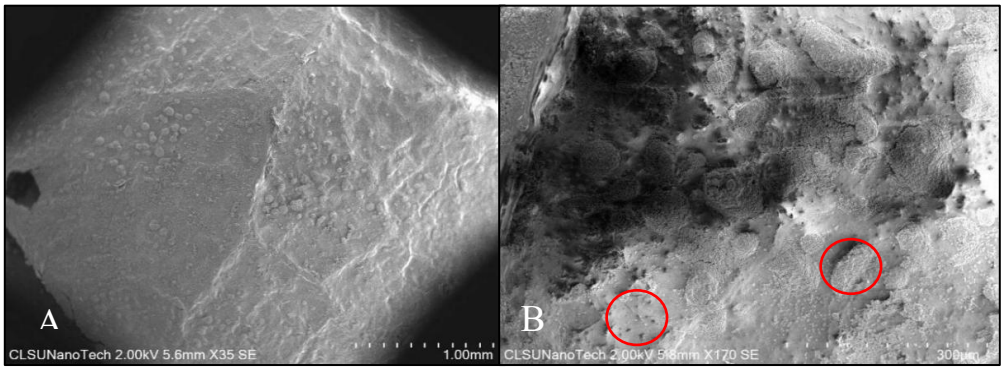


Figure 11. Scanning electron micrograph of leaf litter at day 60: (A) surface of leaf at 1.0 mm, and (B) a closer view of leaf surface with clusters of fungal spores

Discussion

Fungi are important decomposers of organic matter, particularly leaf litter, because of their ability to produce extracellular enzymes that break down complex plant polymers such as cellulose. In the present study, four fungal isolates were obtained from *Samanea* leaf litter, three of which exhibited cellulolytic activity based on their ability to hydrolyze CMC. The formation of clear zones surrounding the fungal colonies after flooding of the CMC agar indicated the hydrolysis of cellulose, consistent with the method described by Hankin and Anagnostakis (1975). CMC agar has also been used to screen cellulolytic fungi isolated from plant litter. Yuan and Chen (2014), for example, utilized CMC agar to evaluate the cellulolytic activity of endophytic fungi isolated from *Pinus massoniana* needle litter. Staining the CMC agar plate with congo red followed by destaining with NaCl facilitated the visualization of cellulolytic activity through the formation of halo surrounding the fungal colony (Waing *et al.,* 2015, Legodi *et al.,* 2019, Belmont-Montefusco *et al.,* 2020, Sun *et al.,* 2020, Reyes *et al.,* 2021). These findings demonstrate the usefulness of CMC agar as a preliminary qualitative method for identifying fungi capable of producing cellulolytic enzymes.

Among the three cellulolytic fungal isolates and identified, *P. citrinum* exhibited the highest enzymatic index of 1.33, followed by *A. caelatus* LFJ3 and *A. caelatus* LFJ4, with enzymatic indices of 1.13 and 1.08, respectively. The enzymatic indices of the three isolates were within the moderate category for cellulase production. The cellulolytic activity of *P. citrinum* observed in the present study is comparable with previous reports of cellulase production by this species. Shubha and Srinivas (2017), for instance, isolated *P. citrinum* from the leaf of *Cymbidium aloifolium* L. and reported an enzymatic index of 1.19, which was lower than the value obtained in the present study. The ability of *P. citrinum* to produce cellulase is also consistent with its reported occurrence in diverse organic substrates. More so, both *A. caelatus* isolates also exhibited positive cellulolytic activity, as demonstrated by the formation of clear zones in the enzymatic assay. This finding is supported by previous reports of cellulolytic enzyme production in *A. caelatus*. Tangunu (2016), for example, purified *A. caelatus* and obtained β-glucosidase from the isolate. The cellulolytic activity demonstrated by the *A. caelatus* isolates in the present study therefore supports the potential role of this species in the decomposition of cellulose-containing plant materials.

The three cellulolytic isolates were characterized using cultural and microscopic morphological characteristics and subsequently confirmed through molecular identification. The cultural characteristics of *P. citrinum* observed in

the present study were consistent with descriptions reported by Saif *et al.* (2020) and Seydametova and Zainol (2021) for preliminary identification of *Penicillium*. *P. citrinum* on its 7th day of incubation grown on PDA had greyish-turquoise color colony with white margin and the reverse side had yellow pigment. Microscopically, *P. citrinum* had biseriated vesicle and flask-shaped phialides. This was supported by Saif *et al.* (2020) which similarly described *P. citrinum* conidia as spherical with smooth to slightly rough surfaces and arranged in distinct columns. Meanwhile, the colony of *A. caelatus* LFJ3 on obverse side has cottony appearance with greenish mature mycelia with dense white mycelia on the periphery. It has uniseriated vesicle and semi-radial arrangement of conidiospores. The cultural characteristics of *A. caelatus* LFJ3 were also comparable with those reported by Pape *et al.* (2019). Moreover, *A. caelatus* LFJ4 exhibited a rough, cottony colony with dark greenish-brown coloration toward the center and lighter green coloration toward the periphery, with pale green pigmentation on the reverse side. The isolate showed both uniseriate and biseriate vesicles with a radial arrangement of conidial structures. The presence of uniseriate and biseriate structures is consistent with characteristics reported for the genus *Aspergillus* by Ibrahim *et al.* (2017). Furthermore, Rahmawati *et al.* (2020) reported *A. caelatus* with its characteristics which resembled the fungal isolate *A. caelatus* LFJ4 in this study.

Although cultural and morphological characteristics provided useful preliminary evidence for fungal identification, molecular analysis was still necessary to further confirm the taxonomic identity of the isolates. Molecular identification using ITS region sequencing of the fungal isolates confirm the identities as *P. citrinum* (KX958025.1), *A. caelatus* LFJ3 (KM613140.1), and *A. caelatus* LFJ4 (MH862672.1), with maximum identities of 100%, 98.81%, and 100%, respectively. These results complemented the cultural and morphological observations and provided molecular support for the identification of the three cellulolytic fungi. The occurrence of molecularly identified *P. citrinum* in diverse substrates, including neem oil cake (Jenitta and Gnanadoss, 2014), leaf litter (Waing *et al.,* 2015), forest soil (Kolanlarli *et al.,* 2019), and decaying fruits (Saif *et al.,* 2020), further demonstrates the broad ecological distribution of this species. Likewise, *A. caelatus* have been isolated from Brazil nuts, dried fruits, and hazelnuts via β-tubulin and calmodulin gene amplification (Gonçalves *et al.,* 2011). Both *A. caelatus* and *A. tamarii* belong to Tamari clade, inside *Aspergillus* section *Flavi* , sharing morphological features and a propensity to colonize soil or plant tissues (Midorikawa *et al.,* 2018, Frisvad *et al.,* 2019, Mahata *et al.,* 2022).

Among the other identified cellulolytic isolates, *P. citrinum* exhibited the highest enzymatic index thus, it was selected for the subsequent *in vitro*

degradation experiment of the *Samanea* leaf litters. Following inoculation and incubation, visible changes in the leaf litter were observed over time. By the 30th day, the leaf litter was visibly colonized by fungal mycelia. After 60 days of incubation, the leaf litter became softer and darker brown, accompanied by denser fungal mycelial growth. These changes suggest progressive fungal colonization and decomposition of the leaf material. The observed changes were comparable with those reported by Yuan and Chen (2014), who observed that leaf litter inoculated with cellulose-degrading fungi became softer and brown and was extensively colonized by fungal mycelia during incubation. Their study further demonstrated a relationship between incubation period and leaf-litter mass loss, with reported mass losses of approximately 14–22% after two months and up to 60% after seven months of incubation. Similarly, Saparrat *et al.* (2010) reported a 4.7% mass loss after 60 days of incubation of *Scutia buxifolia* leaf litter and observed fungal mycelia covering the leaf surface with a velvet-like appearance. The authors associated fungal growth on the leaf surface with the loss of litter biomass.

Although the present study is limited only on documenting visible and microscopic changes rather than quantitative mass loss, the softening and darkening of the *Samanea* leaf litter, together with increased fungal colonization, are consistent with observations associated with fungal-mediated decomposition reported in previous studies. The cellulolytic activity demonstrated by *P. citrinum* in the CMC assay provides additional support for its potential involvement in the degradation of cellulose-containing leaf material.

The SEM observations provided further evidence of progressive fungal colonization of the *Samanea* leaf litter. *Samanea* leaves have previously been reported to possess non-glandular trichomes on their surfaces (Oikawa *et al.,* 2018). After 30 days of incubation, SEM micrographs showed the growth and proliferation of *P. citrinum* mycelia on the leaf surface. An interconnected network of hyphae was observed, with the mycelia adhering to and covering much of the leaf surface, while the trichomes remained visible. These observations indicate that the fungus was actively growing on the leaf-litter substrate during incubation.

At day 60 of incubation, a denser fungal structure appeared and further expansion of the mycelia was evident. Numerous conidiophores and conidia were also visible in the longitudinal sections of the leaf. The increased abundance of fungal structures compared with the earlier incubation period suggests continued fungal development on the leaf-litter substrate. The persistence and proliferation of fungal mycelia are consistent with the ability of saprophytic fungi to utilize decaying plant material as a source of nutrients (Yuan and Chen, 2014).

The association of fungal structures with leaf trichomes is also of interest. Calo *et al.* (2006) reported that trichome density can influence foliar fungal infection by providing sites that facilitate the attachment of fungal spores and hyphae to the leaf surface. The authors further reported that fungal hyphae attached to trichomes can penetrate trichome cells and subsequently contribute to further colonization of plant tissues. In the present study, fungal structures were observed in association with the trichomes at earlier stages of incubation, followed by more extensive fungal development at 60 days.

At higher magnification, clusters of conidia and numerous small, dark-colored, lesion-like structures were visible on the leaf surface. Similar surface alterations have been reported in studies of fungal and enzymatic degradation of plant tissues. This result was supported by the study of Fernandez *et al.* (2024). They had observed changes in the leaf surface of olive leaves exposed to cellulase- and pectinase-related degradation. Wherein, shedding of some upper-surface trichomes were observed. In the present study, the absence or reduced visibility of trichomes at 60 days, together with the increased fungal colonization and surface alterations, may reflect progressive modification and changes on the leaf surface during incubation. However, these SEM observations alone do not establish the specific biochemical processes responsible for the observed structures.

The ability of *P. citrinum* to colonize *Samanea* leaf litter can be associated with the ecological characteristics of filamentous saprophytic fungi. Fungi obtain nutrients through absorption and, in decaying plant substrates, secrete extracellular enzymes that depolymerize complex organic compounds into smaller molecules that can subsequently be absorbed and metabolized. Fernandez *et al.* (2014) described this process as involving the identification of available carbon and nitrogen sources, secretion of appropriate extracellular enzymes, depolymerization and mobilization of complex compounds, and uptake of the resulting soluble substrates.

Fungal colonization is an important early component of leaf-litter decomposition. As leaves senesce and fall to the ground, microorganisms begin to colonize the newly available substrate, and some fungal species may shift from endophytic or plant-associated lifestyles toward saprotrophic activity (Promputtha *et al.,* 2010; Tennakoon, 2021). Leaf-litter biodegradation involves microbial colonization and the subsequent transformation of structural compounds such as lignin and cellulose through microbial activity, particularly that of fungi. Soluble compounds may also be released and transported into the surrounding soil during decomposition.

In the present study, the observed increase in the fungal colonization, changes in the leaf litter texture and color, and modifications of the leaf surface

after 60 days are consistent with active fungal involvement in the decomposition process. The SEM observations particularly demonstrate the establishment and proliferation of *P. citrinum* on the leaf substrate. However, because quantitative measurements of mass loss, cellulose concentration, lignin content, and enzyme activity during the degradation period were not conducted, the extent of actual biochemical degradation cannot be determined solely from the SEM observations.

These findings demonstrated that the leaf litter of *Samanea* harbors fungi with cellulolytic potential, including *P. citrinum* and two isolates identified as *A. caelatus*. Among the isolates, *P. citrinum* exhibited the highest cellulolytic activity and was subsequently able to colonize and produce visible and microscopic changes in *Samanea* leaf litter during 60 days of *in vitro* incubation. The results provide preliminary evidence of the potential involvement of these fungi in the decomposition of cellulose-containing leaf litter.

With this. further studies should evaluate the isolates for additional extracellular enzymes involved in plant biomass decomposition, including laccases and other lignocellulose-degrading enzymes. Quantitative assessment of degradation through percentage mass loss, cellulose and lignin reduction, and changes in carbon and nitrogen content would provide stronger evidence of the extent of biodegradation. Determination of the carbon-to-nitrogen ratio may also provide additional information on changes in the chemical composition of the leaf litter during fungal decomposition. Future experiments may also further examine the effects of temperature and moisture on degradation and evaluate interactions among different fungal isolates through dual-culture experiments. For SEM analysis, removal or gentle scraping of excess fungal mycelia from the leaf surface may also be considered when appropriate to improve visualization of changes in the underlying leaf structures.

Conflicts of interest

The authors declare no conflict of interest.

References

Adebayo, F. O. and Obiekezie, S. O. (2018). Microorganisms in waste management. Research Journal of Science and Technology, 10:28-39.

Baconguis, S. and Pasagda, A. R. (2013). Waste biomass statistics in the Philippines. Canopy International, 39:8-9.

Bani, A., Pioli, S., Ventura, M., Panzacchi, P. *et al.* (2018). The role of microbial community in the decomposition of leaf litter and deadwood. Applied Soil Ecology, 126:75-84.

Belmont-Montefusco, E. L., Nacif-Marçal, L., De Assunção, E. N., Hamada, N. *et al.* (2020). Cultivable cellulolytic fungi isolated from the gut of Amazonian aquatic insects. Acta Amazonica, 50:346-354.

Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R. *et al.* (2016). Future directions: understanding the dominant controls on litter decomposition. Journal of Ecology, 104:229-238.

Calo, L., Garcia, I., Gotor, C. and Romero, L. C. (2006). Leaf hairs influence phytopathogenic fungus infection and confer an increased resistance when expressing a *Trichoderma* -1,3-glucanase. Journal of Experimental Botany, 57:3911-3920.

Dey, P., Singh, J., Scaria, J. and Anand, A. (2018). Improved production of cellulase by *Trichoderma reesei* (MTCC 164) from coconut mesocarp-based lignocellulosic wastes under response surface-optimized condition. Biotech, 9:402.

Djarwanto, S. S. and Tachibana, S. (2009). Screening of fungi capable of degrading lignocellulose from plantation forests. Pakistan Journal of Biological Sciences, 12:669-675.

Fernandez, J., Marroquin-Guzman, M. and Wilson, R. A. (2014). Mechanisms of nutrient acquisition and utilization during fungal infections of leaves. Annual Review of Phytopathology, 52:155-174.

Fernandez, V., Almonte, L., Bahamonde, H. A. and Galindo-Bernabeu, A. (2024). Chemical and structural heterogeneity of olive leaves and their trichomes. Communications Biology, 7.

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S. B., Nováková, A., Chen, A. J., *et al.* (2019). Taxonomy of *Aspergillus* section *flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology, 91-63.

Ghosh, P., Shah, G., Sahota, S., Singh, L. and Vijay, V. K. (2020). Biogas Production from Waste: Technical Overview, Progress, and Challenges. Elsevier eBooks, 89-104.

Goncalves, J. S., Ferracin, L. M., Vieira, M. L. C., Iamanaka, B. T. *et al.* (2011). Molecular analysis of *Aspergillus* section *Flavi* isolated from Brazil nuts. World Journal of Microbiology & Biotechnology, 28:1817-1825.

Gupta, V. K., Steindorff, A. S., De Paula, R., Silva-Rocha, R. *et al.* (2016). The post-genomic era of *Trichoderma reesei*: what's next? Trends in Biotechnology, 34:970-982.

Hankin, L. H. and Anagnostakis, S. L. (1975). The use of solid media for the detection of enzyme production of fungi. Mycologia, 67:597-607.

Ibrahim, M. J., Kabir, J., Kwanashie, C. N. and Salawudeen, M. T. (2017). Identification of *Aspergillus* species in feed fed to caged birds using morphological characteristics in Zaria, Nigeria. Microbiology Research International, 5:16-24.

Jenitta, J. and Gnanadoss, J. J. (2014). Screening, molecular characterization and phylogenetic analysis of *Penicillium* sp producing high levels of protease. International Journal of Current Science, 11:10-17.

Kjoller, A. and Struwe, S. (1982). Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos, 39:389-422.

Kolanlarli, T. K., Asan, A., Şen, B. and Ökten, S. (2019). Biodiversity of *Penicillium* species isolated from Edirne Söğütlük forest soil (Turkey). Directory of Open Access Journals, 10:26-39.

Krishna, M. P. and Mohan, M. (2017). Litter decomposition in forest ecosystems: A review. Energy, Ecology and Environment*,* 2:236-249.

Legodi, L. M., La Grange, D., Jansen van Rensburg, E. L. and Ncube, I. (2019). Isolation of cellulose degrading fungi from decaying banana pseudostem and *Strelitzia alba*. Enzyme Research*,* 2019:10.

Mahata, P. K., Dass, R. S., Pan, A. and Muthusamy, B. (2022). Substantive morphological descriptions, phylogenetic analysis and single nucleotide polymorphisms of *Aspergillus* species from *Foeniculum vulgare*. Frontiers in Microbiology, 13:832320.

Midorikawa, G. E. O., Corrêa, C. L., Noronha, E. F., Filho, E. X. F., Togawa, R. C., Costa, M. M. D. C., Silva-Junior, O. B., Grynberg, P. and Miller, R. N. (2018). Analysis of the transcriptome in *Aspergillus tamarii* during enzymatic degradation of sugarcane bagasse. Frontiers in Bioengineering and Biotechnology, 6.

Oikawa, T., Ishimaru, Y., Munemasa, S., Takeuchi, Y. *et al.* (2018). Ion channels regulate nyctinastic leaf opening in *Samanea saman*. Current Biology, 28:2230-2238.

Osono, T. (2019). Functional diversity of ligninolytic fungi associated with leaf litter decomposition. Ecological Research, 34:30-43.

Pape, P. L., Ximenes, R. M., Ariza, B., Iriarte, J. *et al.* (2019). First case of *Aspergillus caelatus* airway colonization in a chronic obstructive pulmonary disease patient. International Journal of Infectious Diseases, 81:85-90.

Prakash, P. Y. and Bhargava, K. (2016). A modified micro chamber agar spot slide culture technique for microscopic examination of filamentous fungi. Journal of Microbiological Methods, 123:126-129.

Promputtha, I., Hyde, K. D., McKenzie, E. H. C. and Peberdy, J. F. (2010). Can leaf degrading enzymes provide evidence that endophytic fungi become saprobes? Fungal Diversity, 41:89-99.

Rahmawati, N., Sumardi, I. and Dungani, R. (2020). Isolation and identification of fungi inhabiting rubber-impregnated wood, and their role of quality changing the impregnated wood. Bioresources, 15:2839-2849.

Reyes, R. D. C., Parayao, A. M. and Waing, K. G. D. (2021). Identification and evaluation of enzymatic ability of fungal endophytes from *Citrofortunella microcarpa* (Bunge) Wijnands. Studies in Fungi, 6:460-468.

Saif, F. A., Yaseen, S. A., Alameen, A. S., Mane, S. B. *et al.* (2020). Identification of *Penicillium* species of fruits using morphology and spectroscopic methods. Journal of Physics Conference Series*,* 1644:012019.

Saparrat, M. C., Estevez, J. M., Troncozo, M. I., and Arambarri, A. M. *et al.* (2010). In-vitro depolymerization of *Scutia buxifolia* leaf-litter by a dominant *Ascomycota Ciliochorella* sp. International Biodeterioration & Biodegradation, 64:262-266.

Sarsaiya, S., Awasthi, S., Awasthi, M. K., Awasthi, A. *et al.* (2017). The dynamic of cellulase activity of fungi inhabiting organic municipal solid waste. Bioresource Technology, 251:411-415.

Seydametova, E. and Zainol, N. (2021). Morphological, physiological, biochemical and molecular characterization of statin-producing *Penicillium* microfungi isolated from little-explored tropical ecosystems. Current Research in Microbial Sciences, 2:100044.

Shubha, J. and Srinivas, C. (2017). Diversity and extracellular enzymes of endophytic fungi associated with *Cymbidium aloifolium* L. African Journal of Biotechnology, 16:2248-2258.

Sun, S., Weng, Y., Di, X., Liu, Z. *et al.* (2020). Screening of cellulose-degrading fungi in forest litter and fungal effects on litter decomposition. BioResources, 15:2937-2946.

Tangunu, A. (2016). Screening of cellulose and hemicellulose enzyme activities in different *Aspergillus* species. (Master's Thesis). Balıkesir University Institute of Science and Biology.

Tennakoon, D. (2021). Life in leaf litter: Fungal community succession during decomposition. Mycosphere, 12:406-429.

Waing, K. G. D., Abella, E. A., Kalaw, S. P., Waing, F. P. *et al.* (2015). Studies on biodiversity of leaf litter fungi of Central Luzon State University and evaluation of their enzyme producing ability. Current Research in Environmental & Applied Mycology, 5:269-276.

Yuan, Z. and Chen, L. (2014). The role of endophytic fungal individuals and communities in the decomposition of *Pinus massoniana* needle litter. PLoS ONE, 9:e000000.

(Received: 16 May 2025, Revised: 9 September 2026, Accepted: 13 November 2026)