

Baluran Savanna Mold: A Natural Tool for Plastic Biodegradation

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Abstract. Plastic waste poses a serious threat to environmental balance if left unmanaged. Savannas are unique ecosystems with extreme and dry conditions that support the growth of adaptive microorganisms, including molds capable of producing enzymes involved in polymer degradation. However, despite this potential, studies on plastic-degrading fungi from Indonesian savannas remain limited. This study examined the ability of three mold isolates obtained from savanna soil in Baluran National Park to degrade polyethylene (PE) during 15- and 30-day incubations. From an initial set of 30 isolates, three were selected for biodegradation assays. Species identification, based on morphological traits, identified them as *A. niger*, *T. harzianum*, and *T. asperellum*. All isolates demonstrated biodegradation potential, indicated by increased biomass, reduced plastic weight, and decreased pH. The degradation activity was likely driven by oxidative and hydrolytic enzyme production. Among the isolates, *T. asperellum* exhibited the highest degradation efficiency, reaching 14.4% after 15 days and 20.95% after 30 days, while *A. niger* and *T. harzianum* showed lower but still measurable activity. These findings highlight the ecological value of Baluran savanna molds and their potential application as environmentally friendly agents for PE biodegradation.

1 Introduction

Plastic is one of the most widely used synthetic materials in the world due to its lightweight, durability, and versatility. However, these advantages also contribute to serious environmental problems, as plastics are highly resistant to natural degradation.

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According to the Indonesian Ministry of Environment and Forestry, the country generates more than 60 million tons of waste annually, with approximately 15% consisting of plastic waste. A significant portion of this waste ends up in landfills and aquatic ecosystems, causing ecological imbalances and threatening biodiversity.

Among various types of plastic, Polyethylene (PE) is one of the most commonly used, particularly in single-use bags and packaging materials. Its long hydrocarbon chains and hydrophobic nature make PE highly resistant to chemical and physical degradation. Conventional waste management methods such as incineration and recycling remain insufficient, as they often generate secondary environmental issues such as toxic gas emissions and high energy consumption. Therefore, a biotechnological approach, specifically biodegradation by microorganisms, is considered a more sustainable and environmentally friendly alternative.

Several groups of microorganisms, including bacteria and fungi, have been reported to possess the ability to degrade plastics. Fungi are of particular interest because they can produce a variety of oxidative and hydrolytic enzymes, such as laccases, peroxidases, esterases, and cutinases, which are capable of cleaving carbon bonds in plastic polymer structures [1]. Previous studies have demonstrated that fungal genera such as *Aspergillus* and *Trichoderma* can degrade low-density polyethylene (LDPE) and polyethylene (PE) with varying efficiencies depending on environmental conditions and incubation periods.

Baluran National Park, known as the “Little Africa of Java,” hosts a tropical savanna ecosystem characterized by extreme environmental conditions— high temperatures, strong sunlight intensity, and low humidity and nutrient availability. Such conditions foster the growth of indigenous microorganisms that have adapted to environmental stress, including fungi with the potential to produce unique enzymes. Studies on fungi from Indonesian savanna environments remain limited, despite the potential of local microbial resources to contribute to sustainable plastic biodegradation strategies suited to tropical ecosystems.

Despite the ecological uniqueness of Indonesian savannas, research on plastic-degrading fungi from these environments remains very limited. To address this gap, the present study aimed to screen indigenous fungal isolates from the savanna soil of Baluran National Park for their ability to degrade polyethylene (PE). The study also assessed their degradation efficiency and identified the species with the highest biodegradation potential. By doing so, this work contributes to filling the scarcity of data on fungal biodegraders from tropical savannas and provides insights that may support the development of sustainable, microbiology-based approaches for plastic waste management.

2 Material and Method

2.1 Sample Collection and Isolation of Fungi

Soil samples were collected from the savanna area of Baluran National Park, East Java, Indonesia (7°55'– 7°29' S, 114°17'–114°23' E). Approximately 200 gr of soil were taken from a depth of 5-10 cm using sterile spatulas and stored in sterile plastic bags at ambient temperature. Fungal isolation was performed using the serial dilution method on *Potato*

Dextrose Agar (PDA) medium supplemented with 50 µg/mL chloramphenicol to inhibit bacterial growth. The plates were incubated at 30°C for 5–7 days. Morphologically distinct fungal colonies are purified through subculturing on fresh PDA plates.

2.2 Screening of Plastic Degrading Fungi

A total of 30 fungal isolates were obtained and subjected to preliminary screening for plastic degradation potential. Polyethylene (PE) plastic sheets (1 × 1 cm) were sterilized with ethanol 70%, rinsed with distilled water, and air-dried prior to use. Screening was conducted using *Bushnell Haas Broth* (BHB) medium containing 0,1% (w/v) yeast extract and a PE film as the sole carbon source. Each flask was inoculated with 1 mL of spore suspension (OD₆₀₀ = 0.1) and incubated at room temperature (28 ± 2°C) for 15 and 30 days under shaking conditions (120 rpm). Control flasks without fungal inoculation were maintained under identical conditions.

2.3 Measurement of Fungal Biomass and Plastic Weight Loss

At the end of each incubation period, fungal biomass was collected by filtration through Whatman No.1 filter paper, washed with distilled water, and dried at 60° C until constant weight. The biomass dry weight (g/mL) was determined gravimetrically. For the plastic weight loss, PE Films were carefully removed, washed with 2% *Sodium Dodecyl Sulfate* (SDS) to remove attached mycelia, rinsed with distilled water, dried at 60° C, and weighed. The degradation efficiency (%) was calculated using the following equation:

$$\%B = \frac{(W_o - W_i)}{W_o} \times 100\% \quad (1)$$

where W_o is the initial weight and W_i is the final weight after incubation.

The pH of each culture flask was measured at the end of each incubation period (15 and 30 days) using a calibrated digital pH meter. Before measurement, cultures were homogenized and the supernatant was separated by filtration to ensure accurate pH readings.

2.4 Morphological Identification of Fungal Isolates

Fungal isolates showing the highest degradation efficiency were identified based on macroscopic and microscopic characteristics. Colony color, texture, and growth rate were observed macroscopically, while microscopic features such as conidiophore structure, spore shape, and arrangement were examined under a compound light microscope. Species identification was conducted by comparing morphological traits with standard taxonomic keys described by [2].

It is acknowledged that morphological identification alone has limitations, and molecular confirmation (e.g., ITS gene sequencing) would be required for more accurate and robust species verification.

2.5 Data Analysis

The data were analyzed qualitatively using descriptive methods. Observations of fungal growth, changes in biomass, plastic weight reduction, and pH variation were interpreted based on visual and numerical trends without statistical testing. The biodegradation potential of each isolate was assessed comparatively to determine the most effective fungal species in degrading polyethylene plastic.

3 Results and Discussion

3.1 Fungal Isolation and Identification

A total of approximately 30 fungal colonies were successfully isolated from savanna soil collected in Baluran National Park. Based on colony morphology, three representative isolates were selected for biodegradation assays. Morphological identification, based on macroscopic and microscopic characteristics, revealed the isolates as *Aspergillus niger*, *Trichoderma harzianum*, and *Trichoderma asperellum* [3] (Figure 1).

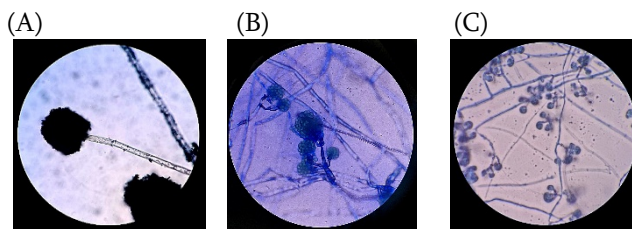


Fig. 1. Microscopic morphology of fungal isolates.

Panel images show diagnostic morphological characteristics employed to confirm species identity: (A) *Aspergillus niger* (K.13); (B) *Trichoderma harzianum* (K.14); (C) *Trichoderma asperellum* (K2K0I2)

These genera are widely recognized for producing oxidative and hydrolytic enzymes, including laccases, peroxidases, esterases, and cutinases, which can break carbon linkages in polyethylene. Such enzymatic activity has been commonly associated with fungal capacity to degrade Polyethylene (PE).

3.2 Polyethylene Degradation Efficiency

All fungal isolates demonstrated the ability to degrade polyethylene films during the 15-30 day incubation periods. Biodegradations was indicated by measurable decreases in plastic weight and increased fungal biomass. Among the tested isolates, *Trichoderma asperellum* exhibited the highest degradation efficiency, achieving 14,4% degradation at 15 days, which further increased to 20,95% at 30 days.

3.3 Biomass production

Mean dry biomass of the three isolates is presented in Table 1. All isolates showed an increase in dry biomass from day 15 to day 30. K2K0I2 (*Trichoderma asperellum*) produced the highest biomass, increasing from 0.01024 g/30 mL at day 15 to 0.01346 g/30 mL at day 30. K.14 (*T. harzianum*) increased from 0.00899 to 0.01035 g/30 mL, while K.13 (*A. niger*) showed the lowest values, from 0.00111 to 0.00687 g/30 mL. (See Table 1.)

Table 1. Dry biomass of fungal isolates after 15 and 30 days of incubation

Isolate code	Species	Mean biomass (g/30mL)	
		Day 15	Day 30
K.13	<i>Aspergillus niger</i>	0,00111	0,00687
K.14	<i>Trichoderma harzianum</i>	0,00899	0,01035
K2K0I2	<i>Trichoderma asperellum</i>	0,01024	0,01346

The observed increase in biomass over time indicates that the fungal isolates were able to utilize components derived from PE degradation or other available carbon sources in the medium to support growth. The higher biomass accumulation in K2K0I2 is consistent with its superior degradation performance (Table 2), suggesting more active metabolism and/or greater enzyme production in this isolate. Conversely, the relatively low biomass of K.13 may reflect slower metabolic adaptation to the PE substrate under the experimental conditions. These patterns imply a correlation between biomass increase and biodegradation activity, although confirmation would require statistical correlation analysis and enzyme assays.

3.4 Polyethylene (PE) degradation efficiency

The biodegradation percentages obtained after 15 and 30 days of incubation are presented in Table 2. All isolates showed increased PE degradation with longer incubation time. K2K0I2 (*T. asperellum*) exhibited the highest degradation efficiency, reaching 14.4% at day 15 and 20.95% at day 30. K.14 (*T. harzianum*) showed moderate activity with 10.05% and 16.46%, respectively. Meanwhile, K.13 (*A. niger*) demonstrated the lowest performance, achieving only 1.24% at day 15 and 5.68% at day 30.

Table 2. Polyethylene (PE) degradation efficiency (%) after 15 and 30 days of incubation

Isolate Code	Species	Mean biodegradation percentage (%)	
		Day 15	Day 30
K.13	<i>Aspergillus niger</i>	1,24	5,68
K.14	<i>Trichoderma harzianum</i>	10,05	16,46
K2K0I2	<i>Trichoderma asperellum</i>	14,4	20,95

The increasing degradation values from day 15 to day 30 indicate that all isolates were able to utilize or transform PE more actively over time. The superior performance of *T. asperellum* (K2K0I2) is consistent with its higher biomass production and may reflect stronger enzymatic activity, particularly in producing oxidative and hydrolytic enzymes associated with PE depolymerization. The moderate degradation efficiency of *T. harzianum* and the relatively low activity of *A. niger* suggest inter-species variation in

metabolic adaptation to PE substrates. These findings align with previous studies reporting that fungi from harsh environments often possess enhanced polymer-degrading capabilities, although confirmation of superiority would require statistical analysis and enzyme assays.

3.5 pH changes in culture media

The pH values of the culture media at day 0, day 15, and day 30 are shown in Table 3. All isolates exhibited a slight decrease in pH after incubation with polyethylene. For isolate K.13 (*A. niger*), the pH decreased from 7.00 to 6.37 at day 15 and 6.30 at day 30. K.14 (*T. harzianum*) showed a similar pattern, with pH decreasing from 7.00 to 6.25 and 6.18, respectively. Meanwhile, K2KOI2 (*T. asperellum*) experienced a pH reduction from 7.00 to 6.22 at day 15 and 6.14 at day 30.

Table 3. pH measurement for the three types of mold isolates in culture media.

Isolate Code	Species	Mean biodegradation percentage (%)		
		Day 0	Day 15	Day 30
K.13	<i>Aspergillus niger</i>	7	6,37	6,30
K.14	<i>Trichoderma harzianum</i>	7	6,25	6,18
K2KOI2	<i>Trichoderma asperellum</i>	7	6,22	6,14

The gradual decline in pH across all isolates indicates the release of organic acids during fungal metabolism, a phenomenon commonly associated with oxidative breakdown of polyethylene chains. The acidic environment supports further depolymerization of PE, consistent with the patterns observed in biomass increase and degradation efficiency. The slightly greater pH reduction in *T. asperellum* aligns with its higher biodegradation performance, suggesting more active metabolic and enzymatic processes compared to the other isolates.

The isolates Sav 2, PR-1.2, and K2KOI2—presumed to be *Trichoderma asperellum*—demonstrated the highest plastic degradation efficiency among all tested isolates. Their performance was notably superior to previous findings. In this study, *T. asperellum* achieved a maximum degradation rate of 20.95% within 30 days, far exceeding the 7.18% and 5.5% reported for *Aspergillus niger* [1,4], the 7.51% recorded for *Trichoderma* sp. [5], and the 1.80% documented for *Trichoderma harzianum* [6]. These differences highlight the strong biodegradation potential of the Baluran-derived isolates compared to previously studied fungal species.

The superior performance of *T. asperellum* aligns well with the supporting physiological parameters measured in this study. Higher biomass production indicates more active fungal propagation driven by the utilization of plastic as a carbon source. In addition, a more pronounced decline in pH throughout incubation reflects intensified metabolic activity, which is strongly associated with enhanced degradation efficiency. Together, the increases in biomass, the elevated degradation percentage, and the greater pH reduction demonstrate that the Baluran-derived isolates possess robust and efficient plastic biodegradation capabilities, especially under extended incubation periods.

The Bekol Savannah in Baluran National Park is one of the driest and most extreme savannah ecosystems in Indonesia. This environment is characterised by high

temperatures, intense solar radiation, low humidity, and limited soil nutrients throughout the year. These extreme ecological conditions encourage microorganisms, including fungi, to develop adaptive mechanisms not found in fungi from more stable environments.

Ecologically, fungi living in dry savanna habitats develop more efficient metabolism and the ability to produce more aggressive enzymes to break down substrates that are difficult to degrade. Fungi from the Bekol Savannah in particular show characteristics of adaptation to oxidative stress and prolonged drought. This adaptation is directly related to increased production of oxidative enzymes such as laccase, peroxidase, esterase, and cutinase, which are known to play an important role in the depolymerisation of plastics such as polyethylene (PE).

4 Conclusion

This study showed that three fungal isolates from the Baluran savanna—*Aspergillus niger*, *Trichoderma harzianum*, and *Trichoderma asperellum*—were able to degrade polyethylene (PE) during 15–30 days of incubation. All isolates exhibited increased biomass, reduced plastic weight, and slight pH decreases, indicating active fungal growth in the presence of PE. Among them, *T. asperellum* showed the highest degradation efficiency, reaching 20.95% after 30 days. These findings demonstrate that Baluran-derived fungi have promising potential for supporting environmentally friendly PE biodegradation efforts.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement

Istiana Putri: Conceptualization, Methodology, Investigation, Data curation, Writing- Original draft preparation. Ni'matuzahroh: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. Agus Supriyanto: Validation, Writing- Reviewing and Editing. Tri Nurhariyati: Validation, Writing- Reviewing and Editing. Moch. Affandi: Validation, Writing- Reviewing and Editing. Salamun: Validation, Writing- Reviewing and Editing. Almando Gerald: Validation, Writing- Reviewing and Editing. Johan Setiawan: Validation, Writing- Reviewing and Editing. Brigita Nur Diyan Agustiana: Validation, Writing- Reviewing and Editing. Dela Dwi Alawiyah: Validation, Writing- Reviewing and Editing. Ana Mariatul Khiftiyah: Validation,

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