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Accelerated Fermentation of Pineapple Peel-Based Eco-Enzyme with *Ganoderma lucidum*: A Novel Bioconversion Strategy for Antidermatophytic

Hasna Ul Maritsa[1], Anggari Linda Destiana[2]*, Uni Baroroh Husnuddin[3], Putri Dwi Mulyani[4], Diah Tri Utami[5,] Aris Abdul Rasid[5]

[1]Department of Natural Sciences, Faculty of Science and Technology, Universitas Jambi
Jl. Jambi – Muara Bulian No.KM. 15, Mendalo Darat, Kec. Jambi Luar Kota, Kabupaten Muaro Jambi, Jambi
Jl. Letjen Suprapto No.33, Telanaipura, Kec. Telanaipura, Kota Jambi, Jambi 36361
[5]Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Jambi
Jl. Letjen Suprapto No.33, Telanaipura, Kec. Telanaipura, Kota Jambi, Jambi 36361
Email: anggari.linda.destiana@unja.ac.id **Corresponding Author*

Abstract

Pineapple peel waste, rich in lignocellulosic compounds and bioactive metabolites, remains underutilized despite its potential. Traditional eco-enzyme production from pineapple peels, molasses, and water (3:1:10 ratio) requires 90 days of spontaneous fermentation, limiting efficiency. This study investigates Ganoderma lucidum, a medicinal white-rot fungus, as a biocatalyst to accelerate fermentation and enhance antidermatophytic activity against *Trichophyton rubrum.* Fermentation substrates were prepared with or without *G. lucidum* mycelial plugs and monitored at 15 and 45 days for pH, aroma, color, and organoleptic changes. Eco-enzyme efficacy was assessed via agar well diffusion assays at concentrations of 20%, 40%, 60%, 80%, and 100%, measuring *T. rubrum* colony diameters.Results showed G. lucidum significantly hastened organic decomposition and secondary metabolite production. By day 45, inoculated samples reached pH 2.85 (vs. 3.2 in controls), exhibited faster aroma stabilization and darker coloration, and achieved complete inhibition (0.0 mm colony diameter) across all concentrations. At day 15, inhibition was already strong (0.7 mm vs. 90 mm in untreated controls). This approach reduces fermentation time by over 50%, yielding a potent antifungal agent from waste. It offers a sustainable bioconversion strategy for eco-enzyme production with therapeutic potential.

Keywords: Bioconversion ,eco-enzyme, *Ganoderma lucidum*, pineapple peel, *Trichophyton rubrum*

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Introduction

The management of agro-industrial waste can be used to obtain bioactive compounds, particularly from tropical fruits such as pineapple (*Ananas comosus*), has become a pressing issue in the development of sustainable bioproducts. The solid-state fermentation of pineapple waste process is a biotechnological alternative for the release of bioactive compounds. Pineapple waste can vary between 60–80% (depending on the variety) and corresponds to the crown, peel, leaves, core, and stems.(Casas-Rodríguez *et al*., 2024) (Hamzah *et al*., 2021) pineapple peel waste are also a potential source for obtaining tannins (Li *et al*., 2014)

Pineapple peel, a major byproduct of the fruit processing industry, contains valuable compounds like bromelain, pectinase, xylanase, flavonoids, and other phenolic compounds with beneficial properties (Casas-Rodríguez *et al*., 2024), (Meena *et al*., 2022), (Castro *et al*., 2025), (Ajayi *et al*., 2022). Bioconversion technologies have led to the creation of eco-enzymes—liquid fermentation products from organic waste mixed with sugar and water. These solutions are rich in active metabolites such as organic acids, enzymes, alcohols, esters, and phenolic compounds, which have antimicrobial, antioxidant, and anti-inflammatory effects (Li *et al*., 2014), (Meena *et al*., 2022), (Castro *et al*., 2025).

Eco-enzymes from pineapple waste have demonstrated strong antibacterial activity against pathogens like *Staphylococcus aureus*, *B. subtilis*, *Propionibacterium acnes*, *Escherichia coli*, and *S. epidermidis*. They also show antifungal activity against dermatophytes such as *Trichophyton rubrum*. These products have diverse applications, including liquid fertilizier, household cleaners, and

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wastewater treatment and sludge treatment processes (Ningrum *et al*., 2024), (Ningrum *et al*., 2024), (Zahira *et al*., 2023), (Pratama *et al*., 2025), (Das *et al*., 2024).

However, the conventional fermentation process for eco-enzyme production is time-consuming, typically requiring up to 90 days, which limits its scalability and industrial application (Ningrum *et al*., 2024),(Zahira *et al*., 2023),(Das *et al*., 2024). To address this limitation, incorporating *Ganoderma lucidum*, a white-rot Basidiomycota fungus, presents a promising strategy. *G. lucidum* is known for its ability to degrade lignocellulosic materials through ligninolytic enzymes (laccase, manganese peroxidase, lignin peroxidase) and for producing over 400 bioactive compounds, including triterpenoids, polysaccharides, and peptides (Sánchez-Hernández *et al*., 2023), (Wu *et al*., 2024).

Several studies have confirmed that extracts of *G. lucidum* exhibit potent antifungal effects against dermatophytes, including *T. rubrum*, *T. mentagrophytes*, and *Candida* species (Sánchez-Hernández *et al*., 2023), (Wu *et al*., 2024), (Castro *et al*., 2025) (Sułkowska-Ziaja *et al*., 2023), (Dhanaraju *et al*., 2022). Moreover, *G. lucidum* thrives in mildly acidic conditions and can enhance organic acid production during fermentation, leading to an environment unfavorable for pathogenic fungi (Ortega-Hernández *et al*., 2023), (Sułkowska-Ziaja *et al*., 2023). This combination of enzymatic breakdown, acidification, and metabolite production can significantly improve the antimicrobial efficacy and reduce the fermentation period of eco-enzyme production.

The decision to utilize shorter fermentation periods of 15 and 45 days represents a significant modification of traditional eco-enzyme production. Previous research by Mavani *et al***.** (2020) indicated that extending fermentation beyond three months provides no substantial benefit in antimicrobial efficacy, as hydrolytic enzyme maturation typically peaks at that stage.

In this study, we introduced a novel approach by incorporating *G. lucidum* to accelerate the accumulation of bioactive metabolites. We aimed to challenge the conventional 90-day requirement by demonstrating that high-level fungicidal activity against *T. rubrum* can be attained in as little as 45 days. This not only highlights the synergistic efficiency of the *G. lucidum* and eco-enzyme combination but also establishes a more time-effective protocol for biopesticide or antifungal applications.

Therefore, this study aims to integrate *G. lucidum* into pineapple peel fermentation to (i) accelerate the fermentation timeline, (ii) increase the production of antifungal metabolites, and (iii) evaluate the antifungal activity of the resulting eco-enzyme, particularly against *Trichophyton rubrum*. This research provides a new, sustainable way to transform agro-waste into valuable antifungal bioproducts.

Methods

Fresh pineapple peels were sourced from Tangkit Baru (Muaro Jambi, Indonesia). The fungal strain *Ganoderma lucidum* was obtained from the Indonesian Culture Collection (InaCC LIPI, Indonesia), while clinical isolates of *Trichophyton rubrum* were provided by the Faculty of Medicine, Universitas Indonesia. Analytical-grade molasses and sterile distilled water were used as fermentation substrates. Growth and assay media included Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) (Merck, Germany).

Culture Rejuvenation

Ganoderma lucidum and dermatophyte fungi *Tricophyton rubrum* were rejuvenated using Potato Dextrose Agar growth media and incubated at an incubation temperature of 28^{o}C for 7 days.

Eco-Enzyme Fermentation Setup

Eco-enzyme-based pineapple waste was made following the method of Ramadhani 2022 *et al* and (Mavani *et al*., 2020) modification which was made with a ratio of 3: 1: 10 to pineapple skin, molasses and distilled water. A total of 60 grams of pineapple skin, 20 grams of molasses and 200 ml of distilled water were mixed into a 250 ml Erlenmeyer flask. Next, the *Ganoderma lucidum* inducer was carried out. *Ganoderma lucidum* that has been rejuvenated and harvested at the age of 7 days, mushroom cultivation of 6 mycelium measuring 6 mm in diameter was put into 200 ml of eco enzymes-based pineapple waste. This fermentation solution was incubated for up to 15 days, and 45 days using a rotary shaker (140 rpm) (Ningrum *et al*., 2024), (Mavani *et al*., 2020).

Antidermatophytic Assay

The antifungal activity of eco enzyme filtrate was evaluated against *T. rubrum* using the pourplate method. Fungal spore suspension (10^{6}

CFU/mL) was prepared in sterile saline and spread evenly onto PDA plates. Furthermore, 1 mL of 15-day and 45-day-old eco enzyme fluid was spread evenly on the PDA that had been planted with *T. rubrum*. The culture was then incubated for 7 days. Antifungal activity was observed based on the presence or absence of *T. rubrum* growth.

Statistical Analysis

All tests were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). One-way Analysis of Variance (ANOVA) was performed to determine significant differences among treatments ($p < 0.05$). Pearson correlation analysis was performed using SPSS to assess differences and correlations between pH values and inhibition zone diameters.

Results and Discussion

Fermentation Kinetics

The accelerated reduction in pH observed in the *G. Lucidum*-fortified group is attributed to the metabolic activity of the fungus during the fermentation process. *G. Lucidum* is known to secrete various organic acids (such as oxalic acid and malic acid) as byproducts of carbohydrate metabolism to modify its environment for better nutrient absorption. When integrated into the eco-enzyme system, the fungus utilizes the available glucose and organic matter, intensifying the production of these acids, which subsequently drives the pH down to 2.9 more rapidly than in the standard eco-enzyme group.

Furthermore, the interaction between *G. lucidum* and the eco-enzyme medium acts as a metabolic trigger. The presence of complex organic substrates in the eco-enzyme induces the fungus to produce specific secondary metabolites as a survival and competitive strategy. Key metabolites, such as lectins and ganoderic acids, are synthesized more robustly under these conditions to inhibit the growth of competing microorganisms. The synergistic effect of a nutrient-rich substrate and a declining pH stimulates the biosynthetic pathways of *G. lucidum*, peaking between days 14–19, and leads to the accumulation of high-density antifungal compounds that effectively eliminate the colony diameter of *T. rubrum*

Changes in pH values during the incubation process are indicators of biochemical activities that occur during fermentation (Sharma *et al.*, 2020). This study observed pH values and pH decreases in two treatments: control without the addition of Ganoderma lucidum and ecoenzymes with the addition of G. lucidum, during the incubation period of 15 and 45 days.

The effect of pH (4.0, 5.0, and 6.0) on acidification and fermentation of fruit and vegetable wastes was investigated using batch and semi-continuous experiments under mesophilic condition. Results showed that fermentation types change with pH variation. The pH of acidification system containing fruit and vegetable wastes could automatically decrease to 3.0 ~ 4.0. At this pH range, a stable ethanol production was observed, at which ethanol-type fermentation was obtained. Based on the results, the fermentation types were classified into ethanol-type, mixed acid-type, propionic acid-type, and butyric acid-type fermentations, which occurred at pH 4.0 ~ 4.5, 4.5 ~ 5.0, 5.0 ~ 5.5, and 5.5 ~ 6.5,

The table shows the change in pH and the percentage decrease during the incubation process for two different treatments: the control and Ecoenzymes treated with G. lucidum, observed at 0, 15, and 45 days of incubation. At the beginning of incubation (day 0), the pH value in both treatments was the same, at 5.30. After 15 days of incubation, a decrease in pH was observed in both treatments, but the decrease in pH in the Ecoenzymes treated with *G. lucidum* was much greater. The pH in the control decreased to 4.71 (a decrease of 11.13%), while in the Ecoenzymes treated with *G. lucidum*, it dropped drastically to 2.99 (a decrease of 43.58%). After 45 days of incubation, the pH in the control decreased slightly to 4.67, for a total decrease of 11.89%. Meanwhile, in the Ecoenzymes treatment treated with G. lucidum, the pH continued to decrease until it reached 2.86, with a total decrease in pH of 46.23%.

Overall, these data indicate that the addition of *G. lucidum* to ecoenzymes resulted in a significantly greater decrease in pH compared to the control, both on day 15 and day 45 of incubation. This indicates that the activity of microorganisms or enzymes from G. lucidum plays a significant role in increasing the pH reduction process during incubation.

The curves in the graph show the change in pH values during the 45-day incubation process under two treatment conditions: Control (without *G. lucidum*), depicted by the dashed blue line; and Ecoenzymes + G. lucidum, depicted by the solid green line.

Key observations from the graph: On day 0, the initial pH value for both treatments was the

same, at around 5.30. In the control treatment, the pH decreased slightly from 5.30 to around 4.71 on day 15 and then stabilized at around 4.67 on day 45. This decrease in pH was relatively small and gradual. In the G. lucidum treatment, there was a drastic decrease in pH from day 0 to day 15, from 5.30 to around 2.99. After that, the pH decreased slightly again to around 2.86 on day 45, indicating a more significant decrease compared to the control.

A substantial decline in pH was observed in the *Ganoderma lucidum*-treated group, decreasing from 5.30 at day 0 to 2.85 at day 45—representing a 46.23% reduction. In contrast, the control group only exhibited an 11.89% decrease over the same period (Table 1 and Figure 1). This trend highlights enhanced acidogenesis and microbial metabolism facilitated by *G. lucidum*, likely due to the biosynthesis of organic acids such as acetic and citric acids, alongside triterpenoids such as ganoderic acid (Ortega-Hernández *et al*., 2023) (Nurlatifah *et al*., 2022),(Zheng *et al*., 2015).

Acidic environments with pH values below 3 are known to be detrimental to dermatophyte proliferation. Specifically, acid stress can impair ergosterol

biosynthesis and disrupt fungal cell membrane integrity, thereby inhibiting the growth of *T. rubrum* (Sánchez-Hernández *et al*., 2023),(Zheng *et al*., 2015). The pH-dependent suppression mechanism is further enhanced by the presence of ganoderic acid and other secondary metabolites produced by *G. lucidum.*

Previous reports have indicated that the production of triterpenoids and polysaccharides in *G. lucidum*

peaks during days 14 to 19 of cultivation, which may coincide with the enhanced bioactivity observed in this study (Ortega-Hernández *et al*., 2023),(Nurlatifah *et al*., 2022),(Naveen Kumar *et al*., 2017).

Therefore, the rapid pH decline not only reflects intensified fermentation but also correlates with increased antifungal potential. The acidic pH not only suppresses fungal growth but may also stabilize bioactive metabolites such as triterpenoids and polysaccharides (Sánchez-Hernández *et al*., 2023),(Nurlatifah *et al*., 2022),(Naveen Kumar *et al*., 2017). These findings confirm that acidification is a crucial indicator of fermentation progress and a predictor of antifungal bioactivity.

The curve illustrates a sharp decline in pH in the *G. lucidum*-inoculated group (from 5.30 to 2.85) versus a modest decrease in the control group (to 4.67), indicating accelerated acidogenesis and microbial metabolism induced by *G. lucidum*. The following is a graph of pH changes during the fermentation process: Blue color shows fermentation without *Ganoderma lucidum* (control) Green color shows fermentation with G. lucidum Conclusion from the graph: The addition of G. lucidum significantly accelerated the decrease in pH (up to pH 2.85 in 45 days), which reflects higher microbial activity and organic acid production.

Table 1. pH Dynamics During Eco-Enzyme Fermentation With and Without *G.lucidum*

Incubation Time (Days)	pH		pH Decrease (%)	
	Control	Ecoenzymes + *G.lucidum*	Control	Ecoenzymes + *G.lucidum*
0	5,30	5,30	-	-
15	4,71	2,99	11.13%	43.58%
45	4,67	2,86	11.89%	46.23%

The data show a significant decrease in pH in the *G. lucidum*-treated group compared to the control, indicating enhanced acidogenesis and and fermentation activity

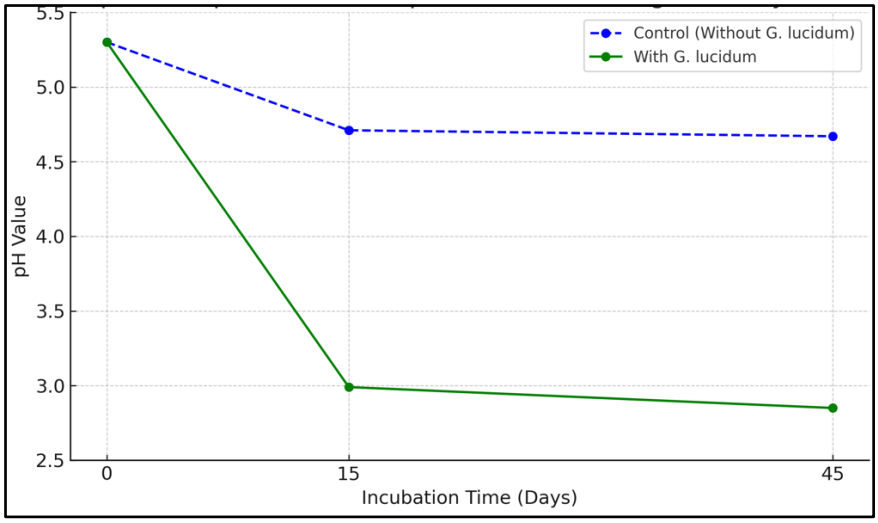

Figure 1. pH Reduction curve of eco-enzyme fermentation with and without *Ganoderma lucidum* over 45 days.

Antidermatophytic Assay

On day 15, both conventional and *G. lucidum*-modified eco-enzymes displayed partial inhibition (0.7 ± 0.1 mm) across concentrations. However, by day 45, complete inhibition was achieved, with zero fungal growth (tabl. 2 and Figure 2). ANOVA confirmed a significant increase in inhibition over time ($p < 0.001$), and Pearson analysis revealed a perfect negative correlation ($r = –1.0$) between pH and inhibition zone diameter.

These findings confirm a fungistatic-to-fungicidal transition supported by time-dependent bioactive accumulation, consistent with metabolite production kinetics reported in *G. lucidum* literature (Li *et al.*, 2014),(Sun *et al.*, 2016). Interestingly, varying eco-enzyme concentrations (20%–80%) had little effect on inhibition at day 15. However, extended incubation to 45 days resulted in total fungicidal activity regardless of concentration (Fig. 3), suggesting time-dependent metabolite accumulation plays a greater role than dosage.

The experimental data reveals that the combination of eco-enzyme and *G. lucidum* achieved complete mycelial inhibition of *T. rubrum* after 45 days of incubation. This finding is significant when compared to commercial antifungal standards. In clinical applications, synthetic agents such as Ketoconazole or Clotrimazole are commonly employed as positive controls due to their proven ability to disrupt fungal cell membrane integrity. The fact that the fortified eco-enzyme reached a level of 'Complete Inhibition' suggests that its bioactive components—specifically the low pH environment, fungal metabolites, and protein-polysaccharide complexes—provide a cumulative effect comparable to these synthetic fungicides. While commercial drugs are purified compounds, this organic eco-enzyme offers a sustainable and low-cost alternative with high efficacy at concentrated levels (20%–80%). It is important to note that this potent fungicidal effect was achieved through a 45-day incubation period of the eco-enzyme and *G. lucidum* mixture. This duration allowed for the optimal development of bioactive metabolites and the stabilization of the acidic environment, which were crucial in reaching the Complete Inhibition stage. These findings suggest that given sufficient interaction time, the fortified eco-enzyme can perform as effectively as synthetic standards in suppressing *T. rubrum* growth. This comparison underscores the potential of eco-enzymes not only as a waste management product but also as a potent bio-fungicidal agent. This duration allowed for the optimal development of bioactive metabolites and the stabilization of the acidic environment, which were crucial in reaching the complete inhibition stage. This is consistent with the biosynthetic timeline of *G. lucidum*'s antifungal compounds, which peak at 14–19 days (Ortega-Hernández *et al.*, 2023),(Nurlatifah *et al.*, 2022) with sustained activity enhanced under acidic conditions**.** Therefore, the extended incubation to 45 days ensured that these metabolites were present in sufficient concentrations to exert a total fungicidal effect. Therefore, the extended incubation to 45 days ensured that these metabolites were present in sufficient concentrations to exert a total fungicidal effect, effectively reducing the colony diameter to zero regardless of the initial dosage.

Table 2. Colony growth of *T. rubrum* in control vs. eco-enzyme-treated groups (mean ± SD; n = 3

Treatment Type	Incubation Time	Concentration	Diameter colony (mm)	Fungal Growth Observation
No treatment (Control)	15 days	0%	90	Full mycelial coverage
Eco-Enzyme	15 days	100%	1.9	Partial inhibition
	45 days	100%	0.7	Partial inhibition
Eco-Enzyme + *G. lucidum*	15 days	20%	0.7	Partial inhibition
		40%	0.7	Partial inhibition
		60%	0.7	Partial inhibition
		80%	0.7	Partial inhibition
	45 days	20%	0.0	Complete inhibition
		40%	0.0	Complete inhibition
		60%	0.0	Complete inhibition
		80%	0.0	Complete inhibition

Complete inhibition observed after 45 days in the *G. lucidum*-group strongly supports a synergistic effect between low pH, fungal metabolites, and protein-polysaccharide complexes. Statistical analysis (one-way ANOVA, $p < 0.001$) confirmed that eco-enzyme bioactivity significantly increases with incubation time. Pearson correlation analysis demonstrated a perfect inverse correlation ($r = 1.0$) between pH and zone of inhibition.

Visual Analysis: Colony diameter vs. Concentration

These results demonstrate that antifungal potency is driven more by incubation time than concentration. This supports the hypothesis that *G. lucidum*-derived metabolites accumulate over time, reaching a threshold sufficient to inhibit *T. rubrum*.

Table 2 demonstrates that *T. rubrum* shows robust growth in the absence of eco-enzyme treatment, full mycelial coverage the media. But, the growth began to be inhibited when treated with the addition of eco-enzymes. In 15 days incubation, *T. rubrum* grown on media with eco enzyme showed a colony diameter of 90 mm, in contras, the innovative eco-enzyme group shows a reduced growth diameter of 0.7 mm across all concentration variations. This indicates that the innovative eco-enzyme effectively inhibits the growth of *T. rubrum*. Furthermore, an incubation period of up to 45 days can enhance its effectiveness in inhibiting *T. rubrum*, as evidenced by the inability of the fungus to grow on agar medium.

This finding supports the potential of the eco-enzyme as an anti-dermatophyte treatment within a shorter harvest incubation period. (Sun *et al*., 2016) noted that *G. lucidum* contains lectin compounds that exhibit antifungal properties against various pathogenic fungi, including *T. rubrum*.

It is important to clarify that while the highly acidic environment at 45 days of fermentation may exceed the optimal pH range for *G. lucidum* biological growth, it plays a strategic role in the treatment's efficacy. The low pH serves to stabilize and enhance the potency of the secondary metabolites, such as lectins and protein-polysaccharide complexes, which were synthesized during the earlier stages of incubation when conditions were more favorable. This suggests that the 45-day result is not a product of active fungal growth, but a result of accumulated metabolite density and the synergistic effect of the acidic eco-enzyme medium. Consequently, the eco-enzyme acts as a vehicle that preserves these antifungal compounds, allowing them to effectively eliminate the colony diameter of *T. rubrum* even after the primary producer (*G. lucidum*) has ceased active proliferation. Additionally, it produces bioactive metabolites such as ganoderic acid, exopolysaccharides, and intracellular polysaccharides over periods of 14–19 days (Casas-Rodríguez *et al*., 2024; Li *et al*., 2014)

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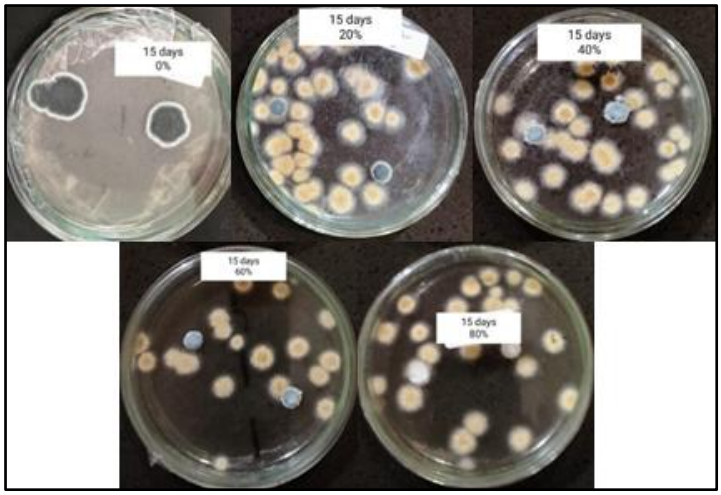

Figure 2. Effect of eco-enzyme and *G. lucidum* during 15 days of incubation on the growth of *T. rubrum*

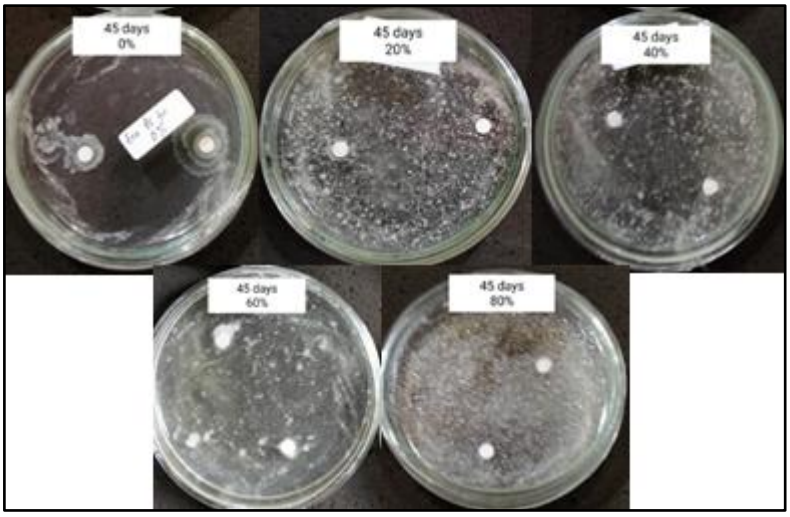

Figure 3. Effect of eco-enzyme and G. lucidum during 45 days of incubation on the growth of T. rubrum

G. lucidum functions optimally at a pH range of 3.5 to 7, with glucose reduction potentially elevating pH (Casas-Rodríguez *et al*., 2024). However, the pH decrease to 2.9, due to the innovative eco-enzyme fermentation system, likely hinders the survivability of *G. lucidum* and allows acidophilic bacteria to dominate. This phenomenon explains the absence of visible *G. lucidum* mycelium at day 45, as supported by Figures 2 and 3. The results reaffirm that *T. rubrum* proliferates actively in untreated media, exhibiting an inhibition zone diameter of 90 mm (full growth), which contrasts sharply with the 0.7 mm colony diameter observed in all concentrations of the innovative eco-enzyme. Moreover, no fungal growth was recorded on day 45, reinforcing the fungicidal nature of the eco-enzyme after extended incubation. Consequently, the 45-day result is not a product of active fungal proliferation, but a result of accumulated metabolite density and the synergistic effect of the acidic medium. The eco-enzyme acts as a vehicle that preserves these antifungal compounds, allowing them to effectively eliminate the colony diameter of *T. rubrum* even after the primary producer, *G. lucidum*, has ceased active growth due to the declining pH.

These observations suggest that the inclusion of *G. lucidum* facilitates shorter fermentation cycles (45 days) while significantly enhancing antifungal efficacy against dermatophytes. As reported by Saroci *et al*. (Meena *et al.*, 2022), conventional pineapple eco-enzyme fermentation without *G. lucidum* requires 90 days to achieve comparable inhibition. Microscopic observations confirmed G. lucidum's presence during early fermentation (day 15) as yellowish colonies, whereas by day 45, no visible *G. lucidum* or *T. rubrum* mycelium remained—supporting the hypothesis that acid-tolerant bacteria dominate at pH < 3 (Sánchez-Hernández *et al*., 2023). These findings highlight the eco-enzyme's potential as a

natural antifungal bioproduct, offering an accelerated alternative to conventional 90-day systems (Zahira *et al*., 2023),(Sułkowska-Ziaja *et al*., 2023).

Conclusion

This study demonstrates that the incorporation of *Ganoderma lucidum* significantly accelerates the fermentation of pineapple peel-based eco-enzymes while enhancing their antidermatophytic activity against Trichophyton rubrum. The optimized fermentation process achieved complete fungal inhibition within 45 days and induced greater acidification and organoleptic changes compared to the control. These findings highlight the dual role of *G. lucidum* as a fermentation accelerator and a bioactive enhancer, offering a promising model for sustainable bioconversion of agricultural waste into high-value antimicrobial products.

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