

In Vitro Antifungal Activity of Eco-Enzyme Derived from Horticultural Organic Waste Against *Candida albicans*

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Abstract

This study aimed to evaluate the antifungal activity of eco-enzyme (EE) derived from various horticultural organic wastes against *Candida albicans*. Two formulations were prepared: Bioz1 (pineapple peel, orange, papaya, starfruit, kuini, and mango) and Bioz2 (pineapple, orange, banana corm, noni, and kuini). Both were fermented for 100 days. The antifungal activity was tested in vitro using the disc diffusion method at concentrations of 1:0, 1:10, 1:50, and 1:100 (EE: distilled water). Results indicated that Bioz1 at 1:50 exhibited the highest inhibition zone (7.38 mm, medium category), while Bioz2 at 1:0 showed the strongest activity (8.16 mm, medium category). Dilution at 1:10 in Bioz2 was the most effective concentration for inhibiting fungal growth. These findings highlight the potential use of eco-enzyme as an environmentally friendly natural disinfectant against pathogenic fungi.

Keywords: eco-enzyme, *Candida albicans*, antifungal activity, organic_waste, horticulture

I. INTRODUCTION

Global agricultural production generates a vast amount of organic waste each year. In Indonesia alone, more than 65 million tons of fruit and vegetable waste are produced annually, much of which is underutilized or disposed improperly (FAO, 2022). This unmanaged waste not only contributes to greenhouse gas emissions but also poses sanitation and environmental challenges. At the same time, *Candida albicans*, an opportunistic fungal pathogen, remains one of the most common causes of fungal infections worldwide. According to the CDC (2020), invasive candidiasis affects more than 250,000 people annually, with mortality rates ranging from 20–40% depending on the severity of infection. The heavy reliance on synthetic antifungal drugs often leads to resistance, high treatment costs, and environmental toxicity.

Therefore, there is an urgent need to explore low-cost, eco-friendly alternatives that simultaneously address the problem of organic waste management and fungal control. Eco-enzyme (EE), introduced by studies have demonstrated its antibacterial properties (Mavani et al., 2020); (Ramadani et al., 2022) and its potential as a natural disinfectant (Rusdianasari et al., 2021). EE is a multifunctional product that can address the problem of large amounts of waste or organic waste, especially in households. Efforts are made to address and maintain the environment, which has positive benefits and can also be used to fertilize the soil. This activity method is carried out through lectures and

discussions, as well as hands-on practice. The results of this EE production training can help communities manage organic waste, both fruit and vegetable waste (Wasito, 2023). EE can function as a substitute for bioactivators currently used to improve soil structure and increase plant growth quality and resistance to *Trichoderma harzianum* and *Aspergillus niger* (Armaniar et al., 2023). Research on EE with applications in agriculture shows that the application of 75% topsoil + 25% compost with Ecoenzyme as a growing medium can stimulate the growth of shallots (Luta et al., 2022). The application of NPK from the fermentation of various types of plant waste at a dose of F3: 750 ml/liter of water/plot and the use of the Bima Brebes variety yielded the best results (Sitepu & Refnizuida, 2023). Ecoenzyme in shallots increases the productivity of shallots and soybeans (Lubis et al., 2022). EE can enhance the growth of annual plants (Warsito et al., 2023). EE can be used to eliminate unpleasant odors, such as those found in carpets, using EE from orange peel at a ratio of 1:30; and for toilet cleaning (preventing blockages and purifying waste systems), pure EE is used without dissolving it in water (Benny et al., 2023)

However, most research has focused on antibacterial or cleaning applications, with limited exploration of its antifungal properties, particularly against *C. albicans*. Only a handful of studies (Lubis et al., 2024) reported partial inhibition effects, leaving a gap in understanding its full potential,

optimum formulation, and effectiveness at different dilutions. Thus, the antifungal efficacy of eco-enzyme remains an underexplored field.

The antifungal properties of eco-enzyme are thought to arise from its rich composition of organic acids (citric, acetic, lactic acids), flavonoids, terpenoids, and phenolic compounds released during fermentation. These compounds can damage fungal cell membranes, disrupt enzyme activity, and inhibit spore germination (Benny et al., 2023). In addition, eco-enzyme's acidic pH creates an unfavorable environment for pathogenic fungi. Comparative studies of fruit-based extracts have highlighted pineapple peel, citrus fruits, and noni as sources of bioactive compounds with antimicrobial activities. This theoretical basis supports the hypothesis that eco-enzyme formulations combining diverse horticultural wastes could yield significant antifungal effects. This study was designed to evaluate the **in vitro antifungal activity of eco-enzyme formulations derived from mixed horticultural organic wastes against *Candida albicans***. Specifically, two formulations—Bioz1 (pineapple peel, orange, papaya, starfruit, kuini, mango) and Bioz2 (pineapple, orange, banana corm, noni, kuini)—were tested at different dilutions. The novelty of this research lies in identifying the most effective dilution levels for antifungal activity and comparing the performance of different fruit-based formulations. Unlike previous studies that primarily focused on antibacterial activity, this study contributes to filling the research gap on the antifungal potential of eco-enzyme, while promoting sustainable waste utilization. The findings provide both scientific insight and practical implications for developing eco-friendly antifungal agents from agricultural waste.

II. RESEARCH METHODOLOGY

Preparation of Eco-Enzyme:

Two formulations of EE were produced. Bioz1 consisted of pineapple peel, orange, papaya, starfruit, kuini, and mango, while Bioz2 consisted of pineapple, orange, banana corm, noni, and kuini. The raw materials were mixed with molasses and water in a ratio of 1:3:10 (molasses:organic waste:water) and fermented in sealed plastic containers for 100 days at ambient temperature (figure 1).



Figure 1. Eco-enzyme fermentation

Antifungal Assay:

The antifungal activity was tested *in vitro* using the disc diffusion method against *Candida albicans*. Sterile paper discs were soaked in EE at different concentrations (1:0, 1:10, 1:50, 1:100, 1:150) and placed on agar plates inoculated with *C. albicans*. After incubation, zones of inhibition were measured.

Data Analysis: The diameter of inhibition zones was measured in millimeters and categorized based on Davis & Stout (1971): weak (<5 mm), medium (5–10 mm), and strong (>10 mm) (Davis & Stout, 1971). Results were compared between Bioz1 and Bioz2 at different concentrations.

III. RESULTS AND DISCUSSION

Figure 2 illustrates the comparative antifungal activity of Bioz1 and Bioz2 at different concentrations

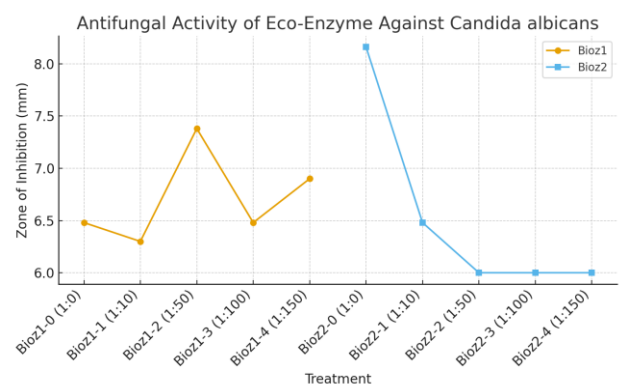


Figure 2. Antifungal activity of eco-enzyme against *C. albicans*

The results indicated that both Bioz1 and Bioz2 eco-enzymes possess antifungal properties against *Candida albicans*. Bioz1 showed the highest inhibition zone at 1:50 dilution (7.38 mm), while Bioz2 exhibited the strongest activity at the pure concentration 1:0 (8.16 mm). Interestingly, the 1:10 dilution of Bioz2 showed consistent inhibitory activity, suggesting that the bioactive compounds may be more effective at certain dilutions rather than in pure form.

These findings align with previous studies that reported antimicrobial properties of eco-enzymes derived from fruit peels. The bioactive compounds such as organic acids, flavonoids, terpenoids, and phenolics in eco-enzymes may disrupt fungal cell walls and inhibit spore germination. The medium inhibition zones observed in this study (5–10 mm) indicate potential as a complementary antifungal agent, although not as strong as synthetic antifungals.

Moreover, the differences between Bioz1 and Bioz2 may be attributed to the variation in raw materials. Pineapple and citrus fruits are rich in citric acid and phenolic compounds, while noni contains iridoids known for antimicrobial properties. The combination of these materials likely influenced the overall activity.

This study highlights the potential of eco-enzyme as a sustainable, low-cost, and environmentally friendly alternative for managing fungal pathogens. However, further research is required to optimize formulations, identify specific active compounds, and evaluate efficacy *in vivo*.

IV. CONCLUSION

Eco-enzyme derived from horticultural organic waste demonstrated antifungal activity against *Candida albicans*. The Bioz1 formulation (1:50 dilution) and Bioz2 formulation (1:0) exhibited the highest inhibitory effects. The dilution of 1:10 in Bioz2 proved the most effective concentration for fungal inhibition. This research suggests that eco-enzyme can be developed as an eco-friendly natural disinfectant for fungal control and sustainable waste utilization.

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