

ISSN: 2184-0261

# Antifungal activity of turmeric rhizome extract against *Ganoderma boninense*

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## ABSTRACT

Basal stem rot (BSR) caused by *Ganoderma boninense* threatens the oil palm industry, particularly in Southeast Asia. This study evaluated methanol extracts of turmeric rhizome (TRE) from four ecotypes (Bangka, Bandung, Palembang, and Surabaya) for antifungal activity against *G. boninense*. TRE showed strong inhibition, with a minimum inhibitory concentration (MIC) of 0.31% and IC<sub>50</sub> values between 0.93% and 1.92%, exhibiting dose-dependent effects. Treated fungal hyphae displayed morphological abnormalities with enhanced chitin and protein deposition. Elevated electrical conductivity in fungal culture media indicated membrane damage and leakage of intracellular contents. These findings highlight the potential of TRE as a sustainable alternative to chemical fungicides for BSR management.

**Received:** August 17, 2024  
**Revised:** November 23, 2024  
**Accepted:** November 23, 2024  
**Published:** December 02, 2024

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**KEYWORDS:** *Ganoderma boninense*, Turmeric rhizome extract, Antifungal properties

## INTRODUCTION

The oil palm industry faces significant challenges from basal stem rot (BSR), a devastating disease caused by the fungal pathogen *Ganoderma boninense*. This disease causes considerable economic losses, particularly in Southeast Asia, where oil palm cultivation is a key agricultural activity (Kamu *et al.*, 2020). Traditional approaches to managing BSR, such as the use of chemical fungicides, have demonstrated limited effectiveness and raised concerns about their environmental impact (Zakaria, 2023). Perennial allelopathic herbaceous plants have emerged as promising protective agents against chronic diseases like *Ganoderma* (Rahmadhani *et al.*, 2018, 2020; Suwandi *et al.*, 2022). Intercropping oil palm with ginger (*Zingiber officinale*) and Java turmeric (*Curcuma xanthorrhiza*) has been shown to reduce *G. boninense* infections (Munandar *et al.*, 2021; Suwandi *et al.*, 2023). The suppression of *Ganoderma* in oil palm intercropped with these rhizomatous herbs is likely attributed to the antifungal properties of their rhizome.

Turmeric (*Curcuma longa*), a perennial herb widely cultivated for its medicinal properties, has long been recognized for its potent antifungal activity. The bioactive compounds in turmeric, particularly curcuminoids, have been shown to inhibit

a range of fungal pathogens, including *G. boninense* (Akter *et al.*, 2018, 2019; Baharon *et al.*, 2019). These compounds are believed to exert their antifungal effects through various mechanisms, such as disrupting cell membrane integrity, inducing oxidative stress, and interfering with fungal metabolic processes (Kumar *et al.*, 2014).

While curcumin has been extensively studied for its antifungal properties, turmeric rhizomes contain a complex mixture of bioactive compounds that may contribute synergistically to their overall antifungal activity. To explore this further, this study aims to evaluate the antifungal efficacy of methanol extracts from different turmeric ecotypes against *G. boninense*. Specifically, we investigated the effects of these extracts on fungal growth, morphology, and cellular integrity.

This study explores the antifungal effects of TRE against *G. boninense* using a range of concentrations to determine minimum inhibitory concentration (MIC), IC<sub>50</sub> values, and their impact on fungal morphology and culture medium conductivity. These parameters provide insights into the mode of action of TRE and its potential for practical applications in oil palm disease management. The results underscore the importance of plant-based antifungal agents in developing sustainable solutions to combat agricultural pathogens.

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## MATERIALS AND METHODS

### Plant Extraction

Fresh turmeric rhizomes from different ecotypes, including Bangka (Bangka Island), Bandung (West Java), Palembang (South Sumatra), and Surabaya (East Java), were collected from local markets in their respective regions. The rhizomes were thoroughly washed first with tap water and then with distilled water, after which they were grated into fine pieces. A 100 g portion of the shredded rhizome was soaked in 200 mL of methanol and placed on a shaker at 150 rpm for 72 hours. The resulting supernatant was collected and filtered through the Whatman No. 1 filter paper. The filtered methanol extract, designated as a 100% concentration, was stored at 4 °C until further use.

### Antifungal Effect of Rhizome Extracts on *Ganoderma boninense* Growth

To assess the antifungal activity of the turmeric rhizome extracts (TRE), various concentrations (0.31%, 0.62%, 1.25%, 2.5%, and 5%) were incorporated into the MEA medium. Five-millimeter mycelial disks from a 5-day-old *G. boninense* culture were inoculated onto the treated and control (1% methanol) media. The plates were incubated at 28 °C in the dark. Colony diameter was measured daily until the control reached the edge of the plate. The radial growth rate (RGR) was calculated as the slope of the linear regression between colony radius and incubation time (Upasani *et al.*, 2016). Each treatment was replicated five

times. The percentage inhibition of *G. boninense* growth was calculated using the formula:

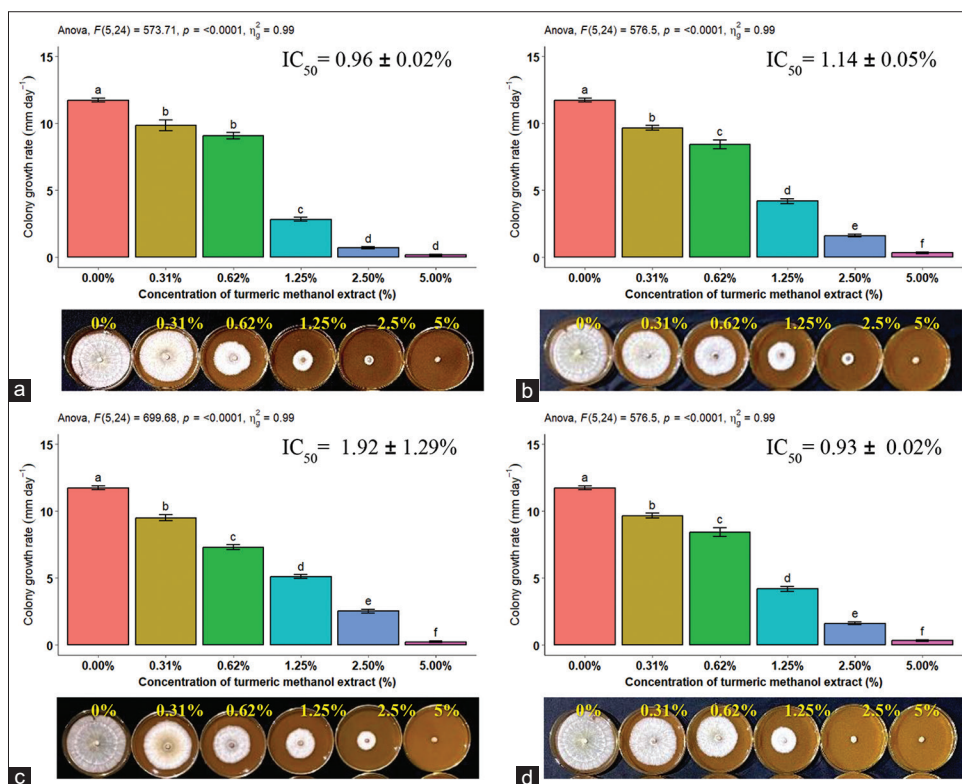
$$\text{Inhibition (\%)} = [(RGR \text{ of control} - RGR \text{ of treatment}) / RGR \text{ of control}] \times 100$$

### Microscopic Observations and Electrical Conductivity of *Ganoderma* Culture

Ten-day-old *G. boninense* cultures treated with various concentrations of turmeric rhizome extract (TRE) were collected from the colony margins and stained with 0.05% lactophenol cotton blue. The stained mycelia were examined under a light microscope at 400x magnification to observe morphological changes, including hyphal structure, branching patterns, and septation. Additionally, a 10-day-old *G. boninense* culture grown on MEA medium treated with rhizome extracts was gently macerated in 10 mL of distilled water. The electrical conductivity (EC) of the resulting solution was measured using a LAQUAtwin EC-22 conductivity meter. Each treatment was replicated five times, with three measurements taken for each replicate.

### Data Analysis

The effect of extract concentration on the RGR and EC within an ecotype of turmeric was compared using ANOVA and HSD post test. The  $IC_{50}$  value is the concentration of the inhibitor that causes a 50% reduction in the RGR calculated using DRC package. Data analysis was performed using Rstudio 2024.09.0.



**Figure 1:** Dose-dependent inhibition of *Ganoderma boninense* growth by turmeric rhizome methanol extracts from different ecotypes: a) Surabaya, b) Palembang, c) Bangka and d) Bandung

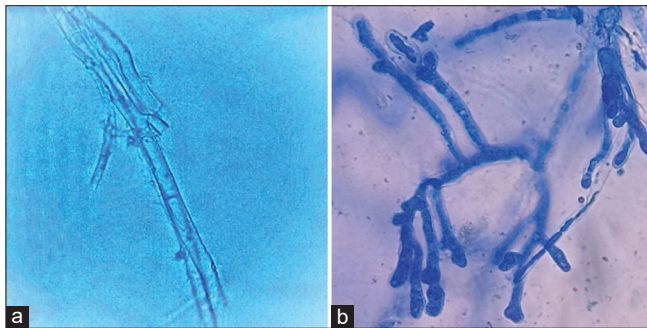
## RESULTS

### Antifungal Effect of Rhizome Extracts on *G. boninense* Growth

Methanol extracts of turmeric rhizome (TRE) exhibited antifungal properties against *G. boninense*. All tested ecotypes demonstrated inhibitory effects, with a minimum inhibitory concentration (MIC) of 0.31% (3.1 µL/mL). The maximum inhibition observed ranged from 97.3% to 99.1%. Increasing TRE concentrations led to a dose-dependent increase in inhibition, with IC<sub>50</sub> values of 0.96%, 1.44%, 1.92%, and 0.93% for Surabaya, Palembang, Bangka, and Bandung ecotypes, respectively (Figure 1).

### Microscopic Observations and Electrical Conductivity of *Ganoderma* Culture

Microscopic observations revealed morphological abnormalities in *G. boninense* hyphae treated with TRE. Compared to



**Figure 2:** Morphological abnormalities and enhanced lactophenol cotton blue staining in *Ganoderma boninense* hyphae treated with turmeric rhizome extract compared to untreated control. a) Untreated control and b) Treated with turmeric rhizome extract

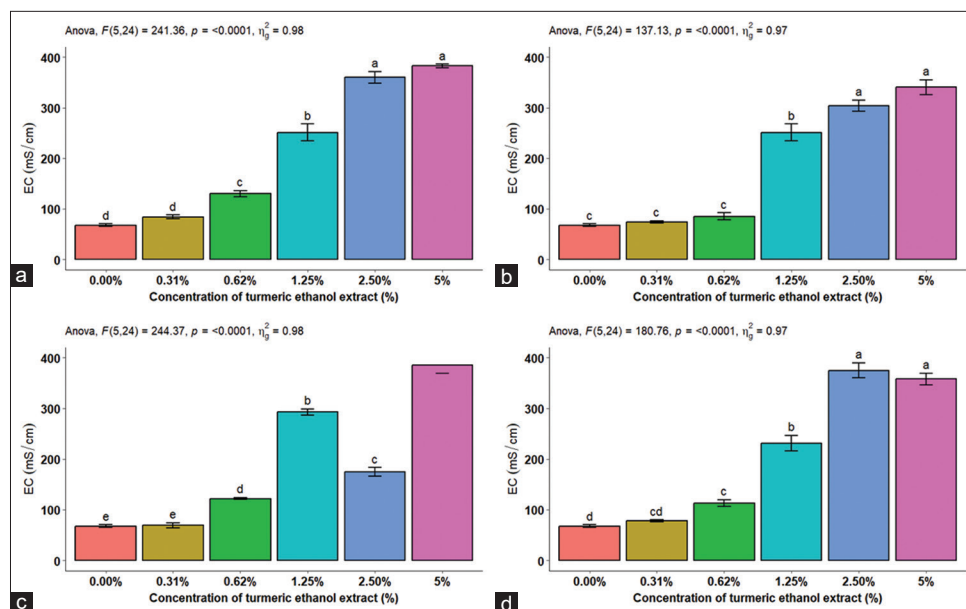
the control, treated hyphae were shorter, thicker, and more irregularly shaped. Increased branching, septation, and swollen tips were also observed. The increased intensity of lactophenol cotton blue staining indicated enhanced chitin and protein deposition in the hyphal wall. Overall, TRE treatment disrupted the normal growth pattern of the fungus (Figure 2).

The electrical conductivity (EC) of the *Ganoderma* culture media increased significantly with increasing TRE concentrations. A three-fold increase in EC was observed at a concentration of 1.25% for all TRE ecotypes (Figure 3). This marked increase in EC correlated well with the significant inhibition of *G. boninense* growth rate.

## DISCUSSION

The results of this study demonstrate the potent antifungal activity of turmeric rhizome extracts (TRE) against *G. boninense*, a major pathogen causing basal stem rot in oil palm. The observed inhibitory effects, particularly on mycelial growth and morphology, suggest that TRE interferes with the fungal growth process. The observed dose-dependent inhibition, with an MIC of 0.31% (3.1 µL/mL) and IC<sub>50</sub> values ranging from 0.93% to 1.92% (9.3 to 19.22 µL/mL), highlights the efficacy of TRE compared to other plant-derived antifungal agents. For instance, curcumin, a major bioactive compound in turmeric, exhibited MIC values ranging from 0.25 to 2 mg/mL against various *Candida* strains (Neelofar et al., 2011).

Microscopic analysis revealed that TRE treatment induced morphological abnormalities in *G. boninense* hyphae, including shortened, thickened, and irregularly shaped cells with increased branching and septation. These alterations, along with enhanced chitin and protein deposition in the hyphal wall, suggest that TRE interferes with essential cellular processes such as cell wall



**Figure 3:** Increase in electrical conductivity (EC) of *Ganoderma boninense* cultures with rising concentrations of turmeric rhizome methanol extracts from different ecotypes: a) Surabaya, b) Palembang, c) Bangka and d) Bandung

synthesis and membrane integrity. These findings are consistent with the known mechanisms of action of curcuminoids, which have been reported to induce apoptosis, chitin accumulation, and cell wall damage in fungi like *Candida albicans* (Kumar et al., 2014) and *Sporothrix schenckii* (Huang et al., 2016).

The significant increase in electrical conductivity (EC) of the culture medium following TRE treatment indicates membrane damage and leakage of intracellular contents in *G. boninense*. This observation aligns with previous studies demonstrating increased EC in fungal cultures treated with antifungal agents such as essential oils (e.g., geraniol and citral) against *Aspergillus flavus* and *Aspergillus ochraceus* (Tang et al., 2018). The correlation between electrical conductivity and membrane integrity of *G. boninense* is further supported by research indicating that increased conductivity often reflects cellular damage and compromised membrane function (Yuan et al., 2024). The release of intracellular ions and metabolites into the surrounding medium likely contributes to the elevated EC values.

The findings from this study provide strong evidence for the potential of turmeric rhizome extracts as a natural and sustainable alternative to synthetic fungicides for the management of basal stem rot disease in oil palm. Further research is needed to identify the specific bioactive compounds responsible for the antifungal activity and to optimize their application in agricultural settings. Additionally, studies on the long-term effects of TRE on soil microbial communities and plant health are essential to assess its environmental impact.

## CONCLUSIONS

This study demonstrates the potent antifungal activity of turmeric rhizome extracts (TRE) against *G. boninense*, the causal agent of basal stem rot (BSR) in oil palms. TRE from all tested ecotypes effectively inhibited fungal growth in a dose-dependent manner, with a minimum inhibitory concentration (MIC) of 0.31% and  $IC_{50}$  values ranging from 0.93% to 1.92%. Microscopic analysis revealed significant morphological disruptions in fungal hyphae, including increased branching, septation, and cell wall abnormalities, while elevated electrical conductivity (EC) indicated membrane damage and leakage of intracellular contents. These findings underscore the potential of TRE as a natural and sustainable alternative to synthetic fungicides for BSR management. Further research is needed to identify the bioactive compounds responsible for the antifungal effects, optimize their application in agricultural settings, and assess long-term impacts on soil health and the broader environment.

## ACKNOWLEDGEMENT

This work was financially supported by DIPA of Public Service Agency of Universitas Sriwijaya 2024 SP DIPA-023.17.2.677515/2024 in accordance with the Rector's Decree Number: 0016/UN9/SK.LP2M.PT/2024.

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