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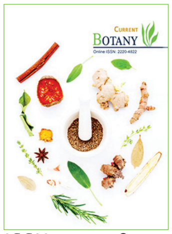

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Antifungal properties of cardamom (*Elettaria cardamomum*) root exudate against *Ganoderma boninense*

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ABSTRACT

Ganoderma boninense, the causal agent of basal stem rot (BSR), poses a significant threat to oil palm plantations, resulting in severe economic losses and limited effective control options. This study investigates the antifungal properties of root exudates from three cultivars of cardamom (*Elettaria cardamomum*) as a sustainable alternative for BSR management. Root exudates were collected from eight cardamom samples and tested in vitro against *G. boninense* at concentrations of 1.25%, 5%, and 20% using malt extract agar (MEA) medium. Fungal growth inhibition was assessed by measuring colony diameter and electrical conductivity (EC) to evaluate membrane integrity. All exudate samples exhibited varying degrees of antifungal activity, with maximum inhibition across the tested concentrations ranging from 13.4% to 39.5%. The degree of inhibition depended on both the sample and concentration of the exudate, with no clear correlation between cultivar origin and antifungal efficacy. Increased EC values in treated media suggest that the inhibition mechanism involves hyphal membrane damage. This study is the first to report the antifungal activity of cardamom root exudates against *G. boninense*, highlighting their potential as natural agents for sustainable disease management in oil palm plantations.

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INTRODUCTION

Basal stem rot (BSR), primarily caused by the pathogenic fungus *Ganoderma boninense*, poses a significant threat to the productivity and economic viability of oil palm plantations (Zakaria, 2023). BSR disease affects the xylem tissue of the trunk, disrupting the distribution of water and nutrients within the tree. Symptoms include yellowing and necrotic leaves, unopened spears, reduced crown size, and a drooping lower frond appearance (Kurihara *et al.*, 2022). Although this disease predominantly affects mature oil palms, particularly those over 25 years old, it has also been reported in younger palms and even seedlings, indicating a wider threat to oil palm cultivation (Flood *et al.*, 2022). BSR can lead to severe economic losses, with reported yield reductions of up to 68% of the attainable yields for infected palms (Kamu *et al.*, 2021).

Controlling *G. boninense* is particularly challenging due to its saprophytic lifestyle, its persistence in the environment, and its spread via wind, animals, and human activity (Khoo & Chong, 2023). Existing management strategies such as fungicide application, sanitation practices, and removal of infected plants are often ineffective, largely due to environmental concerns, side effects, and high costs (Zakaria, 2023). The difficulty is further compounded by the hemi-biotrophic infection strategy and robust survival mechanisms of the pathogen (Flood *et al.*, 2022).

Rhizomatous plants with allelopathic properties have shown potential as an alternative approach for managing *G. boninense* (Suwandi *et al.*, 2023). These plants release bioactive compounds through root exudates, decomposing plant material, volatile emissions from leaves, stems, and roots, as well as surface wash-off. Such exudates can influence the growth and survival of soilborne pathogens (Munandar *et al.*, 2021; Suwandi *et al.*, 2023, 2024; Karlina *et al.*, 2024). The suppression of *G. boninense* in oil palms intercropped with rhizomatous plants may be attributed to the antifungal effects of their root exudates. Root exudates contain various organic compounds, including carbohydrates, organic acids, amino acids, and secondary metabolites, which can modulate soil microbial communities and influence phytopathogenic fungi. These interactions may be either inhibitory or stimulatory and occur through direct or indirect mechanisms. Allelopathic effects are typically selective, affecting only specific target organisms (Hussien & Abbas, 2023; Sun *et al.*, 2023; Wu *et al.*, 2025).

Cardamom (*Elettaria* spp.), a rhizomatous plant belonging to the Zingiberaceae family is well known for its essential oils, which are rich in bioactive compounds with antioxidant, antitumor, and antibacterial properties (Dinata *et al.*, 2024). A study by Khuat *et al.* (2022) showed that seed extract from *E. cardamomum* inhibited the mycelial growth of *Fusarium oxysporum* by up to 17.4%, with efficacy increasing at higher concentrations. *Elettaria cardamomum* essential oil (EO) contains many bioactive compounds, especially α-terpinyl acetate (56.5%), limonene (12.6%), and mentha-2,4(8)-diene (7.65%). These compounds have antifungal properties against different clinical *Candida* strains, significantly lowering virulence factors like biofilm formation and exoenzyme production (Noumi *et al.*, 2022). While those previous studies have reported the antifungal effects of cardamom extracts or essential oils derived from seeds and other plant parts, the biological activity of root exudates from cardamom plants against *G. boninense* has not been explored. Therefore, this study aims to evaluate the antifungal activity of root exudates from several cardamom cultivars against *G. boninense*.

MATERIALS AND METHODS

Collection of Cardamom Rhizomes and Preparation of Root Exudates

Three cardamom cultivars were used in this study: a hybrid cultivar (LGH) from Pangandaran, West Java; a local cultivar (LGL) from Pangandaran, West Java; and another hybrid cultivar (LGHs) from Tangerang, West Java. The rhizomes were planted in 10-liter pots filled with compost. Plants were fertilized every two weeks with liquid fermented cow manure compost. Three-month-old plants were carefully uprooted, and their roots were washed under running water to remove soil. Six plants, with a combined root and rhizome weight of approximately 250 grams, were soaked in 500 mL of distilled water. Continuous aeration was provided using an aquarium aerator to optimize the release of root exudates.

Following soaking, the solution was filtered in three stages: through an aquarium filter to remove coarse particles, through a Whatman No. 1 filter paper to remove finer particles, and finally through a 0.22 μm PVDF filter. The resulting solution was considered a 100% root exudate concentration and stored at 4 °C until use. Eight root exudate samples were prepared: five from the Pangandaran hybrid cultivar (LGH1–LGH5), two from the Tangerang hybrid cultivar (LGHs6 and LGHs7), and one from the Pangandaran local cultivar (LGL8).

Antifungal Effect of Root Exudates on *Ganoderma boninense* Growth

Antifungal activity was tested *in vitro* at exudate concentrations of 0%, 1.25%, 5%, and 20%. The test medium consisted of Malt Extract Agar (MEA), prepared with 20 g malt extract and 20 g agar per liter. The medium was sterilized in an autoclave at 121 °C and 1 atm for 20 minutes. Each exudate concentration was mixed into the medium. The media were homogenized, poured into dishes, and allowed to solidify. The control (0%) consisted of MEA with distilled water. Each treatment was replicated in five Petri dishes.

A 5 mm plug of *G. boninense* (5-day-old culture) was inoculated onto each plate and incubated at room temperature (27 °C) in the dark. Antifungal activity was evaluated based on colony growth inhibition and electrical conductivity (EC) of the media. Colony diameters were measured daily for 5-7 days until control colonies fully covered the plate. The growth rate (mm/day) was determined using linear regression between colony diameter and observation days (Upasani *et al.*, 2016). Inhibition was calculated as:

$$\text{Inhibition (\%)} = [(\text{Control Growth} - \text{Treatment Growth}) / \text{Control Growth}] \times 100$$

EC was measured using a Horiba Laquatwin EC22 meter. The media were divided into four parts, each mixed with 10 mL of distilled water and soaked for 60 minutes. The soaking solution was then applied to the EC sensor, and readings were recorded once stable.

Data Analysis

Data was analyzed using analysis of variance (ANOVA) in Excel and R (version 4.1.2). If significant differences were observed, the means were further compared using the Honestly Significant Difference (HSD) test at a 5% significance level.

RESULTS

Colony Growth Inhibition

Cardamom root exudates from three cultivars (LGH1-LGH5, LGHs6-LGHs7, and LGL8) inhibited *G. boninense* colony growth at concentrations of 1.25%-20%. The morphology of treated colonies did not differ from controls. Aerial mycelium was similar in both treated and control colonies, with no observable discoloration (Figure 1). Inhibition patterns varied depending on sample and concentration. Average inhibition ranged from 3.2% (LGH3) to 24.3% (LGH2). LGH1 and LGH5 exudates only inhibited growth at 5%. LGH4 and LGHs6 inhibited growth at both 1.25% and 20%, with stronger inhibition at 20%. LGHs7 inhibited at 1.25% and 5%. LGH2 inhibited growth at 5% and 20%. Interestingly, LGH3 inhibited growth by 14.8% at 1.25% but promoted *G. boninense* growth by 7.8% at 20%.

Maximum growth inhibition across concentrations of 1.25-20% ranging from 13.4% (LGH1 5%) to 39.5% (LGH4 20%). Three samples of exudates (LGH1 5%, LGH3 1.25%, LGHs6 1.25%) showed less fungicidal activity by causing a maximum inhibition less than 20%. The other five samples (LGH2, LGH4, LGH5,

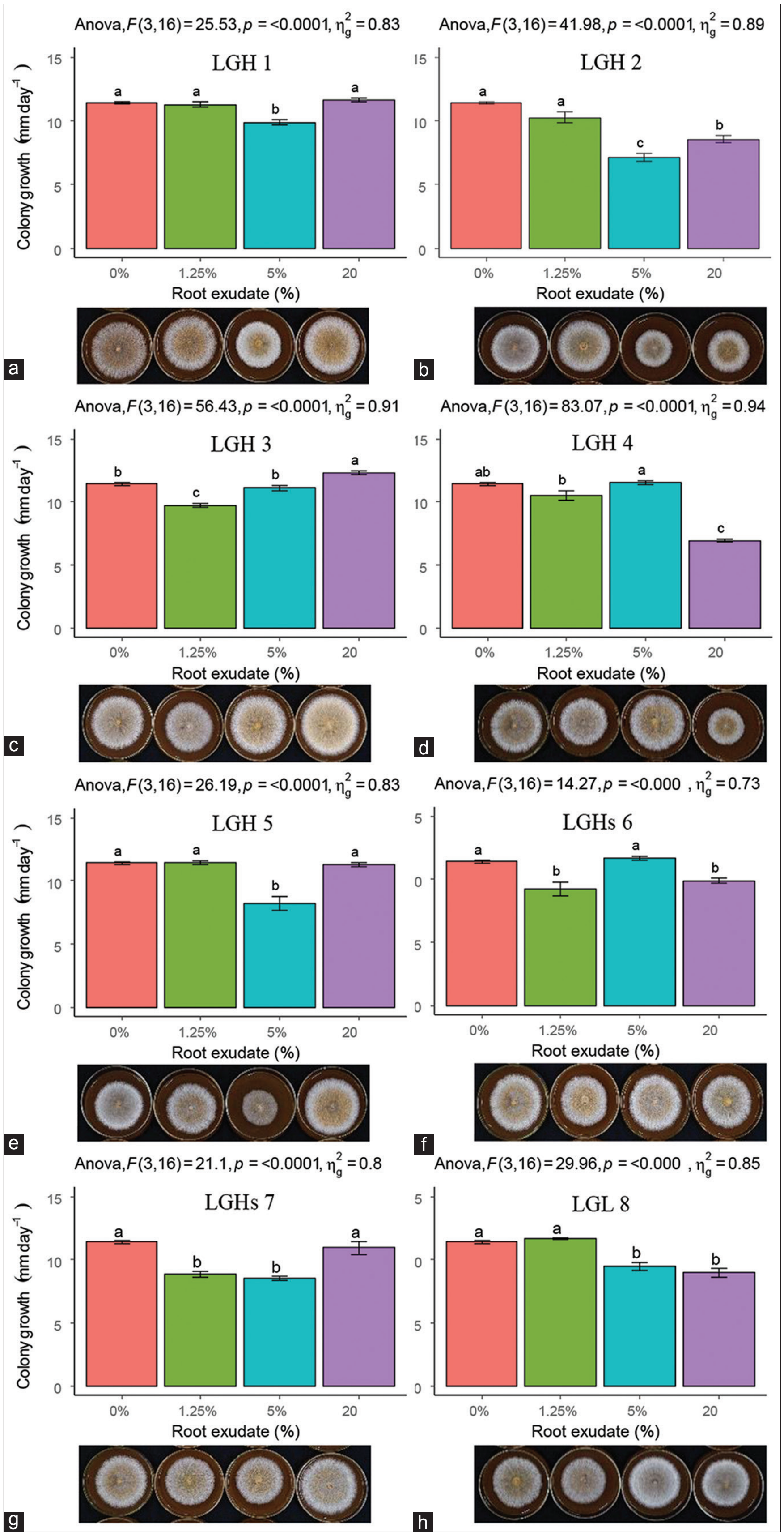

Figure 1: (a-h) Growth of *Ganoderma boninense* colony on MEA supplemented with cardamom root exudate at concentration 0% (control), 1.25, 5, and 20%

LGHs7, LGL8) produced a higher fungicidal property with a maximum inhibition more than 20%. Antifungal activity of the root exudate samples varied between samples within the same variety. There was no association between the origin of the variety and inhibition activity.

Electrical Conductivity of Media

EC measurements revealed electrolyte leakage from fungal hyphae due to exudate treatment. Increases in EC indicate elevated ion concentrations caused by cell membrane damage.

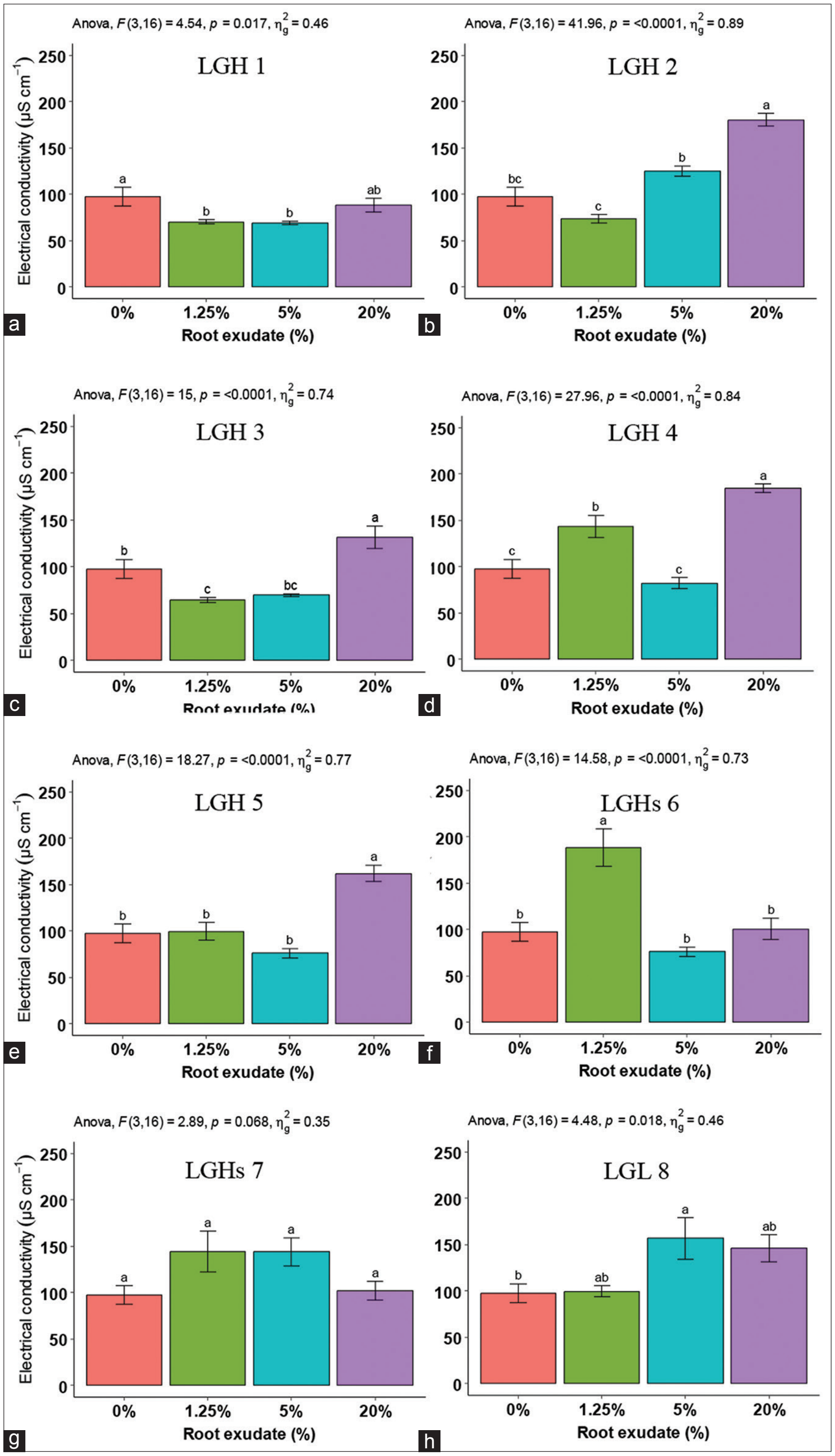

Figure 2: (a-h) Electrical conductivity of *Ganoderma boninense* colony on MEA supplemented with cardamom root exudate at concentration 0% (control), 1.25, 5, and 20%

EC values increased by more than 25% in some treatments (Figure 2), for example: LGH2: EC increased by 22.1% (5%) and 46.1% (20%); LGH4: 32.2% (1.25%) and 47.4% (20%); LGHs6: 48.4% (1.25%) and 3.4% (20%); LGHs7: 32.6% (1.25%) and 32.4% (5%); LGL8: 38.1% (5%) and 33.5% (20%) (Figure 2). These EC increases correlated with treatments that significantly inhibited *G. boninense* colony growth.

DISCUSSION

Cardamom root exudates at concentrations of 1.25%-20% inhibited the colony growth of *G. boninense*, with varying levels of effectiveness depending on both the sample and the concentration used. Interestingly, higher concentrations did not consistently result in stronger inhibition. In some cases (LGH1, LGH3, LGH5, LGHs6, LGHs7), the 20% concentration even reduced or eliminated antifungal activity. The variation in antifungal effectiveness was observed not only among different cultivars but also among samples from the same cultivar, indicating that antifungal activity may be influenced by individual plant characteristics rather than solely by cultivar origin. No clear association was found between cultivar origin and inhibitory activity. This study is the first to report antifungal activity of cardamom (*E. cardamomum*) root exudates against *G. boninense*. Similar antifungal effects have been previously reported from the root exudates of other rhizomatous plants, such as galangal and turmeric (Rahmadhani *et al.*, 2018), and taro (*Xanthosoma sagittifolium*) (Suwandi *et al.*, 2022).

This study is the first to report the antifungal activity of cardamom root exudates against *G. boninense*. Most prior antifungal studies on cardamom have focused on extracts or essential oils derived from seeds or other plant parts. For instance, acetone extracts of *E. cardamomum* seeds have been found to inhibit the colony growth of *Candida albicans* (Younus, 2023). The essential oil from *E. cardamomum*, rich in α-terpinyl acetate, limonene, and mentha-2,4(8)-diene, exhibited antifungal activity against multiple *C. albicans* strains (Noumi *et al.*, 2022). However, compared to the essential oil from other plants, the essential oil from *E. cardamomum* that contained α-Terpineol acetate (43.7%) and Cineol (33.10%) exhibited lower antifungal activity against *Candida* species. Several studies have shown that the essential oil from *E. cardamomum* had significant antibacterial effects rather than antifungal (Teneva *et al.*, 2016; Abdullah *et al.*, 2017).

The significant increases in electrical conductivity (EC) observed in media treated with inhibitory exudates suggest damage to the cell membranes of *G. boninense* hyphae. This finding supports the hypothesis that growth inhibition is caused by hyphal membrane disruption, a common mechanism of antifungal action in plant-derived products. For example, rosemary essential oil has been reported to inhibit *Colletotrichum gloeosporioides* by compromising membrane integrity, as indicated by a more than twofold increase in EC (Yuan *et al.*, 2024). Similarly, citronellal was shown to inhibit *Penicillium digitatum* by disrupting ergosterol biosynthesis, leading to membrane leakage and fungal cell death (OuYang *et al.*, 2021). These findings highlight the potential of cardamom root exudates as a natural antifungal agent for managing *G. boninense* in oil palm plantations. Further investigation is warranted to identify the specific bioactive compounds responsible for this activity and to evaluate their effectiveness under field conditions.

CONCLUSIONS

Cardamom root exudates have antifungal properties that effectively inhibit the growth of *G. boninense*, with the level of inhibition varying by sample and concentration. The inhibition mechanism appears to involve membrane damage in fungal hyphae, as evidenced by increased electrical conductivity in treated media. These results suggest that cardamom root exudates could serve as a natural antifungal agent for managing *G. boninense* in oil palm plantations.

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