



In Vitro Efficacy of Gingerol-Containing Emprit Ginger (*Zingiber officinale* var. *amarum*) Extract Against *Fusarium oxysporum*

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Received: June 2026; Revised: July 2026; Accepted: August 2026; Published: September 2026

Abstract: This study aimed to evaluate the antifungal activity of emprit ginger (*Zingiber officinale* var. *amarum*) extract containing 6-gingerol against *Fusarium oxysporum* under *in vitro* conditions and to determine the concentration with the highest inhibitory effect. The experiment was conducted using a completely randomized design with six treatments consisting of a negative control, emprit ginger extract at concentrations of 0.5%, 1.5%, 2.5%, and 5%, and a synthetic fungicide as the positive control, with five replications for each treatment. The 6-gingerol content was quantified using High-Performance Liquid Chromatography (HPLC), while antifungal activity was assessed using the poisoned food technique on Potato Dextrose Agar (PDA) medium. Data were analyzed using analysis of variance (ANOVA), followed by the Honestly Significant Difference (HSD) test at a 5% significance level. The results revealed that emprit ginger extract contained 6-gingerol at 8.082 µg/mL. Growth inhibition of *F. oxysporum* increased with increasing extract concentrations, reaching the highest inhibition (56.49%) at 5% extract concentration. Under the tested *in vitro* conditions, this treatment showed higher inhibition than the positive control (43.26%). The extract also induced morphological alterations, including reduced mycelial density, pigmentation changes, and restricted colony expansion. These findings indicate that emprit ginger extract containing 6-gingerol has potential as a botanical biofungicide candidate for suppressing *F. oxysporum*. Further validation through *in vivo* and field-scale studies is required before practical application.

Keywords: Emprit ginger; 6-gingerol; *Fusarium oxysporum*; botanical biofungicide; antifungal activity

How to Cite: Maharani, J., Jayanti, R. M., Hutagalung, S., & Herawati, M. M. (2026). In Vitro Efficacy of Gingerol-Containing Emprit Ginger (*Zingiber officinale* var. *amarum*) Extract Against *Fusarium oxysporum*. *Bioscientist: Jurnal Ilmiah Biologi*, 14(3), 1190–1202. <https://doi.org/10.33394/bioscientist.v14i3.21261>



<https://doi.org/10.33394/bioscientist.v14i3.21261>

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INTRODUCTION

Fusarium oxysporum is one of the most important soil-borne fungal pathogens responsible for Fusarium wilt disease in a wide range of economically important crops. This pathogen exhibits a broad host range, infecting various agricultural commodities, including tomato, chili pepper, potato, common bean, yardlong bean, banana, and vanilla (Pinaria et al., 2020). The successful establishment and persistence of *F. oxysporum* in agricultural systems are mainly attributed to its ability to produce long-lived survival structures, such as chlamydospores, which enable the pathogen to remain viable in soil for extended periods even in the absence of host plants (Manang et al., 2024; Michielse & Rep, 2009). Furthermore, the pathogen can be disseminated through contaminated soil, irrigation water, agricultural tools, and infected plant residues, facilitating its rapid spread within cultivation areas (Heriyanto, 2019). These biological characteristics make Fusarium wilt one of the most difficult diseases to manage in crop production systems. Depending on host susceptibility, pathogen population, and environmental conditions, Fusarium wilt can cause severe yield reduction and, in some cases, complete crop failure (Yanti et al., 2018).

Current management strategies for *F. oxysporum* largely depend on the application of synthetic fungicides. Several active compounds, including mancozeb, prochloraz manganese chloride complex, and combinations of trifloxystrobin and tebuconazole, have been used to suppress fungal diseases in agricultural systems (Millenia, 2022). Although synthetic fungicides provide rapid and effective disease suppression, their intensive and repeated application may result in several undesirable consequences, including the development of fungicide-resistant pathogen populations, environmental contamination, chemical residue accumulation, and adverse effects on beneficial and non-target organisms (Singkoh & Katili, 2019; Lucas et al., 2015). Therefore, the development of alternative disease management strategies that are effective, environmentally sustainable, and compatible with integrated pest management programs is increasingly important.

Plant-derived biopesticides represent a promising alternative for reducing dependence on synthetic fungicides. Botanical pesticides contain diverse secondary metabolites, including phenolics, flavonoids, terpenoids, alkaloids, and other bioactive compounds, which may exhibit antifungal activity through multiple mechanisms of action (Lengai et al., 2020). Compared with synthetic pesticides, plant-based products generally originate from renewable resources, are more biodegradable, and may pose lower risks of persistent environmental residues (Lengai et al., 2020). Indonesia, as one of the countries with high plant biodiversity, provides numerous potential sources of botanical pesticides, including soursop, lemongrass, betel, papaya, neem, and ginger (Yusup et al., 2021). However, the effectiveness of botanical extracts depends strongly on plant species, bioactive compound composition, extraction method, and application concentration. Therefore, chemical characterization and biological evaluation are essential steps for developing reliable plant-based antifungal products.

Among potential botanical resources, ginger (*Zingiber officinale* Roscoe), particularly emprit ginger (*Zingiber officinale* var. *amarum*), has attracted considerable attention due to its rich content of biologically active secondary metabolites. Emprit ginger is widely cultivated in Indonesia, easily accessible, and economically valuable. The rhizome contains several bioactive compounds, particularly phenolic compounds and terpenoids, with 6-gingerol recognized as one of the major bioactive constituents associated with antioxidant, antimicrobial, and antifungal activities (Ahnafani et al., 2024). Previous studies have demonstrated that ginger-derived compounds can inhibit fungal development through several mechanisms, including disruption of cell membrane integrity, alteration of cellular metabolism, and suppression of mycelial growth (Radice et al., 2022). Therefore, quantification of 6-gingerol is an important parameter for characterizing ginger extract quality and supporting the evaluation of its antifungal potential.

The antifungal potential of plant extracts against *Fusarium* species has been widely investigated. Several studies have demonstrated that botanical extracts containing phenolic compounds, flavonoids, and terpenoids can inhibit fungal mycelial growth and spore germination (Lengai et al., 2020). For example, extracts of *Piper aduncum* have been reported to suppress radial mycelial growth of *F. oxysporum*, with their antifungal activity associated with specific bioactive compounds identified in the extract (Cárdenas-Laverde et al., 2021). Similarly, *Origanum elongatum* extracts showed strong inhibitory effects against *F. oxysporum* mycelial growth and spore germination, while also reducing *Fusarium* wilt severity under plant-based evaluations (Hari et al., 2024). Extracts from *Rosmarinus officinalis* have also demonstrated antifungal activity against *F. oxysporum* under both *in vitro* and *in vivo* conditions (Li et al., 2026).

Ginger-derived products have also shown promising antifungal properties against several *Fusarium* species. Yamamoto-Ribeiro et al. (2013) reported that ginger essential oil inhibited the growth of *Fusarium verticillioides* and reduced fumonisin production. Similarly, Romoli et al. (2022) demonstrated that ginger essential oil suppressed mycelial growth and mycotoxin production of *Fusarium graminearum*. Ginger extract has also been reported to inhibit the mycelial growth of *F. oxysporum* (Prasath et al., 2023). These findings suggest that ginger represents a promising source of antifungal compounds; however, antifungal effectiveness may vary depending on ginger variety, extraction procedure, chemical composition, and application concentration.

Despite increasing evidence regarding the antifungal activity of ginger against *Fusarium* species, several knowledge gaps remain. Most previous studies have evaluated ginger extracts or essential oils based primarily on biological activity without directly linking antifungal performance with quantified levels of specific bioactive compounds. Information regarding the antifungal efficacy of emprit ginger (*Zingiber officinale* var. *amarum*) extract characterized by its 6-gingerol content against *Fusarium oxysporum* remains limited. In addition, comparative evaluations involving different extract concentrations, synthetic fungicide controls, and morphological characterization of fungal colonies under *in vitro* conditions are still insufficient.

Therefore, this study aimed to quantify the 6-gingerol content of emprit ginger extract using high-performance liquid chromatography (HPLC) and evaluate the antifungal efficacy of different extract concentrations against *F. oxysporum* under *in vitro* conditions. We hypothesized that increasing concentrations of emprit ginger extract containing 6-gingerol would progressively inhibit the growth of *F. oxysporum*. The novelty of this study lies in integrating chemical characterization of 6-gingerol with biological evaluation of antifungal activity, including concentration-response assessment, comparison with synthetic fungicide (Previcur-N) as a positive control, and morphological observation of fungal colony development. This integrated approach provides a more comprehensive evaluation of the potential of emprit ginger extract as a botanical fungicide for sustainable management of *Fusarium* wilt disease.

METHOD

Experimental Design and Research Site

The study was conducted from August 2025 to March 2026. Emprit ginger extraction was carried out at the Chemistry Laboratory, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana. The antifungal efficacy evaluation of emprit ginger extract against *Fusarium oxysporum* was performed at the Plant Protection Laboratory, Faculty of Agriculture and Business, Universitas Kristen Satya Wacana.

The experiment was conducted using an experimental method with a Completely Randomized Design (CRD) consisting of a single treatment factor, namely the concentration of emprit ginger extract containing gingerol compounds. The experiment was designed to evaluate the effect of different extract concentrations and controls on the growth response of *Fusarium oxysporum*. Quantitative data were obtained based on fungal colony diameter measurements, which were subsequently analyzed statistically to determine the antifungal efficacy of emprit ginger extract against *Fusarium oxysporum*.

Emprit Ginger Extraction

Fresh emprit ginger rhizomes were washed thoroughly with running water and cut into small pieces. The samples were dried in an oven at 49–55°C for two days until

dried simplicia were obtained. The dried samples were subsequently ground into powder using a grinder (Guo et al., 2017).

A total of 10 g of ginger powder was extracted by maceration using 50 mL of 60% ethanol for 24 h at room temperature (Srikandi et al., 2020). After maceration, the filtrate was separated from the residue, and the solvent was removed using a rotary evaporator at 40°C until a concentrated extract was obtained. The resulting extract was used for the determination of 6-gingerol content and antifungal activity evaluation.

Determination of 6-Gingerol Content Using High-Performance Liquid Chromatography (HPLC)

The 6-gingerol content of emprit ginger extract was determined using High-Performance Liquid Chromatography (HPLC). The extract was weighed according to the analytical requirements and dissolved in methanol in a volumetric flask. The solution was sonicated for 10 min until homogeneous, diluted to a predetermined volume, and filtered using a 0.45 µm membrane filter before HPLC analysis.

A 6-gingerol standard solution was prepared in methanol to obtain a stock solution with a concentration of 1000 mg/L. The stock solution was further diluted as required to construct an external calibration curve for quantification.

The HPLC analysis was performed using a Knauer HPLC system equipped with a C18 column (150 × 4.6 mm; 5 µm particle size) with detection at 254 nm (Hussein & Joo, 2018). Identification of 6-gingerol was performed by comparing the retention time of the sample peak with that of the standard compound. Quantification was conducted based on the peak area obtained from the external standard calibration curve.

In Vitro Antifungal Activity Assay of Emprit Ginger Extract against *Fusarium oxysporum*

The antifungal activity of emprit ginger extract against *Fusarium oxysporum* was evaluated using the poisoned food technique (toxic media method) by incorporating the extract into Potato Dextrose Agar (PDA) medium before solidification (Kalhor et al., 2022).

For each treatment, 50 mL of sterile PDA medium was prepared. The PDA medium was cooled to approximately 50–55°C before the addition of emprit ginger extract to maintain the liquid state of the medium and minimize possible degradation of active compounds due to high temperature. The extract was incorporated into the PDA medium according to the designated treatment concentration while maintaining a final volume of 50 mL. The treatment arrangement for the antifungal assay is presented in Table 1.

Table 1. Treatment arrangement for the efficacy evaluation of emprit ginger extract against *Fusarium oxysporum*

Treatment	Media composition	Description
K–	50 mL PDA	Negative control (without extract and fungicide)
P1	49.5 mL PDA + 0.5 mL emprit ginger extract	Emprit ginger extract treatment
P2	48.5 mL PDA + 1.5 mL emprit ginger extract	Emprit ginger extract treatment
P3	47.5 mL PDA + 2.5 mL emprit ginger extract	Emprit ginger extract treatment
P4	45.0 mL PDA + 5.0 mL emprit ginger extract	Emprit ginger extract treatment
K+	40 mL PDA + 10 mL Previcur-N solution	Positive control

Note: The Previcur-N solution was prepared by dissolving 2 g of Previcur-N in 10 mL of distilled water.

A pure culture of *Fusarium oxysporum* was prepared, and a fungal plug with a diameter of 0.8 cm was inoculated onto PDA medium supplemented with emprit ginger extract according to each treatment. The treatments consisted of negative control

(0%), emprit ginger extract concentrations of 0.5%, 1.5%, 2.5%, and 5%, as well as a positive control using synthetic fungicide Previcur-N (Bayer) containing propamocarb hydrochloride (722 g/L) as the active ingredient. Each treatment was performed with five replications.

The supplemented PDA media were poured into Petri dishes and allowed to solidify before inoculation. Subsequently, *Fusarium oxysporum* was cultured on the prepared media.

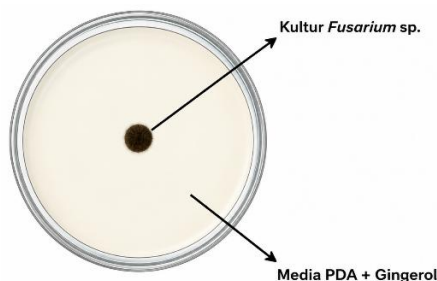


Figure 1. Illustration of gingerol efficacy against *Fusarium oxysporum*

The inoculated plates were incubated for 12 days at 28°C. Fungal growth was observed by measuring colony diameter, and the percentage of growth inhibition was calculated using the following formula:

$$\text{Efikasi (\%)} = \frac{C - T}{C} \times 100\%$$

Note:

E = percentage of treatment efficacy (%)

C = growth intensity in the control treatment

T = growth intensity in the extract treatment

The inhibition data were analyzed using analysis of variance (ANOVA) to determine significant differences among treatments. When significant differences were detected, the analysis was continued using the Honestly Significant Difference (HSD/BNJ) test at $\alpha = 0.05$ to compare differences among treatments.

RESULTS AND DISCUSSION

Analysis of Emprit Ginger Extract (*Zingiber officinale* var. *amarum*) Containing Gingerol

The quantification of 6-gingerol in emprit ginger extract was conducted using HPLC at 254 nm, and the chromatographic profile is shown in Figure 2.

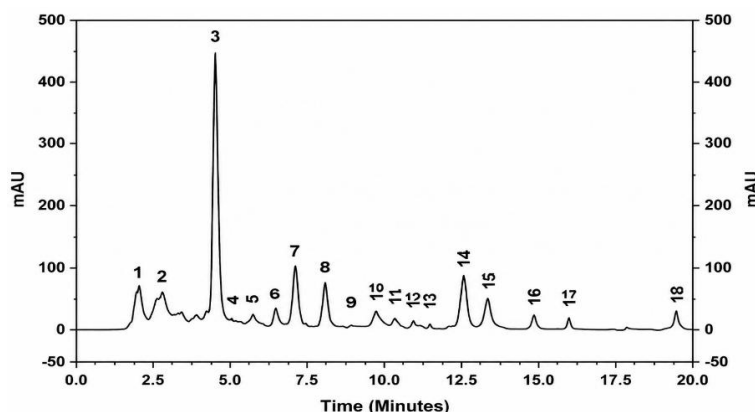


Figure 2. HPLC chromatogram of emprit ginger extract at a 5× dilution factor showing gingerol identification (peak 3, RT = 4.817 min, peak area = 5,610,161 mAU·min) analyzed at $\lambda = 254$ nm

The HPLC chromatogram showed several peaks detected within a retention time range of 0–20 min. The dominant peak appeared at a retention time (RT) of 4.817 min with a peak area of 5,610,161 mAU·min. Based on comparison with the reference standard, this peak was identified as 6-gingerol. Quantitative analysis revealed that the 6-gingerol concentration in emprit ginger extract was 8.082 µg/mL. The presence of a dominant 6-gingerol peak indicates that this compound represents one of the major bioactive constituents in the analyzed ginger extract.

Gingerol, particularly 6-gingerol, is widely recognized as the primary pungent phenolic compound in fresh ginger rhizomes and serves as an important chemical marker associated with ginger biological activities. This compound has been reported to possess antioxidant, anti-inflammatory, antimicrobial, and antifungal properties due to its phenolic structure and ability to interact with cellular targets (Semwal et al., 2015; Mao et al., 2019). The detection of 6-gingerol as the predominant compound in this study is consistent with previous findings by Tabboon *et al.* (2021), who reported that 6-gingerol is a major constituent of *Zingiber officinale* and can be effectively identified and quantified using HPLC analysis.

The 6-gingerol concentration obtained in this study (8.082 µg/mL) indicates that the extraction procedure applied was effective in recovering bioactive compounds from emprit ginger rhizomes. However, variations in gingerol content among studies are commonly observed and may be attributed to differences in plant genotype, cultivation conditions, geographical origin, maturity stage, harvesting time, and post-harvest processing. Previous studies have demonstrated that phytochemical composition in ginger is strongly influenced by genetic and environmental factors, resulting in variations in the concentration of gingerol derivatives among different samples (Prasad & Tyagi, 2015; Mao et al., 2019).

The extraction process is an important factor determining the recovery and stability of ginger bioactive compounds. In this study, maceration followed by solvent evaporation was applied for extract preparation. Maceration was selected because it involves relatively mild extraction conditions and lower temperatures, which can reduce degradation of thermolabile phenolic compounds. Similar findings have been reported by Ghasemzadeh *et al.* (2016), who demonstrated that extraction conditions significantly affect the recovery of phenolic compounds and antioxidant activity of ginger extracts. Furthermore, Maghraby *et al.* (2023) emphasized that appropriate extraction techniques are essential for maintaining the stability of bioactive compounds, including gingerol, and ensuring the quality of plant-derived extracts.

The drying process of ginger rhizomes also plays a critical role in maintaining gingerol stability. In the present study, the raw material was dried at 40–55°C to minimize thermal degradation and preserve the content of gingerol compounds. During drying or heating processes, 6-gingerol can undergo dehydration reactions that convert it into shogaol, a structurally related compound with different biological properties. Increasing drying temperatures has been reported to accelerate this conversion process, resulting in decreased gingerol levels and increased shogaol formation (Semwal et al., 2015; Dalsasso et al., 2022). Therefore, controlled drying conditions applied in this study may have contributed to maintaining 6-gingerol stability in the final extract.

The presence of 6-gingerol and other phytochemical constituents in ginger extract provides a scientific basis for its biological activity. Ginger rhizomes contain various secondary metabolites, including shogaol, zingerone, zingiberene, flavonoids, and volatile oil components, which may contribute to antimicrobial and antifungal effects through synergistic interactions (Mao et al., 2019; Radice et al., 2022). The

biological activity of plant extracts is therefore not solely dependent on a single compound but is often the result of combined effects among multiple metabolites acting through different cellular mechanisms (Gusmiarni & Des, 2021).

Overall, HPLC analysis confirmed the presence of 6-gingerol as a dominant bioactive compound in emprit ginger extract. These results demonstrate that the extraction and sample preparation procedures used in this study were capable of preserving important ginger phytochemicals. The confirmed presence of 6-gingerol provides a strong foundation for evaluating the antifungal potential of emprit ginger extract against *Fusarium oxysporum* in subsequent experiments.

Efficacy of Emprit Ginger Extract against *Fusarium oxysporum*

The antifungal efficacy of emprit ginger extract containing 6-gingerol against *Fusarium oxysporum* was evaluated based on colony diameter and percentage inhibition of fungal growth after 12 days of incubation at 28°C. Six treatments were evaluated, consisting of a negative control, a positive control using synthetic fungicide (*Previcur-N* containing propamocarb hydrochloride 722 g/L as the active ingredient), and emprit ginger extract at concentrations of 0.5%, 1.5%, 2.5%, and 5%. Each treatment was conducted with five replications.

Table 2. Effect of emprit ginger extract on colony growth and growth inhibition percentage of *Fusarium oxysporum*

Treatment	Mean colony diameter (cm)	Standard deviation (cm)	Growth inhibition (%)
Negative control	7.86	0.23	0.00% e
Positive control	4.46	0.30	43.26% b
0.5% extract	6.56	0.40	16.54% d
1.5% extract	5.30	0.91	32.57% c
2.5% extract	4.60	0.51	41.47% b
5% extract	3.42	0.58	56.49% a

Note: Values are presented as mean colony diameter and standard deviation (SD) from five replications (n = 5). Different letters within the same column indicate significant differences based on Tukey's honestly significant difference (HSD) test following ANOVA at a 5% significance level ($p < 0.05$).

The application of emprit ginger extract inhibited the growth of *Fusarium oxysporum* at all tested concentrations. The inhibition percentage increased progressively from 16.54% at 0.5% extract concentration to 56.49% at 5% concentration (Table 2). Correspondingly, fungal colony diameter decreased from 6.56 ± 0.40 cm at 0.5% extract concentration to 3.42 ± 0.58 cm at 5% concentration. The positive control treatment resulted in 43.26% inhibition, with an average colony diameter of 4.46 ± 0.30 cm, whereas the negative control exhibited unrestricted fungal growth, producing the largest colony diameter (7.86 ± 0.23 cm).

The observed inhibitory response demonstrates a clear concentration-dependent relationship between emprit ginger extract application and suppression of *F. oxysporum* growth. Increasing extract concentration resulted in greater inhibition, indicating that higher amounts of bioactive compounds were available to interact with fungal cells. Similar dose-dependent antifungal activity has been reported in previous studies, where increasing concentrations of ginger extract enhanced inhibition of *Fusarium* species and other phytopathogenic fungi under *in vitro* conditions (Okwuosa et al., 2021). This response is commonly observed in botanical extracts because the antimicrobial effect is strongly associated with the concentration of active secondary metabolites that diffuse into the growth medium (Nazzaro et al., 2013).

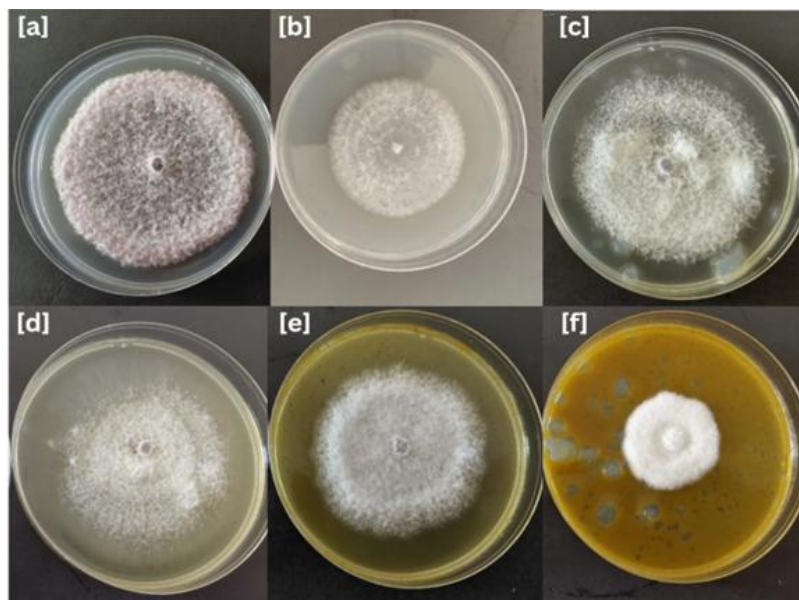


Figure 3. Growth of *Fusarium oxysporum* colonies on PDA medium supplemented with emprit ginger extract containing gingerol at different concentrations after 12 days of incubation at 28°C: (a) negative control, (b) positive control, (c) 0.5% extract concentration, (d) 1.5% extract concentration, (e) 2.5% extract concentration, and (f) 5% extract concentration

The visual differences in fungal colony development among treatments are presented in Figure 3. The negative control showed rapid fungal expansion, with mycelial growth covering almost the entire PDA surface. In contrast, colonies treated with emprit ginger extract exhibited restricted radial growth, and the inhibition became more apparent as extract concentration increased. The 5% extract treatment produced the smallest colony diameter among all treatments, whereas the positive control also limited fungal development compared with the untreated control. These observations confirm the quantitative inhibition results and demonstrate that increasing extract concentration effectively suppressed *F. oxysporum* growth.

The antifungal activity of emprit ginger extract is likely associated with the presence of multiple bioactive compounds, particularly 6-gingerol, which was confirmed through HPLC analysis in this study. Ginger rhizomes contain several phytochemicals, including gingerols, shogaols, zingerone, flavonoids, and terpenoid compounds, which contribute to antimicrobial activity through complementary mechanisms (Semwal et al., 2015; Mao et al., 2019). The antifungal activity of ginger extract is therefore considered a result of synergistic interactions among different metabolites rather than the effect of a single compound.

6-Gingerol, as the major phenolic constituent of ginger, has been reported to exhibit antimicrobial activity by affecting fungal cell membrane integrity, increasing membrane permeability, and inducing leakage of intracellular components. Phenolic compounds can interact with membrane lipids and proteins, resulting in structural disruption and impaired cellular functions (Hyldgaard et al., 2012; Shalaby et al., 2023). In addition, ginger-derived compounds may interfere with essential metabolic processes, including enzyme activity, oxidative balance, and biosynthesis pathways required for fungal growth.

The stronger inhibition observed at higher extract concentrations may also be explained by the multitarget mechanisms of ginger bioactive compounds. Shalaby et al. (2023) reported that gingerol and related phenolic compounds can disrupt fungal

membrane structure, inhibit metabolic enzymes, and induce oxidative stress, ultimately resulting in reduced fungal viability. Furthermore, Zhou *et al.* (2024) demonstrated that phenolic compounds from plant sources can act against fungal pathogens through multiple cellular targets, providing broader inhibitory effects compared with compounds acting through a single pathway.

The reduction in colony diameter was accompanied by visible morphological changes in fungal growth. Colonies treated with emprit ginger extract showed slower radial expansion and thinner mycelial structures compared with the negative control. These morphological alterations suggest that the extract not only inhibited fungal growth rate but also affected normal mycelial development. Similar morphological responses have been reported in fungi exposed to plant-derived antimicrobial compounds, where disruption of membrane function and cellular metabolism resulted in abnormal mycelial growth patterns (Nazzaro *et al.*, 2013; Radice *et al.*, 2022).

Interestingly, the 5% emprit ginger extract treatment exhibited higher inhibition than the synthetic fungicide treatment under the tested *in vitro* conditions. However, this finding should be interpreted carefully because direct comparisons between botanical extracts and synthetic fungicides are influenced by differences in active compound composition, formulation, stability, application rate, and mode of action. Synthetic fungicides generally contain purified active ingredients with specific targets, whereas plant extracts contain complex mixtures of compounds that may vary in concentration and biological activity.

Therefore, although emprit ginger extract demonstrated strong antifungal activity under laboratory conditions, further evaluation under greenhouse and field conditions is required before practical agricultural application. Environmental factors, plant interactions, pathogen infection pressure, and extract formulation may influence its effectiveness in natural conditions. Previous studies have emphasized that *in vitro* screening represents an essential initial step in evaluating botanical fungicides, but subsequent validation under plant and field conditions is necessary to confirm their practical potential (Köhl *et al.*, 2019; Suswanto *et al.*, 2022).

Overall, the findings demonstrate that emprit ginger extract (*Zingiber officinale* var. *amarum*) possesses significant antifungal activity against *Fusarium oxysporum*. The inhibition of fungal growth and alteration of colony morphology are likely attributed to the synergistic activity of 6-gingerol and other secondary metabolites present in the extract. The strong inhibitory effect observed at 5% concentration highlights the potential of emprit ginger extract as a botanical antifungal agent. Nevertheless, additional studies involving *in vivo* evaluation, comprehensive metabolomic profiling, toxicity assessment, and formulation optimization are required to support its development as an environmentally sustainable biofungicide for *Fusarium* wilt management.

CONCLUSION

Based on the findings of this study, emprit ginger extract (*Zingiber officinale* var. *amarum*) containing 6-gingerol exhibited antifungal activity against *Fusarium oxysporum* under *in vitro* conditions. High-performance liquid chromatography (HPLC) analysis confirmed the presence of 6-gingerol in the extract at a concentration of 8.082 µg/mL. Increasing extract concentrations resulted in higher inhibition of *Fusarium oxysporum* growth, with the 5% extract concentration showing the highest inhibition rate (56.49%). Under the tested *in vitro* conditions, this concentration exhibited greater inhibition than the positive control containing propamocarb hydrochloride. In addition to reducing colony growth, emprit ginger extract induced morphological changes in

fungus colonies, indicating inhibition of mycelial development. These findings suggest that emprit ginger extract has potential for development as a botanical biofungicide for controlling *Fusarium oxysporum*. However, further investigations, particularly through *in vivo* and field evaluations, are required to validate its effectiveness and applicability as an environmentally friendly alternative for plant disease management.

RECOMMENDATION

Further studies are recommended to evaluate the effectiveness of emprit ginger extract (*Zingiber officinale* var. *amarum*) containing gingerol under greenhouse and field conditions to determine its capacity to control Fusarium wilt disease directly in cultivated plants. Future research should also focus on developing more stable biofungicide formulations, determining the minimum effective concentration, and assessing phytotoxicity to host plants to ensure safe and efficient application.

In addition, comprehensive investigations into the mechanisms of action of emprit ginger bioactive compounds, either individually or through synergistic interactions with other phytochemicals, are necessary to support the development of optimized biopesticide products. Several factors may influence the effectiveness of the extract, including variations in gingerol content due to differences in harvesting stage and processing methods, stability of active compounds during extraction and storage, and the limitation of *in vitro* assays, which may not fully represent the complexity of field conditions. Therefore, further comprehensive research is required to confirm the efficacy, stability, and practical feasibility of emprit ginger extract as a biofungicide for sustainable agricultural systems.

ACKNOWLEDGMENT

The authors would like to express their gratitude to all parties who contributed to the successful completion of this research. This study was supported by funding from the Directorate of Research and Community Service (Direktorat Riset dan Pengabdian Masyarakat) in 2025.

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