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BIOLOGICAL CONTROL OF LEAF SPOT DISEASE (*Phyllosticta zingiberi*) ON RED GINGER (*Zingiber officinale* Rosc.) USING BIOCONTROL AGENTS

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ABSTRACT

Leaf spot disease is one of the major constraints in red ginger (Zingiber officinale Rosc.) cultivation, causing significant economic losses to farmers and reducing national export value. The disease is suspected to be seed-borne, as the pathogen can survive in infected planting materials. This study aimed to evaluate the effectiveness of the biocontrol fungi Trichoderma koningii, T. harzianum, Gliocladium spp., and G. virens in suppressing Phyllosticta zingiberi, the causal agent of leaf spot disease.. Additionally, the study assessed different application methods of biocontrol agents in controlling the disease. The study was conducted at the Experimental Field of the Faculty of Agriculture, Universitas Amir Hamzah, Medan Estate Village, Percut Sei Tuan District, Deli Serdang Regency, at an altitude of approximately 25 m above sea level, from January to August 2010. The study consisted of two main stages: (1) identification of the causal pathogen of leaf spot disease in red ginger, and (2) in vitro antagonistic testing of biocontrol agents against P. zingiberi. The results showed that the application of antifungal suspension of Gliocladium virens on leaves was relatively more effective in inhibiting the growth of P. zingiberi compared to other tested biocontrol agents..
Keywords: Biological control; *Phyllosticta zingiberi*; red ginger; biocontrol agents; leaf spot disease; *Gliocladium virens*

INTRODUCTION

Red ginger (*Zingiber officinale* Rosc.) is an important medicinal and industrial crop widely utilized as a spice, traditional medicine, and export commodity. In Indonesia and other tropical countries, ginger has high economic value and export potential. However, increasing demand has not been accompanied by optimal production and productivity due to limitations in cultivation practices and plant health management (FAO, 2022; Rahman et al., 2021).

Plant diseases caused by pathogenic microorganisms remain one of the most critical constraints in ginger production systems. Major diseases affecting ginger include bacterial wilt caused by *Ralstonia solanacearum*, rhizome rot caused by *Fusarium oxysporum*, and leaf spot disease caused by Phyllosticta zingiberi (Sharma et al., 2020; Gupta et al., 2022). Leaf spot disease is particularly destructive as it reduces leaf area, interferes with photosynthesis, and may lead to severe yield losses or plant mortality. The pathogen can spread through infected rhizomes, airborne dispersal, and rain splash, indicating both seed-borne and environmentally transmitted infection pathways (Kumar et al., 2019; Singh et al., 2021).

Previous studies and field observations have shown that leaf spot symptoms typically appear at approximately three months after planting, resulting in significant yield reduction. The persistence of the pathogen in soil and planting materials further complicates disease management and contributes to repeated outbreaks in ginger cultivation systems (Gupta et al., 2022).

Currently, disease control in ginger cultivation is largely dependent on chemical pesticides. However, excessive and continuous use of synthetic pesticides can result in environmental pollution, pathogen resistance, and harmful effects on non-target organisms and human health (Aktar et al., 2020; Nicolopoulou-Stamati et al., 2016). Therefore, the development of environmentally friendly and sustainable disease management strategies is essential.

Biological control using antagonistic microorganisms has emerged as a promising and sustainable alternative for plant disease management. Fungal biocontrol agents such as Trichoderma spp. and Gliocladium spp. are widely recognized for their ability to suppress plant pathogens through mechanisms such as competition, antibiosis, induction of plant resistance, and mycoparasitism (Harman et al., 2021; Woo et al., 2014).

Previous research has demonstrated the potential of biological control agents against *Phyllosticta zingiberi* under laboratory conditions. Test Potential of Selected Biological Control Fungi Against *Phyllosticta zingiberi* Under Laboratory Conditions by Martha Adiwaty Sihaloho, Hapsoh, and Hasanuddin, 2026 reported that several antagonistic fungi exhibited inhibitory effects on the growth of *P. zingiberi* in vitro. The findings indicate that *Gliocladium spp.* and *T. harzianum* are the most effective biological control agents for managing leaf spot disease in red ginger. However, further evaluation is required to assess their effectiveness under different application methods and conditions closer to field environments.

Based on these considerations, this study aimed to evaluate the effectiveness of several biocontrol fungi, namely Trichoderma koningii, *Trichoderma harzianum, Gliocladium spp.,* and *Gliocladium virens*, in controlling leaf spot disease caused by Phyllosticta zingiberi on red ginger, as well as to assess the effectiveness of different application methods of these biocontrol agents.

This study aims to evaluate the effectiveness of selected biocontrol fungi and their application methods in controlling leaf spot disease in red ginger under field conditions.

MATERIALS AND METHODES

Study Site and Duration

. The study was conducted from January to August 2010 at the Experimental Field of Amir Hamzah University, Indonesia

Materials and Equipment

The materials used in this study included red ginger planting materials (Zingiber officinale Rosc.), rice straw compost components (bio-activator MOD-71, rice straw, animal manure, rice bran, and palm sugar), rice husk, topsoil, organic fertilizers, ABG leaf and flower nutrients, coconut water, suspensions of biocontrol agents *(Trichoderma koningii, T. harzianum, Gliocladium spp., and Gliocladium virens*), water, and bamboo baskets (30 × 50 cm).

Experimental Procedures

Compost Preparation

Rice straw compost was prepared by activating the bio-activator (MOD-71) with sugar and water, followed by incubation for 24 h. Composting materials (rice straw, soil, bran, and manure) were arranged in layers and sprayed with the activated solution. The compost was turned after one week and allowed to decompose for three weeks until ready for use.

Screen House Preparation

The experiment was conducted in a simple screen house (6 m × 28 m) constructed using bamboo frames, covered with transparent plastic roofing and black mesh walls. Land and Experimental Setup were the experimental area was cleared of weeds and prepared into plots (100 cm × 50 cm) with 50 cm spacing between plots and 100 cm between replications. Bamboo baskets were arranged on the plots over a brick base.

Planting Material Preparation

Mature red ginger rhizomes were washed, soaked in coconut water for 1 h, air-dried, and cut into pieces containing two buds each. The rhizomes were then pre-sprouted in a nursery for approximately one month. Nursery and Seedling Establishment. The nursery (2 m × 3 m) was shaded to maintain high humidity. Rhizomes were placed on jute sacks with buds facing upward and covered with the same material until sprouting.

Growing Media Preparation

Topsoil was sieved and mixed thoroughly with straw compost and rice husk at a ratio of 3:1:1 (v/v). The mixture was filled into bamboo baskets up to three-quarters of their volume and incubated for one week prior to planting. And Pathogen Inoculation *Phyllosticta zingiberi* inoculation was performed by spraying 10 mL of conidial suspension (10^7 conidia mL^{-1}) per basket.

Transplanting

Uniform seedlings were transplanted into baskets (three plants per basket). The baskets were temporarily covered with coconut fronds to protect seedlings from direct sunlight. And than Application of Biocontrol Agents were biocontrol agents (*Trichoderma koningii*, *T. harzianum*, *Gliocladium* spp., and *G. virens*) were applied according to treatment. The first application was conducted after removal of the cover, followed by repeated applications at three-day intervals. Spray volume was adjusted to ensure uniform coverage of the leaf surface.

Research Methodology

The experiment was arranged in a factorial Randomized Complete Block Design (RCBD) consisting of two factors. Factor I: Type and concentration of biocontrol agents (13 levels) :

A_0 : Control (*sterile water*)
K_1 : *Trichoderma koningii* at 10^6 conidia mL^{-1}
K_2 : *Trichoderma koningii* at 10^7 conidia mL^{-1}
K_3 : *Trichoderma koningii*: 10^8 conidia mL^{-1}
H_1 : *Trichoderma harzianum* 10^6 conidia mL^{-1}
H_2 : *Trichoderma harzianum* 10^7 conidia mL^{-1}
H_3 : *Trichoderma harzianum* 10^8 conidia mL^{-1}
G_1 : *Gliocladium spp* 10^6 conidia mL^{-1}
G_2 : *Gliocladium spp* 10^7 conidia mL^{-1}
G_3 : *Gliocladium spp* 10^8 conidia mL^{-1}
V_1 : *Gliocladium virens* 10^6 conidia mL^{-1}
V_2 : *Gliocladium virens* 10^7 conidia mL^{-1}
V_3 : *Gliocladium virens* 10^8 conidia mL^{-1}

Factor II: Application method (2 levels)
T: Soil application
D: Foliar application
Thus, a total of 26 treatment combinations (13 × 2) were obtained.

Experimental Units

Each treatment was replicated three times, resulting in 78 experimental plots. Each plot consisted of 2 baskets, with 3 plants per plot. The total number of baskets used was 156 units. The spacing between plots was 50 cm, and between blocks was 100 cm.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) based on the following linear model:

$$Yijk = \mu + \rho i + \alpha j + \beta k + (\alpha\beta)jk + \varepsilon ijk$$

where:

- YijkY_{ijk}Yijk = observed value in block *i*, treatment *j*, and application method *k*
- μ\muμ = overall mean
- ρi\rho_iρi = effect of block *i*
- αj\alpha_jαj = effect of biocontrol agent treatment
- βk\beta_kβk = effect of application method
- (αβ)jk(\alpha\beta)_{jk}(αβ)jk = interaction effect
- εijk\varepsilon_{ijk}εijk = experimental error

If significant differences were detected, means were further compared using Duncan’s Multiple Range Test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

Incubation Period

The incubation period of leaf spot disease caused by *Phyllosticta zingiberi* under different treatments is shown in Table 1.

Table 1. Mean incubation period of leaf spot disease caused by *Phyllosticta zingiberi* on red ginger (*Zingiber officinale* Rosc.)

Treatment	Symptom appearance	Incubation period (days after inoculation)
A0T	yes	16.08
A0D	yes	18.67
K1T	yes	10.92
K1D	yes	10.92
K2T	yes	23.83
K2D	yes	4.50
K3T	yes	23.83
K3D	yes	13.50
H1T	yes	10.92
H1D	yes	13.50
H2T	yes	16.75
H2D	yes	4.50
H3T	yes	11.58
H3D	yes	4.50
G1T	yes	11.58
G1D	yes	1.92
G2T	yes	9.67
G2D	yes	1.92
G3T	yes	14.17
G3D	yes	5.75
V1T	yes	7.08
V1D	no	0.00
V2T	yes	6.42
V2D	no	0.00
V3T	yes	7.08
V3D	yes	1.92

Table 1 shows that the incubation period of leaf spot disease caused by *Phyllosticta zingiberi* varied among treatments, indicating different levels of effectiveness of biocontrol agents and application methods. The longest incubation period was observed in treatments K2T and K3T (*Trichoderma koningii* applied via soil at concentrations of 10^7 and 10^8 conidia mL^{-1}), reaching 23.83 days after inoculation. This suggests that *T. koningii* effectively delayed pathogen infection, likely through competition for nutrients and colonization of the rhizosphere. In contrast, the shortest incubation period was recorded in treatments V1D and V2D *(Gliocladium virens* applied via foliar application), where no symptoms were observed (0.00 days), indicating complete suppression of disease development under these treatments. Other treatments, particularly those involving *Gliocladium spp. and Trichoderma harzianum*, showed intermediate incubation periods.

The variation in incubation period reflects differences in antagonistic mechanisms among biocontrol agents, such as competition, antibiosis, and mycoparasitism. Trichoderma spp. are known to inhibit pathogen establishment through rapid growth and competition in the root zone, while Gliocladium virens produces antifungal compounds that directly suppress pathogen growth (Harman et al., 2021; Woo et al., 2014). These results indicate that both the type of biocontrol agent and the method of application play important roles in delaying disease onset in red ginger.

Disease Incidence

Analysis of variance showed that biocontrol agents significantly affected disease incidence during the main observation period. The highest disease incidence was recorded in the control treatment (A0), reaching 3.86%, while the lowest incidence was observed in *Gliocladium virens* at 10^7 conidia mL^{-1} (V2), with only 0.71% infection.

The effectiveness of *G. virens* in reducing disease incidence is likely related to its ability to produce antifungal metabolites such as gliotoxin and viridin, which inhibit pathogen growth. Previous studies have demonstrated that *Gliocladium* spp. suppress plant pathogens through antibiosis and mycoparasitism (Woo et al., 2014; Harman et al., 2021).

Effect of Application Method

The application method significantly influenced disease incidence. Foliar application resulted in lower disease incidence (1.62%) compared to soil application (3.05%). This suggests that foliar application is more effective, likely because *P. zingiberi* primarily infects leaf tissue, making direct application more efficient.

Interaction Effect

No significant interaction was observed between biocontrol agents and application methods for disease incidence. However, numerically, the lowest disease levels were consistently observed in treatments involving G. virens and foliar application, indicating a strong practical effect.

Disease Intensity

Biocontrol treatments significantly reduced disease intensity compared to the control. The highest disease intensity was observed in the control (3.72%), while the lowest was recorded in Gliocladium spp. at 10^7 conidia mL^{-1} (G2), with 1.18%.

The reduction in disease intensity suggests that increasing conidial concentration enhances antagonistic activity. This is consistent with previous findings that higher inoculum density of biocontrol agents improves pathogen suppression efficiency (Gupta et al., 2022).

Among the tested agents, Trichoderma harzianum and G. virens also showed strong suppression effects. These fungi are known to act through multiple mechanisms, including: (a) competition for nutrients; (b) production of antifungal compounds; (c) induction of plant systemic resistance (Harman et al., 2021; Sharma et al., 2020)

Effect of Application Method on Disease Intensity

Application method significantly affected disease intensity. Foliar application resulted in lower intensity (1.59%) compared to soil application (2.43%). This confirms that targeting the infection site (leaf surface) enhances the effectiveness of biocontrol agents.

Interaction on Disease Intensity

A significant interaction between biocontrol agents and application method was observed at later stages. The lowest disease intensity was consistently recorded in treatments V1D and V2D (0.00%), followed by G2D and G3D. This indicates that *G. virens* and Gliocladium spp. applied via foliar spray were the most effective treatments.

The results demonstrate that biological control agents, particularly *Gliocladium virens*, are effective in suppressing leaf spot disease in red ginger. The superiority of *G. virens* is attributed to its strong antagonistic activity, including antibiotic production and rapid colonization.

The findings also highlight the importance of application method, where foliar application proved more effective than soil application. This aligns with the infection biology of *P. zingiberi*, which primarily attacks leaf tissues.

Additionally, variability in treatment response may be influenced by pathogen virulence, environmental conditions, and host susceptibility. The use of susceptible ginger variety (Jahira 1) likely contributed to disease development across treatments, emphasizing the need for integrated disease management strategies combining resistant varieties and biological control.

Overall, the study confirms that biocontrol agents, especially *G. virens*, offer a sustainable alternative to chemical control, supporting environmentally friendly plant disease management.

CONCLUSION

The results of this study demonstrate that biocontrol agents significantly influence the suppression of leaf spot disease caused by *Phyllosticta zingiberi* in red ginger (*Zingiber officinale* Rosc.). Among the tested agents, Gliocladium virens showed the highest effectiveness in reducing disease incidence and intensity, particularly when applied through foliar application. Application method also played an important role, where foliar application was more effective than soil application in suppressing disease development. This is likely due to the direct contact of biocontrol agents with the infection site on leaf surfaces.

Overall, the use of biocontrol fungi, especially *Gliocladium virens*, can be considered a promising and environmentally friendly alternative for managing leaf spot disease in red ginger cultivation.

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