Effectiveness of Essential Oil Formula and Silica Against Diamond Shape Leaf Spot Disease in Red Ginger

M P Sari¹, D Wahyuno¹, D Florina¹, D Manohara¹, and Hoerudin²

¹Indonesian Spice and Medicinal Crops Research Institute, IAARD, Jalan Tentara Pelajar 3, Bogor 16111, West Java, Indonesia.
²Indonesian Agricultural Postharvest Research and Development, IAARD, Jalan Tentara Pelajar 12, Bogor 16111, West Java, Indonesia.

Corresponding author email: marlina_puspita434@yahoo.co.id

Abstract. Diamond shape leaf spot disease caused by Pyricularia zingiberi is an essential disease in red ginger. This current research aims to identify the potency of essential oil formula and silica fertilizer against the diamond shape leaf spot disease. Efficacy of clove oil, lemongrass oil, and mancozeb as a control against the colony growth was tested by inoculating the fungus on PDA medium amended with 50, 100, 150, 200, 250, and 300 ppm of the tested fungicides. Compared to lemongrass oil, clove oil (LC50: 70.79 ppm) is more effective in suppressing the growth of P. zingiberi. In a greenhouse, the leaves of five months old red ginger plants were inoculated by spraying 10⁵ conidia mL⁻¹ suspensions of P. zingiberi. Foliar application of clove oil base pesticide and silica was carried out a month before pathogen inoculation, arranged in the factorial randomized complete design. The disease severity was measured monthly, and the data were analyzed using ANOVA. Disease severity on the plant treated by either clove oil base pesticide or silica was 9.08% and 8.71% lower than control (20.21%). Further research is still needed. Treatment with clove oil base pesticide or silica shows potency in reducing the disease severity.

Keywords: Pyricularia zingiberi, clove, lemongrass, mancozeb

1. Introduction

Red ginger is a potential medicinal plant but susceptible to pests and diseases, including leaf spot disease, bacterial wilt, parasitic nematode attacks, and insect pests. Leaf spot disease is the main disease in ginger. In suitable environmental conditions, this fungus’s attack becomes a severe problem, especially in the field. In Indonesia, leaf spot disease is caused by some plant parasitic fungi, namely Phyllosticta zingiberi [1], Pyricularia zingiberi [4], and Phakopsora elettariae [2]. The results of the ginger pest survey in Central Java, West Java, and Bengkulu conducted by [3] from the characteristics of the visible symptoms there are variations in the types of fungi that attack in each location with disease incidence ranging from 1.4 up to 92%. Until now, there have been no reports about the yield loss because of this disease. Pyricularia is the dominant fungus infecting the field. Plants under five months of age are susceptible to leaf spot disease [3]. However, red ginger shows more resistance against P. zingiberi than white ginger [4]. But until now, none of the red ginger varieties that 100% resistant to leaf spot disease [5]. In the field, disease control still relies on chemical control with fungicides.

The use of botanical fungicides in controlling plant pests and diseases has long been carried out. The potential of essential oils as botanical fungicides has been widely proven. Essential oils are also degraded...
faster so that they are more environmentally friendly and relatively safer against natural enemies. Besides using botanical fungicides, the addition of silica to red ginger was also carried out in this study to see its effect on the emergence of leaf spot disease. Silica is known to increase plant resistance to diseases caused by fungi and bacteria [6]. In research on rice plants, silica (Si) was able to suppress the intensity of leaf spot attack [7]. Silica treatment can induce the formation of phytoalexin compounds in rice leaves so that they become more resistant to leaf spot disease caused by Pyricularia grisea [8; 22]. Secondary metabolites such as lignin and phenol have an essential role in plant resistance. Silica plays a role in phenol metabolism and lignin biosynthesis in plant cell walls [9]. This current research aims to determine the effectiveness of essential oil formula and bio-silica fertilizer against the diamond shape leaf spot disease on red ginger.

2. Material and methods
This research was carried out in January–December 2020 in the plant disease laboratory and greenhouse of the Indonesian Spices and Medicinal Crop Research Institute (ISMCRI-IAARD) in Bogor.

2.1. Fungal inoculum preparation
The isolate used in this study was Pyricularia zingiberaceae (Pyr-J2), an isolate belonging to the plant pathology property of ISMCRI. The isolate was collected from a leaf of red ginger, which had been identified morphologically and molecularly confirmed in the previous study [4]. The isolate was propagated on a PDA medium for further testing.

2.2. In vitro test
The botanical fungicides tested in this research activity are based on essential oils content in each formula. The efficacy of essential oils (lemongrass and cloves) and mancozeb (contact fungicide) on the growth of fungal colonies (Pyr-J2 isolate) were tested by the food poisoning technique [10]. One thousand ppm stock solution of each treatment (clove oil, lemongrass oil, and mancozeb) was made by adding the appropriate weight or volume of the fungicide to 100 ml of sterile distilled water. The fungal colonies were inoculated onto a PDA medium with fungicide concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, then incubated for seven days at room temperature. The diameter of colonies growth was measured after seven days of incubation; the inhibition percentage was calculated based on the following formula: \( I \% = \left( \frac{D_0 - D_1}{D_0} \right) \times 100 \), where \( D_0 \) is the diameter of control colony, \( D_1 \) is the diameter of the colony treated with fungicide concentrations. Lethal concentrate \( 50 \) (LC\(_{50}\)) from each tested fungicide was obtained by performing probit-based analysis.

2.3. In vivo test
In vivo test was carried out in greenhouse using red ginger plants aged less than five months. The treatment design consisted of 2 factors, the application of fungicides and the foliar application of silica, arranged in a factorial Randomized Complete Block Design with three replications. Fungicides tested consisted of control, clove oil base fungicide, and mancozeb, applied with an active ingredient concentration of 200 ppm. The silica factors consisted of 3 concentration levels, 0 mL.L\(^{-1}\), 10 mL.L\(^{-1}\), and 20 mL.L\(^{-1}\).

All plants were artificially inoculated by spraying a suspension of pathogens (Pyr-J2 isolate) with a density of \( 10^5 \) conidia/mL on plants then covered with plastic for two days, and the inoculated plants were incubated further in the greenhouse for 7 to 10 days. The fungal preparation and the fungal application method for artificial inoculation followed the procedure of [4]. Fungicides treatment was carried out one week after pathogen inoculation.

The silica fertilizer used is the product of Indonesian Agricultural Postharvest Research and Development (ICAPOSTRD-IAARD) which is applied twice (interval one month) before pathogens inoculation by spraying onto the adaxial leaves (lower leaves surface). This product is made from simple extraction of silica nanoparticles from rice husk using technical grade solvent [11]. The liquid formula of silica fertilizer content is 9% Si and K\(_2\)O 6%.
Clove oil base fungicide used is the product of Indonesian Spices and Medicinal Crop Research Institute (ISMCRI-IAARD). This product consisted of nanoemulsion of clove oil, prepared using a low energy spontaneous diffusion technique [12].

Observations were carried out for three months with a responsive design, namely disease intensity, weight, and gingerol rate of rhizome at the end of the observation. Disease intensity is calculated using the following formula according to Waller et al. 2002 [28].

\[ DS = \frac{\sum (nv)}{z \times N} \times 100\% \]

DS = disease severity (%)

n = number of symptomatic leaves by category (score 0-6)

v = scale value (score) of each category

z = scale value (score) of the highest infection category (=6)

N = total number of observed leaves (n0 + n1 + …. + n6)

Scoring method according to IRRI 2002 [13] with modifications:

Score 0 = No spots
Score 1 = Spot area in one leaf <4%
Score 2 = Spot area in one leaf 4-10%
Score 3 = Spot area in one leaf 11-25%
Score 4 = Spot area in one leaf 26-50%
Score 5 = Spot area in one leaf 51-75%
Score 6 = Area of spots in one leaf >75%

Fresh red ginger is harvested from 8 month-old plants, washed using running water. The clean red ginger rhizome was dried at 20°C for 24 hours and then baked for 18 hours at 60°C. The dried simplicia was analyzed for its gingerol content in the ISCMRI testing laboratory using the Thin Layer Chromatography method by [24].

2.4. Data analysis

The data obtained were analyzed statistically by analysis of variance (ANOVA). For the real difference test, Duncan's Multiple Range Test was used at the 5% level.

3. Results and discussion

3.1. In vitro test

Clove oil showed the best inhibitory effect on colony’s growth of \( P \) zingiberi (Pyr-J2 isolate). At a concentration of 150 ppm clove oil, the mycelium of the fungus was no longer able to grow (Figure 1). In the mancozeb treatment, the diameter of the fungal colonies was inhibited and significantly different from the control. However, increasing the concentration level of mancozeb up to 300 ppm did not significantly reduce the colony diameter, although the growing mycelium seemed to be getting thinner (Figure 3). In a similar pattern of the mancozeb treatment, the colony of the Pyr-J2 isolate was not significantly different between the 50 ppm and the 300 ppm at medium with lemongrass oil.

The data from the in vitro test were analyzed by probit to determine the LC50 value also showed that the lowest LC50 value was clove oil of 70.79 ppm (Figure 2). This indicates that at the laboratory level, clove oil has a higher potential than lemongrass oil to suppress the colony's development of \( P \) zingiberi of red ginger.

Clove oil contains eugenol, which is known to have antifungal activity [14]. Previous research showed the ability of clove oil to inhibit the development of plant pathogens, including \( Fusarium \) oxysporum f.sp. cubense [15], \( Rhizoctonia \) solani [16], and \( Fusarium \) oxysporum f.sp. vanilla [17]. The impact of clove oil and mancozeb on the viability of conidia was also tested. At a concentration of 250 ppm, clove oil was able to inhibit the germination of \( P \) zingiberi conidia by 100% (Figure 4). Starting from the concentration of 50 ppm; mancozeb already inhibits 100% spore germination. It seems that mancozeb's mode of action on \( P \) zingiberi is more affecting the spore germination than the colony’s
growth. In line with [18] and [19], mancozeb is a contact fungicide that has a mode of action inhibiting spore germination of various fungi by releasing ethylene bisisothiocyanate sulfidae (EBIS) after contact with water and decomposes back into ethylene bisisothiocyanate (EBI), which is toxic to enzymes in the cytoplasm and mitochondria of fungi.

Either clove oil or mancozeb has the potency to suppress the diamond shape leaf spot disease, both of them were tested further in the greenhouse.

**Figure 1.** Diameter of *Pyricularia* (Pyr-J2) colony on media with various fungicide concentration levels. The same letters on each bar graph are not significantly different according to Duncan's test at the 5% level. ■Control ■Clove ■Lemongrass ■Mancozeb.

**Figure 2.** Colony growth trend of *P zingiberi* (Pyr J2) in various fungicide treatments. (---) Clove Oil (---) Mancozeb (-----) Lemongrass Oil.
Figure 3. Colony growth of the fungus *P. zingiberi* (Pyr J2) in various fungicide treatments. A= Clove oil, B = Lemongrass oil, C = Mancozeb.

Figure 4. Conidia viability of *P. zingiberi* in various treatments. The vertical line on each bar represents a 5% range of values. ■Control □Clove oil ■Mancozeb. The vertical line on each bar represents a 5% range of values.

3.2. *In vivo test*

Based on the result of the in vitro study, clove oil base pesticide was selected for a further test at the greenhouse study. Mancozeb was also used as a comparison. The disease severity of combinations of treatments consisted of pesticide and silica fertilizer levels. The results showed the gingers plants applied with a combination of mancozeb and silica in the highest level had lower disease severity with a percent inhibition of 78.1% (Figure 5). However, there is no significant interaction among the tested treatments. Plants that were applied with clove oil base pesticide had lower disease severity compared to control but not significantly different. Based on Gas Chromatography Spectrometry Mass Assay, clove oil base fungicide used in this test contained 16.97% Caryophyllene, 1.93% Humulene, 1.19% Caryophyllene Oxide, 69.96% Eugenol, and 7.10% Glycerin. Besides eugenol, caryophyllene is also known to have antifungal activity. Report from [27], β-caryophyllene can promote plant growth, induce plant defense genes, and be active against particular plant pathogenic fungi.
More research is still needed; the application interval and accuracy significantly affect the success of disease control with botanical fungicides. Air, sunlight, moisture, and high-temperature exposure can break down the botanical fungicides constituents and degrade their effectiveness as fungicides [20]. Weather conditions also have a significant role in applying botanical fungicides since they degrade quickly [21].

![Figure 5. Diamond shape leaf spot disease severity on various treatments. The vertical line on each bar represents a 5% range of values. Data transformed using Arcsin (\(\sqrt{\text{data}}/100\))x [180/\(\pi\)] when the statistical analysis was performed.](image)

The fresh weight of eight-month-old ginger of each treatment indicated that there is no significant difference among the combinations of treatments tested. Both applications of clove oil base fungicide and silica did not affect the production of red ginger rhizome. In addition to the rhizome weight, the gingerol content in the rhizome was also analyzed. The levels of gingerol in the treated plants with silica and botanical fungicide did not differ significantly compared to the control one. This indicates that the treatments did not affect the quality of the ginger rhizome produced. It is essential for a method of plant disease control needs to be not only effective in decreasing the disease but also have a minimum side effect on the plant.

Some research mentioned that silica could enhance plant resistance against fungal infection. The effectiveness of silica to suppress P griseae causing rice blast disease has been reported before [9, 22, 23]. Even though the absorption and development mechanism of silica in red ginger is still unknown yet, both red ginger and rice belong to monocotyledons [25]. The role of silica against fungal hyphae penetration was initially attributed to a physical barrier fortifying the cell wall. However, several studies have shown that the action of silica on plants also involves plant metabolism [26]. Foliar application of silica on red ginger did not show a phytotoxic effect, both on plant or rhizome produced. However, more research is still needed for elucidating the effect of silica on the disease severity of diamond shape leaf spots since there is a decreasing trend in disease severity of red ginger plants applicated with silica.
4. Conclusion
Clove oil base fungicide and foliar application of silica have the potency to reduce the diamond shape leaf spot disease severity. There was no correlation between the application of fungicides treatment with foliar application of silica in reducing the disease severity. Synthetic fungicide (the ai. of mancozeb) still showed the best inhibitory effect to diamond shape leaf spot disease, either in vitro or in vivo test.

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