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Rhizome essential oil and fractions of *Elettariopsis slahmong* CK. Lim against *Colletotricum gloesporioides* in red dragon fruit *Hylocereus polyrhizus*

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ABSTRACT

Biopesticides are the effective formulated form of active ingredients such as plant extracts (essential oils) to control pests and diseases, that are highly effective, target specific and could reduce harm to ecological chain. From previous study, 1000 ppm of wild zingiberaceae *Ellettariopsis slahmong* C.K. Lim leaf oil dan A/1 fraction were able to supress the growth of *Colletotricum gloesporioides* to up to 100% (*in-vitro*). *C. gloesporioides* is a fungus pathogen causing antrachnose disease in red dragon fruit (*Hylocereus polyrhizus*). The objective of this study was to examine the effectiveness of *E. slahmong* rhizome oil, B/2 and D/4 essential oil (EO) fractions, using the similar method as in previous research; describe the chemical compounds of *E. slahmong* EO from its rhizome oil, B/2 and D/4 fractions; and compare these chemical compounds with compounds in EO from leaf oil, A/1 and C/3 fractions (the subject of previous research). Research was conducted at the laboratory of disease and pest of Laing Research Station of Bogor Research Institute For Spice and Medicinal Plant, Solok West Sumatra Indonesia. The research method followed three stages: (a) suppressing colony diameter using rhizome oil, B/2 and D/4 fraction (concentration levels: 0, 100, 250, 500 and 1000 ppm), (b) suppressing colony biomass using rhizome oil, B/2 and D/4 fraction (concentration levels: 0, 100, 250, 500 dan 1000 ppm), and (c) suppressing colony diameter using volatile compounds from rhizome oil, B/2 and D/4 fraction (dose level: 0; 0,01 ml; 0,025ml; 0,05ml and 0,1 ml/petri dish). Tests (a) and (b) used Completely Randomized Design (CRD). Research results concluded that rhizome oil, B/2 and D/4 fractions have inhibitor effect capacity and are potential raw materials for bio-fungicide. All three tests proved that at concentration level of 1000ppm, *C. gloesporoides* diameter and biomass growth could be completely suppressed (100%). However, B/2 fraction had the most effective results for inhibitor potential effect and dose usage under 1000 ppm. For chemical compound identification, wild zingiberaceae mostly consists of tridecenal (39,81%) and 2-decanoic acid (27,78%).

Keywords: *Elettariopsis slahmong*, *Colletotricum gloesporioides*, red dragon fruit, inhibitor effect, fraction.

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INTRODUCTION

Essential oils (EO) from *Elettariopsis sp* have the chemical compounds which functions as an antioxidant, antibacterial, antifungi and anti-insect (30, 31, 7, 23, 25, 26, 22). One of the *Elettariopsis* plants, which is currently developed as biopesticide *Elettariopsis slahmong* C. K. Lim (24, 26), is a wild plant from the zingiberaceae group which was first discovered in Malaysia by Lim (18). It is also known as the 'stink bug plant' (29,30), gaining its nickname from its strong odour which Lim (18) further names as 'the smelly leaves' since the odor comes from its leaves. From GC-MS fractination, it was discovered that there were seven fractions from *E. slahmong* essential oils (25). From these seven fractions, fraction A/1 and *E. slahmong* leaf essential oil (EO) were able to completely suppress the growth of *C. gloesporioides* (in-vitro), the cause of antrachnose disease in red dragon fruit. This study will examine the biopesticide effectiveness of *E. slahmong* EO from its rhizome, B/2 and D/4 fractions, by applying the same method in the previous research of Nasir dan Nurmansyah (25).

There have been numerous reports of severely damaged red dragon fruit (*H. polyrhizus*) plantations caused by antrachnose disease in Indonesia (9, 25, 4). In other country such as Malaysia (19), similar reports have emerged. Despite the lack of the pathogen's name, heavy attacks with similar symptoms have also been described in several red dragon fruit plantations in Sumatra by Jumjunidang *et al* (12, 13). The repercussions are also severe, *C. gloesporioides* attacks can lower production up to 80% (4). Even worse, in Bintan Island (Riau Island Province) and in Padang (West Sumatra Province), there were plantations which were destroyed completely and finally abandoned by their owners (24, 25). In Indonesia, specifically Sumatra, there has not been any reports on the success of chemical pesticides in controlling this antrachnose disease. This is proven by the increasing destruction of red dragon fruit plantation areas on the island.

National programs for controlling plant's pests and diseases in Indonesia has been focusing on utilizing local plant genetic resource for biopesticide in the attempt to reduce the use of chemical pesticides. The use of chemical pesticides in crop pest control around the world has caused tremendous damage to the environment, pest resurgence, pest and pathogen resistance, lethal effects on non target organisms and very high cost (1, 8, 17, 2) It is estimated that financial cost of the damage to the environment and social economy caused by chemical pesticides in the world is about $8.1 billion a year (17). Biopesticides which are higly effective, target specific and can reduce environmental risks are considered to be the best alternative to chemical pesticides (11, 17).

The reason behind this study, which is to test the biopesticide effect on EO originating from *E. slahmong* plant parts and fractions is because each part of this plant consists of different chemical compounds (30, 31, 25). These could also have the potential to generate different biopesticide effects (5, 14, 7, 27, 23, 25). The best result of this study and the previous research by Nasir dan Nurmansyah (25) in controlling *C. gloesporioides* will be developed to be tested on red dragon fruit whether in green houses or in the field.

METHODOLOGY

The research was conducted at the laboratory of pest and plant disease of Bogor Research Institute for Spice and Medicinal Plants, Laing Research Station, in West Sumatra, Indonesia. The main material for this research, wild zingiberaceae, originated from Bonjol forest in the District of West Pasaman, West Sumatra.

Distilation and fractionation:

E. slahmong rhizome oil and fractions were obtained using previous methods by Nasir dan Nurmansyah (25). Fractions B/2 and D/4 were used to examine antifungal potency against *C. gloesporioides.*

Pathogen isolate:

The isolate of *C. gleosporioides* originated from antrachnose infected red dragon fruit isolate, the similar isolate from previous research (25). The isolates were multiplied from Patato Dextrosa Agar (PDA) and for this study, they are used after reaching the age of 9 days.

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Antifungal testing

Colony diameter growth suppression:

The preparation of the test medium followed methods used previously by Nasir dan Nurmansyah (25). *C. gleosporioides* were cut into 6 mm in diameter pieces with a corkborer and then placed in the center of the medium. Afterwards, it was incubated at the temperature of 28º C for 9 days. The test was conducted using Completely Randomized Design (CRD) factorial with four treatments each. Replications were rhizome oil, B/2 and D/4 as factor I, concentration levels of 0, 100, 250, 500 dan 1000 ppm as factor II.

Colony biomass suppression:

Firstly, liquid medium were prepared using Nasir dan Nurmansyah methods (25). Previously cut *C. gleosporioides* (6 mm in diameter) were inoculated into the medium and then incubated at the temperature of 28º C for 9 days. This test also used CRD factorial with four treatments each. The treatments were: EO of rhizome, B/2 and D/4 fractions as factor I and concentration levels of 0, 100, 250, 500 dan 1000 ppm as factor II. The fungus colony which grew in the medium were extracted and dried in an oven at 80º C for 48 hours. Afterwards, the biomass of the colony was measured.

Volatile compound testing:

To examine the effectiveness of volatile compounds, this study applied the same method used by Nasir and Nurmansyah (25) on EO of wild zingiberaceae rhizome, B/2 and D/4 fractions.

Inhibitor potential:

Colony inhibition growth was measured according to Awang *et al* (3) by using the formula of:

$$I = \frac{C - T}{C} \times 100\ \%$$

I = Inhibition of colony growth.
C = Colony diameter/colony biomass of control.
T = Colony diameter/colony biomass of treatment.

RESULT AND DISCUSSION

All three biopesticide components of *E. slahmong* (rhizome oil, B/2 and D/4 fractions) are able to restrain the growth *C. gloesporioides*, this is indicated by Table 1 below.Treatment with B/2 fraction presented the highest inhibitor effect (40,11%), which was significantly different with results from treatment with rhizome oil and D/4 fraction.

Table 1. Effect of rhizome EO, fraction EO and concentration levels towards *C. gleosporioides* colony diameter growth (DAI 9)

Treatment	Colony diameter (mm)	Inhibitor potential (%)
Biopesticide:		
Rhizome oil	52,50	39,57 b
B/2 fraction	52,25	40,11 a
D/4 fraction	54,65	37,36 c
Concentration level		
0 ppm(K+)	85,00	2,15 e
100 ppm	80,08	8,21 d
250 ppm	66,25	24,06 c
500 ppm	34,33	60,65 b
1000 ppm	0,00	100,00 a
control (without treatment)	87,25	0,00 -
CV (%)	-	1,67

Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT. K+ (emulsifier and dissolven). DAI (days after inoculations).

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Table 2 shows that rhizome oil, fraction B/2 and D/4 could already suppress *C. gloesporoides* colony growth at the dosage of only 100 ppm, with the inhibitor rate ranging from 6,87 - 9,16%. The highest inhibitor rate (100%) from all three treatments resulted from the use of 1000 ppm concentration level. However, the highest inhibitor rate for concentration level between 100 - 500 ppm came from treatment using fraction B/2, which is significantly different from results of rhizome oil and fraction D/4.

Table 2. Interaction between EO of rhizome and fractions, concentration levels and *C. Gleosporioides* colony diameter growth (DAI 9)

Treatment	Colony diameter (mm)	Inhibitor potential (%)
Rhizome oil		
0 ppm	85,00	2,58 i
100 ppm	79,75	8,59 g
250 ppm	65,50	24,92 e
500 ppm	32,25	63,03 c
1000 ppm	0,00	100,00 a
B/2 fraction		
0 ppm	85,00	2,58 i
100 ppm	79,25	9,16 g
250 ppm	65,75	24,64 e
500 ppm	31,25	64,18 b
1000 ppm	0,00	100,00 a
D/4 fraction		
0 ppm	85,00	2,58 i
100 ppm	81,25	6,87 h
250 ppm	67,50	22,63 f
500 ppm	39,50	54,72 d
1000 ppm	0,00	100,00 a

*Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT. K+ (emulsifier and dissolven). DAI (days after inoculations).*Table 2 is here.

Obvservation on colony biomass is presented in Table 3. It identifies treatment using fraction B/2 generated the best result of colony biomass supression-resulting in the lightest colony biomass at 41.10 mg and an inhibitor rate of 54.96%. This result was significantly different with the other two treatments (rhizome oil and fraction B/2). The higher the level of concentration (1000 ppm), the higher the inhibitor rate becomes (100%).

Table 3. Effect of rhizome and fractions EO, concentration levels and *C. gleosporioides* colony biomass growth (DAI 9)

Treatment	Colony biomass (mg)	Inhibitor potential (%)
Biopesticide:		
Rhizome oil	42,00	53,97 b
B/2	41,10	54,96 a
D/ 4/	45,35	50,30 c
Concentration level:		
0 ppm(K+)	87,75	3,83 e
100 ppm	65,58	28,12 d
250 ppm	43,42	52,41 c
500 ppm	17,33	81,00 b
1000 ppm	0,00	100,00 a
Control (without treatment)	91,25	0,00 -
CV (%)	-	2,81

Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT. K+ (emulsifier and dissolven). DAI (days after inoculations).

Similar to results shown in Table 1, 2, and 3, Table 4 also presented that the best results from interaction between EO of rhizome and fractions, concentration levels and *C. gleosporioides* colony biomass growth were generated from B/2 fraction which showed the lightest colony biomas and the highest inhibitor rate. Although further research is needed, it is presumed that dosage use of B/2 fraction between 500 ppm and 1000 ppm (750 ppm) could already result in a 100% inhibitor effect.

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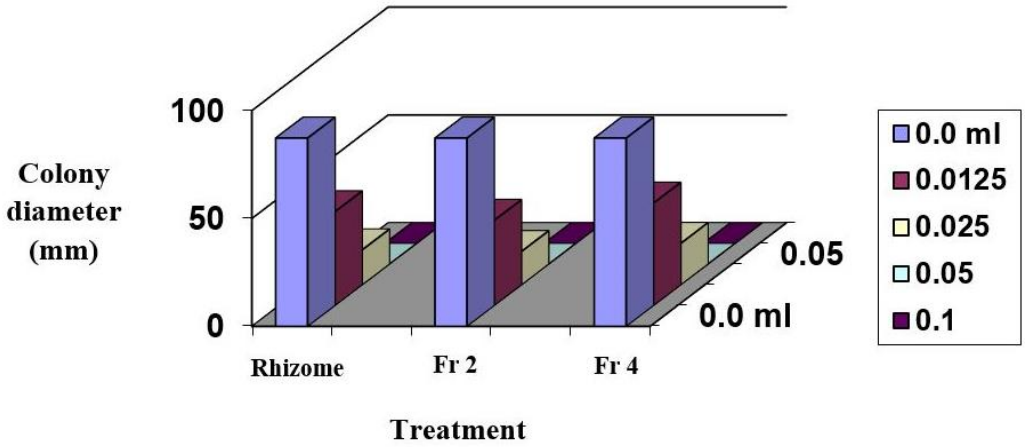
Table 4. Interaction between EO of rhizome and fractions, concentration levels and *C. gleosporioides* colony biomass growth (DAI 9)

Treatment	Colony biomass (mg)	Inhibitor potential (%)
Rhizome oil		
0 ppm	87,75	3,83 i
100 ppm	64,25	29,59 g
250 ppm	41,50	54,52 d
500 ppm	16,50	81,91 b
1000 ppm	0,00	100,00 a
B/ 2 fraction		
0 ppm	87,50	3,83 i
100 ppm	62,25	31,78 f
250 ppm	40,75	55,34 d
500 ppm	14,75	83,83 b
1000 ppm	0,00	100,00 a
D/4 fraction		
0 ppm	87,50	3,83 i
100 ppm	70,25	23,01 h
250 ppm	48,00	47,40 e
500 ppm	20,75	77,26 c
1000 ppm	0,00	100,00 a

Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT. K+ (emulsifier and dissolven). DAI (days after inoculations).

This study also discovered that volatile compounds from rhizome oil, B/2 and D/4 fractions could act as colony diameter growth inhibitors of *C. gleosporioides*. On the ninth day after inoculation, the highest inhibitor effect was shown by EO from B/2 fraction. Treatment by applying 0,0125ml EO/petri dish from rhizome oil, B/2 and D/4 fractions were able to suppress the colony diameter growth consecutively as much as 50,14%, 54,73%, dan 45,56%. Furthermore, each treatment of 0,05ml/petridishes were able to reach 80% inhibitor effect on the diameter growth of *C. gleosporioides* (Picture 1). According to Wong *et al* (30), aldehyd predominated compound in leaf and rhizome of *E. slahmong* has a great role in producing volatile odour. Nurmansyah (28) similarly reported the growth inhibitor effect of volatile compounds in cintronella oil and its fractions, where the use of 0,10 dan 0,075 ml/petri dish were able to suppress the growth of *Phytophthora palmivora* up to 100%.

Picture 1. Effect of rhizome and fraction EO volatile compounds towards *C. gleosporioides* colony diameter growth (9 DAI) (EO = essential oil, Fr2 =fraction B/2, Fr 4= fraction D/4).



From GC-MS analyses, the main compounds in rhizome oil, B/2 and D/4 fractions are presented in Table 5. Whereas primary compounds in leaf oil, A/1 and C/3 fractions from previous research conducted by Nasir and Nurmansyah (25) is presented in Table 6. Wong *et al* (30, 29) discovered difference in abundant compounds found in the leaf and the rhizome of the *Elettariopsis* group (YK. Kam). This research also discovered difference in primary chemical compounds in leaf oil and rhizome oil (Table 5 dan 6). Moreover, through GC-MS analyses, research from Wong *et al* (30) discovered 32 chemical compounds in *E. slahmong*

leaf oil, while this research discovered 80 chemical compounds using the same method. For this reason, it is suspected that the occurrence of different compounds are affected by agroecology dan other micro-climate influences, thus also affect to plant secondary metabolite.

Table 5. GCMS analyses result: major components in wild zingiberaceae rhizome essential oils and its fractions

Essential oil/fractions	Major Components
Rhizome oil	2-Tridecenal (39,81%), 2-decanoic acid (27,78%), 2-octenal (7,56%), nonanoic acid (3,85), nonanal 1,33%, Eucalyptol (2,13%), octanal 1,09 % and more than 40 other compounds (< 1%).
B/2 fraction	2-Tridecenal (26,57%), decanal (25,65%), 2-octenal (7,72%), octanal 6,86%, 2-decenyl acetat (5,44%), dedecenal (2,35%) and more than 30 other compounds (< 1%).
D/4 fraction	2-Tridecenal (19,41%), 2-dimethyl(3cloropropyl)sililoxymethyltetra (16,99%), 2-octenal (16,08%), 1-ethyl-1-(undec-10-enyl)oxy-1-silacyclopenta (16,99%), decanal (2,87%), octanal (2,84%), 6-tridecene (2,79%) more than 30 other compounds (< 1%).

Table 6. GCMS analyses result: main components *E. slahmong* leaf and fraction EO

Wild Zingiberaceae oil/ fraction	Main Components
E. slahmong leaf EO	2-decanoic acid (48,04%), nonanoic acid (9,18%), 2-octenal (8,97%), nonanal 2,96%, octanal 1,20% and 75 other components (< 1%).
A/1 Fraction	2-decanoic acid (31,65%), 2-octenal (17,01%), decenal (12,40%), 6-tetradecene (4,08%), nonanoic decen-1-ol acid (1,37%), and 51 other components (< 1%).
C/3 Fraction	(18,82%), decanal (12,95%), decenal (10,55%), octanal (8,42%), 6-tetradecene (3,24%), octenal (1,56%) and 66 other components (< 1%).

Compared to control, all treatments (rhizome oil, B/2 and D/4 fractions) exhibit inhibitory potential against *C. gleosporioides* (shown in Table 1, 2, 3, 4 and Picture 1), similar to results from Nasir dan Nurmansyah's previous research (25). From various research that has been conducted, it has been reported that the chemical compounds in essential oils such as 2-tridecenal, 2-decanoic acid, 2-octenal, nonanoicacid, decanal dan nonanal have antimicrobe characteristic (10, 17, 16, 14). The growth of the *Saccharomyces cerevisiae* fungus can be maximally deactivated by 2-tridicenal, whereas 2-octenal, which has the characteristic of a fungicide, can obstruct the growth of a microbe cell by suppressing the ATP hydrolic activity through the plasma membrane (10, 17). According to Kumar *et al* (16), 2-decanoic acid and its ester have substantial antifungal potency. At 100 ppm, decanoic acid and nonanoic acid are able to eliminate spores from basidiomycetes which cause white and brown roots (6). These conclusions are further supported by Knobloch *et al* (15) who stated that the chemical compounds in essential oils are antifungal, is able to penetrate the fungus' cell wall, can obstruct metabolism within the cell and at a specific concentration level could result in the fatality of the pathogen such as proven against *C. gleosporioides*. The chemical compounds mentioned above were also found in *E. slahmong* essential oils, presented in Table 5 and 6.

Despite the lack of detailed research on chemical compounds' biopesticide effectiveness contained in *E. slahmong,* this study and also previous research by Nasir and Nurmansyah (25), proved that all treatments of *E. slahmong* (leaf oil, rhizome oil and its fractions) have the potency to suppress the growth of *C. gleosporioides* (Table 5 and 6). Specifically in this research, the most promising biopesticide against *C. gleosporioides* were shown by B/2 fraction, whereas in the previous research it was the A/1 fraction (25). Research on the comparative effectiveness between these two fractions, A/1 and B/2, will be developed on red dragon fruit seedlings. This is an initial study to compare the effectiveness of *E. slahmong* chemical compounds (in fractions, rhizome and leaf oils) against plant-pathogen.

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