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Natural Product of Wild Zingiberaceae *Elettariopsis slahmong*: Biopesticide to Control the Vector of Banana Blood Disease Bacterium in West Sumatera, Indonesia.

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ABSTRACT

Banana is one of the most important food crops in Indonesia. Its production is greater than any other agricultural commodity. With a population of 230 million in 2010, banana was consumed up to three million tons in this country. However, Banana Blood Disease Bacterium (BDB), one of the most devastating banana pathogens in the world, which is only found in Indonesia, threatens not only the growth of this plant but also the lives and the livelihoods for most of the Indonesian society. BDB is caused by a lethal bacteria, *Ralstonia solanacearum* Phylotype-4, which infects a wide range of bananas, from bananas used for consumption to wild bananas. In West Sumatera, the disease killed 1.40% of bananas in 1998, and then increased dramatically to 37.9% in 2003. The total banana production dropped to 62% in this province. The search for controlling the vector has led to the pre-investigation of Wild Zingiberaceae *Elettariopsis slahmong* C.K.Lim which has a stink bug odor similar to a methidathion insecticide. The plant was collected around the conservation area of Lembah Anai in West Sumatra. The goal of this study was to investigate the effectiveness of natural insecticides compound contained in *E. slahmong* against *D. melanogaster*. This study tested the effect of *E. slahmong* on the mortality, antifeedant and repellent levels against *Drosophilla melanogaster*, the vector of BDB. The essential oil of *E. slahmong* was obtained by steam distillation of fresh rhizomes, pseudo stems and leaves. We found that the extract of *E. slahmong* significantly affected the mortality of *D. melanogaster* of 30-40% and also acted as an antifeedant (with success rate of 73-93%) and repellent (with success rate of 99-99.6%). The long term objective of this study is to develop green biopesticide to control BDB in Indonesia, based on an environmentally friendly pest management.
Keywords: BDB, *E. slahmong*, green-biopesticide, food crop, *D. melanogaster*

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INTRODUCTION

Although chemical pesticides play a significant role in agricultural development, the use of this type of pesticide has proven to be problematic: it causes pollution. In Indonesia it is a costly option with more than US$ 6 billion per year spent on this pesticide (Beritasatu.com, 2012). One of the best solutions to reduce pollution caused by the use of chemical pesticides is biopesticide. Based on these facts, the search for alternative ways for natural compounds which may have toxic, antifeedant and repellent characteristics has led to the investigation of wild Zingiberaceae (Nasir *et al*., 2009). The idea of using this plant for biopesticide comes from its distinct odor which is similar to methidathion. Methidation was the active component of an insecticide which was marketed largely in Indonesia some twenty five years ago.

To date, cultivated Zingiberaceae species have been used in everyday human lives (Chan *et al*., 2007; Dan *et al.,* 2007; Ibrahim *et al.,* 2007; Larsen, 2007; Aguinaldo, 2007) but only as spices for meat and fish-based cuisines in Indonesia, Malaysia and Thailand (Lim, 2003; Nasir, 2010). However, the role of wild Zingiberaceae as potential bioinsecticide, has not been tested. In this study, the "stink bug" oil of wild Zingiberaceae *Elettariopsis slahmong* C. K. Lim was used as biopesticide to control *Drosophilla melanogaster*. This is a pioneer work using *E. slahmong* for biopesticide.

D. melanogaster is one of the eight vectors of *Ralstonia sonalacearum* Phylotype 4 (Mairawita *et al*., 2012). *R. solanacearum*, the causal agent of BDB, is one of the most destructive bacteria of banana in the world which is only found in Indonesia (Baharuddin, 1994; Buddenhagen, 1995; Eden-Green and Sastraatmadja, 2000). This pathogen reduces banana production by up to 100% (Sulyo 1994; Nasir *et al.,* 2005; Nasir, 2011). This becomes a major problem when banana provides millions of job opportunities, dominates 35% of all fruit production, contributes to 52.6% of total fruit exports and is one of the most important crops of national food security, health and economic programs in the country (IAARD, 2005).

When BDB destroyed banana plantations in West Java (±35%) and South Sulawesi (±60%) in the mid of 1980s (Sulyo, 1994), the livelihoods of millions of banana growers in other parts of Indonesia, who depend on banana as sources of food and income, were threatened. Within five years, from 2000 to 2005, economic loss caused by BDB (together with Fusarium wilt) in Indonesia was Rp 80 billion (equivalent to US$ 4 million for those specific years) and more than 30.000 ha banana plantations were destroyed (Daryanto, 2002; Nasir *et al*., 1999; Nasir *et al.*, 2005). In West Sumatra, the percentage of banana destroyed by BDB increased dramatically from 1.40% in 1998 to 37.9 % in 2003 (Nasir *et al.,* 2005). It was calculated that total banana production within this five year period dropped to 62% in this province (Taylor, 2005). The situation since 2008-2009 appears to have worsened in West Sumatra, when eight vectors were detected as the active vectors of BDB (Mairawita *et al.,* 2012). Contrast with other studies stating that the distribution of BDB is only 25 km per year (Ploetz *et al.,* 2003), BDB distribution in Sumatra was reported to be a stunning 200 km per year (Setyobudi and Hermanto, 1999). We assume that the vectors have a significant role in speeding pathogen distribution. There is no significant method available to control both the vector and the pathogen to date.

METHODOLOGY

This study evaluates the effectiveness of *E. slahmong* essential oil: 1. on the level of mortality of *D. melanogaster*, 2. as a potential antifeedant of *D. melanogaster*, 3. as a potential repellent against *D. melanogaster*. Feeding inhibitions of *D. melanogaster* were observed by treating bait (topping application) with the essential oil. Treatments including control were conducted in five groups. *D. melanogaster* were starved for six hours before exposure. Work was developed in 300 ml transparent plastic bottles (tpb).

Isolation of essential oils

E. slahmong was collected from The Forest of Research and Education for Biology, Andalas University in Padang, West Sumatra, Indonesia. Plants were cleaned from soil and rinsed with water. Fresh material of the plants were separated into leaves, pseudostems and rhizomes which were then cut (0.5 cm long). Distillation process was conducted for seven hours to obtain oils from each part of the plant. Methanol was used for dilution of the oils.

Bait for *D. Melanogaster*

Rice flour (300 g), agar powder (20 g) and ripe banana (chopped: 500 g) were mixed into 1,500 ml of water. The mixture was cooked until it reached 100^0C, then cooled before kept in the refrigerator. Before used for bait, it was sterilized by using an autoclave for fifteen minutes.

Rearing of *D. Melanogaster*

D. melanogaster was placed in a 300 ml tpb (which already contained untreated bait) and then left for twenty four hours. After twenty four hours, the lid of the bottle was then covered with a gauze bandage. *D. melanogaster* was then reared for the treatments. *D. Melanogaster*: level of mortality, antifeedant and repellent potential

Treatment for each observation (level of mortality, antifeedant and repellent potential) used fifteen individuals of reared *D. melanogaster*. They were placed into tpb which contained 50 g of bait treated with 10 ppm of essential oils which was distilled from leaves, pseudostems and rhizomes. Data from each observation were taken after starved *D. melanogaster* stayed in tpb. The behavior of starved *D. melanogaster* was observed after one hour of exposure in tpb. Data were collected after twenty four hours of exposure, every day for five days.

Observation of the level of mortality

To calculate the level of mortality of *D. melanogaster*, *D. melanogaster* and bait in the tpb were sprayed once, according to treatment, with *E. slahmong* essential oil. Oil was sprayed onto the top of tpb, directed to the bottom inside part of tpb.

The percentage of mortality was calculated by using: $\% \text{ mortality} = \frac{\text{Number of death of } D.\ melanogaster}{\text{Total number of } D.\ melanogaster} \times 100\%$

Observation of the antifeedant characteristic

The effectiveness of *E. slahmong* as an antifeedant was observed by comparing the different percentage of the weight's bait (at the beginning and at end of the study) with control:

$$\% \text{ antifeedant} = \frac{\text{beginning weight of bait} - \text{end weight of bait (at the end of study)}}{\text{beginning weight of bait}} \times 100\%$$

Observation of the repellent characteristic

Study on the repellent was conducted by using two connected tpbs. The top neck of the two tpbs was connected (wrapped) by using sticky tape which created a tunnel between these two bottles. One of the tpb contained treated bait and the other was empty. Every 50g of treated baits produced from leaves, pseudostems and rhizomes were placed in separated tpbs. Fifteen individuals of starved *D. melanogaster* were then also transferred into these tpbs. Observation of the repellent characteristic was evaluated by calculating the amount of *D. melanogaster* which moved to the empty tbp in an attempt to avoid the treated bait.

RESULTS

Essential oils

Steam distillation of leaves (447.5 g), pseudostems (415 g) and roots (345 g) of *E. slahmong* produced 3.5 ml, 1.8 ml and 4.6 ml essential oils respectively. The pure oils distilled from leaves, pseudo stems and rhizomes have shown its different toxics.

Mortality

It was observed that the rate of mortality increased from day to day. Essential oils extracted from rhizome three resulted in the highest level of mortality (Figure 1). Even though there was a fluctuation of results, the average effectiveness was above 30% at the end of the observation day. The increasing rate of mortality was also found in the essential oils produced by pseudo stem. The highest mortality rate was caused by oils extracted from pseudo stem number three and five which were 30% (Figure 2).

Figure 1: Mortality rate of *Drosophilla melanogaster* treated with 10ppm extract (rhizome) of *Elettariopsis slahmong*

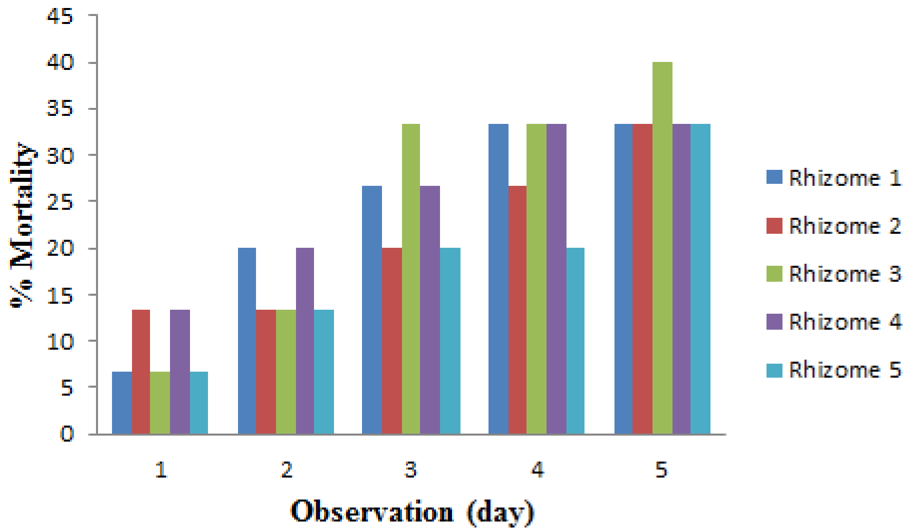
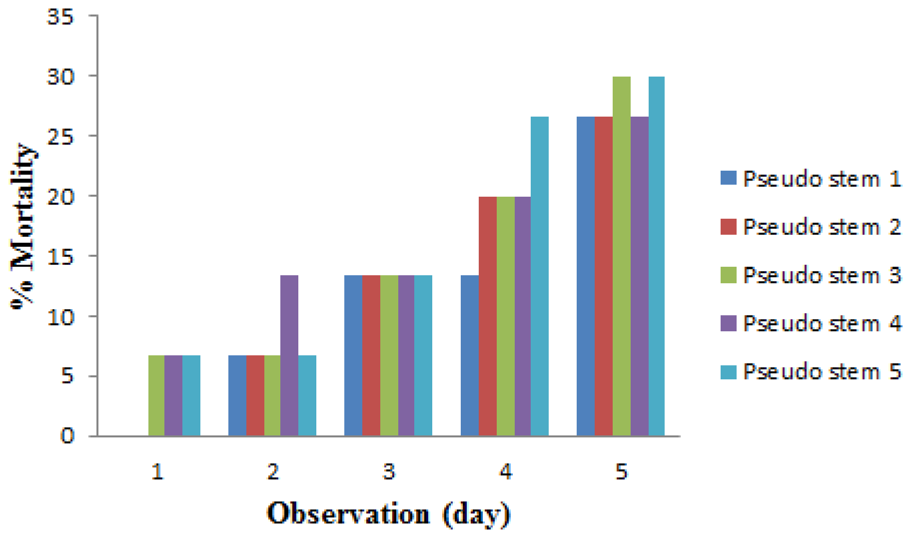


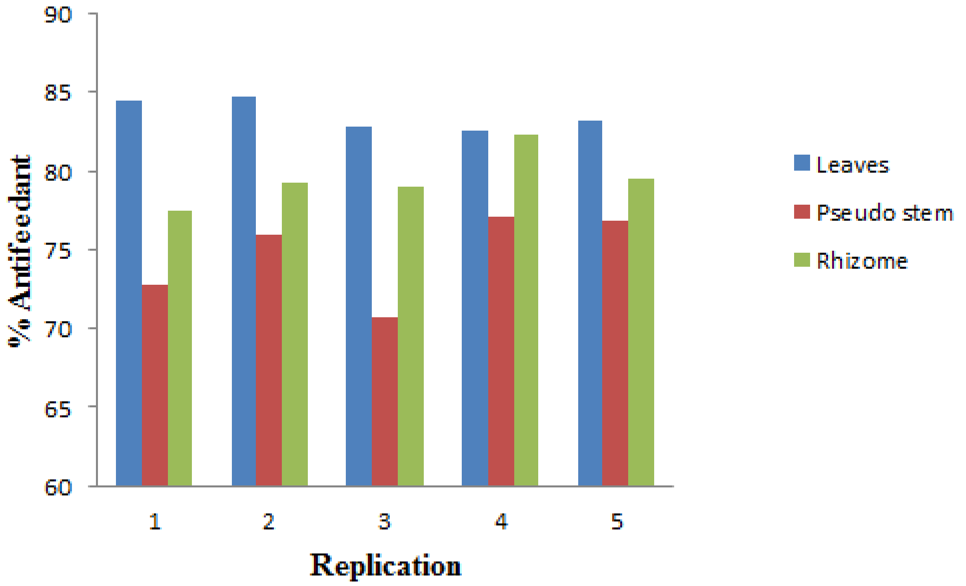
Figure 2: Mortality rate of *Drosophilla melanogaster treated with 10ppm extract (pseudo stem) of Elettariopsis slahmong*



Antifeedant

Data have shown that the essential oils extracted from the leaves, pseudo stems and rhizomes of *E. slahmong* have different effects on *D. melanogaster* (Figure 3). Antifeedant reaction to essential oils extracted from leaves was higher than oils from pseudo stems and rhizomes (above 80%), with low fluctuation among days of observation, decreasing slightly from the beginning to the end of observation days. The lowest effectiveness was found in the oils produced by pseudo stems; however an increase was seen at the end of observation days (up to 78%). For oils distilled from rhizomes, the highest result was found on day four (above 80%) then slightly decreased to 80% at the end of the observation.

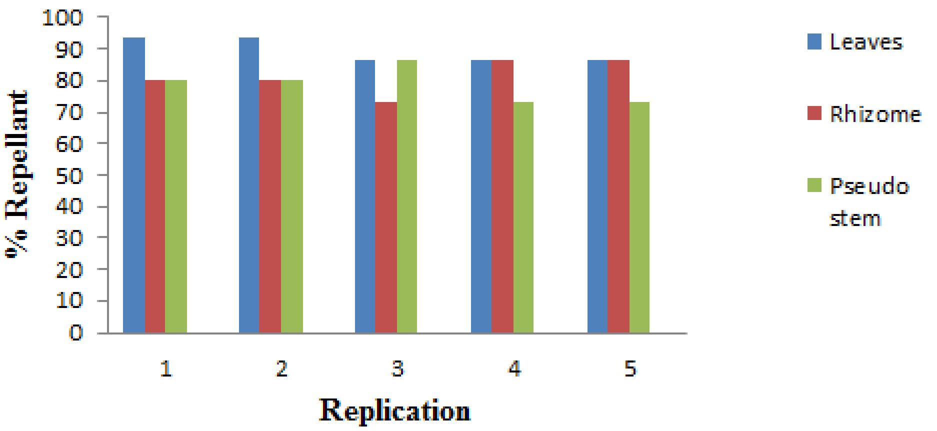
Figure 3: Comparison of the effectiveness of extract (leaves, pseudostems, and rhizomes) of *Elettariopsis slahmong* against *Drosophilla melanogaster*



Repellent

The highest effect of the repellant on *D. melanogaster* was from essential oils produced by leaves and pseudo stems (above 95%). At the end of the observation days, essential oils from pseudo stems have similar effectiveness with the oil produced by leaves. The lowest effectiveness recorded in the treatment was essential oils extracted from the rhizomes (Figure 4).

Figure 4. Comparison of the effectiveness of *Elettariopsis slahmong* extract (leaves, pseudo stems and rhizomes) against *Drosophilla melanogaster*.



DISCUSSION

All parts of wild Zingiberaceae *E. slahmong* have the potential to produce natural product for biopesticide. These findings are very significant considering the fact that the antifeedant and repellent results on *D. melanogaster* were higher than 80%. As pesticide, the result presents a large potential for a controlling system. In terms of concept of environmentally friendly ecosystem, the characters of this oil in controlling the vector are quite safe due to the low number of insects killed. We hope that in the future we are able to produce an *E. slahmong*-based product that is insect friendly. This desire is based by the fact that some insects are important to other food crops. Therefore our long term objective is that *E. slahmong* biopesticide works as a repellent of targeted insects and not as an exterminator. We are still not able to identify the essential components of *E. slahmong* which are potential for biopesticide. However, together with Kagoshima University in Japan, we are currently focusing on this area.

According to Picheansoonthon and Yupparach (2007), essential oil is the major chemical constituent in all parts of the Elettariopsis species. Terpenoid, a component in the oil of *E. slahmong* that produces the odor of the species, predominantly consists 46% of 2-octenal and 29% of 2-decenal. Different species of Elettariopsis have different odor due to the different composition and quantity of the terpenes. Amongst the Elettariopsis species, *E. slahmong* produce a significant stink bug odor (Lim, 2003) which has a similar odor with a chemical pesticide containing methadation which was marketed largely in Indonesia. These characters are assumed to have significant effects on the insects. Current study has only mentioned that oils from leaves and rhizomes have the potential to be used in antibacterial activities. This study is the first report mentioning the impact of the oils to control insect.

In this study, the best result in controlling *D. melanogaster* was essential oil extracted from the leaves of *E. slahmong*. It is assumed that natural biopesticide components from the leaves are more toxic than the oil extracted from pseudo stems and rhizomes. Field study also found that stink bug odor from the leaves was more pungent than pseudo stems and rhizomes. According to Chan *et al*., (2007), total phenol content and antioxidant activity in leaves of other wild species of Zingiberacea (*Etlingera*) were seven to eight times higher than rhizomes. The fluctuation of the effectiveness in this study is caused by the factors of growth environment which affects the quality of the components (leaves, pseudo stems and rhizomes) used in this study (Lim 2003, Chairgulprasert *et al*., 2008, Chan *et al*., 2007).

CONCLUSION

Based on its characters, the essential oils extracted from the leaves of *Elattariopsis slahmong* have a high potential to be used for biopesticide.

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