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Piper betle as bio-pesticides for house flies (musca domestica)

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Abstract---*Piper betle* is a native Indonesian vines with essential oil composition consisting of *phenol compounds* and *phenolic derivatives of propenyl, eugenol, carvacrol, chavicol, cavibetol, alylpirokatekol, cavibetol acetate, alylpirokatekol acetate, cineol, estragole, methylene, p-cyan sesquiterpenes*, and *flavonoid compounds*; which works as a nerve poison and results in the rapid death of insects. The study used a Factorial design with CRD Structure, 2 replications. It aims to determine the effect of *Piper betle* leaf extract on the death of *Musca domestica*, at 6 variations of concentration (0%, 5%, 10%, 15%, 20%, and 25%) and 4 variations of contact time (15, 30, 60, and 120 minutes). The research subject was *Musca domestica* aged 5 days which was developed from residential areas, with a total of 10 treatments each. The analysis technique uses ANOVA with SAS 9.4. The research proves that *Piper betle* can be used as an alternative bio-pesticides to *Musca domestica*. The influencing variables are the concentration of *Piper betle* extract (P-value <0,000), contact time (P-value <0,000), and concentration and contact time interactions (P-value = 0,0007). Concentration shows a very strong association with the death of *Musca domestica* and all three variables show that the Power of Test is close to 100%.

Keyword---Musca domestica, Piper betle, Concentration, Contact time, Bio-pesticides.

Introduction

Diarrheal is still a global problem with high morbidity and mortality, especially in developing countries, and as one of the main causes of high rates of morbidity and mortality of children in the world. It is estimated that more than 10 million

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children less than 5 years die each year (Aug. 2011). In Lampung Province, the incidence of morbidity (diarrhea rate) for all age groups from 2014-2015 tended to increase, from 9.8 per 1,000 population to 21.4 per 1,000 population (Lampung, 2016; Lampung Province Health Profile, 2016). Several factors that directly or indirectly become a driver of diarrhea are agent, vector, host, environment and human behavior. Diarrhea can occur due to consuming contaminated food, one important aspect is the presence of flies (Agtini, 2011; Kusariana, 2013; Sucipto, 2011).

Flies that are often found in residential neighborhoods are house flies (*Musca domestica*). *Musca domestica* breeds rapidly and generally perishes in human and animal feces and other organic materials such as meat, fruit, fish, and fresh and decayed plants (Acharya, 2015; Attaullah et al., 2019; Budiman & Suyono, 2011; Geden, 2012; Klauck et al., 2018). In addition to improving hygiene and environmental sanitation, efforts to control and eradicate can be done directly by physical, biological, and chemical methods. Chemical control using insecticide has harmful effects on non-target insects, as well as human and environmental exposure. Therefore, it is necessary to find plant-based insecticide as an alternative replacement. Bio-insecticide is a group of insecticides derived from plants, such as *pyrethrum, pyrethrin, nicotine, rotenone, limonene, azadirachtin*, and others. The use of bio-insecticides, besides helping to reduce the population of flies, is also safe for humans and the environment (Acharya, 2015; Anisah & Sukesi, 2018; Attaullah et al., 2019; Geden, 2012; Klauck et al., 2018; Yunianti Lapida, 2016).

Indonesia is rich in various types of plants that can be used as natural pesticides. More than 24,000 plant species belonging to 255 families are reported to contain pesticides. The advantage of bio-pesticides is that they do not easily cause resistance. Plants have potential as insecticides because they contain bioactive compounds such as *saponins, flavonoids, alkaloids, tannins, and alkenyl phenols* (Kusariana, 2013; Rohimatun & Suriati, 2011). Green betel (*Piper betle*) is a native Indonesian vines that can reach a height of 15 m. The composition of essential oils consists of phenol compounds and phenolic derivatives of propenyl, eugenol, carvacrol, chavikol, cavibetol, alylpirokatekol, cavibetol acetate, alylpirokatekol acetate, cineol, estragole, methyl ether, p-cymene, karyofilene, cadin and cretin; these compounds work as nerve poisons and cause rapid knockdown and insect death (Adi Parwata, Susanah Rita, & Yoga, 2009; Armianty & Mattulada, 2014; Attaullah et al., 2019; Geden, 2012; Putu, Kusuma, & Muderawan, 2016).

Materials and Methods

This study using Factorial Design with Completely Random Design Structure, 2 times replication. It aims to determine the effect of *Piper betle* leaf extract on the death of *Musca domestica*, in several variations of concentration (5%, 10%, 15%, 20%, 25%, and 0% as a control) and contact time (15 minutes, 30 minutes, 60 minutes , and 120 minutes). The research subject was *Musca domestica* aged 5 days which was breed from residential areas, with 10 flies each treatment.

- Rearing *Musca domestica*
  The study was conducted at the Entomology Laboratory, Department of Environmental Health, Politeknik Kesehatan Tanjungkarang. Cages of 40 cm x 40 cm x 60 cm were used for the rearing of house flies. The cages are covered with mesh gauze and have sleeves on the front and back. The forearm is used for the introduction of milk solution, sugar solution, and oviposition tray. A cotton pad soaked in a sugar solution (3%) is placed in a cage to provide sugar and water. Adult food consisting of glucose (50%) and MacConkey agar powder (50%) are prepared. Sugar solutions and food are provided every day. Fresh milk soaked in cotton has been equipped with flies that have only appeared for three days to increase egg production and then they are given a solution of milk sugar in the same manner as mentioned above. For larval rearing, a sterilized wheat bran mixture (38 g), milk powder (2 g) and 60 ml of water is used as described by (Attaullah et al., 2019; Pavela, 2008).
- Ekstrak *Piper betle*
  Piper betle leaves are obtained from traditional markets in Bandar Lampung City, and then identified from the Department of Biology, Faculty of Mathematics and Natural Sciences, Lampung University. To remove dust, the plants are thoroughly cleaned using destilated water, then dried in the shade for fifteen days. Crushed dried plants were again in dry-oven at 60^0C for 20 minutes. For extraction, 100 g of the sample is mixed with 300 ml of ether and then the mixture is subjected to a Rotary Shaker for 24 hours at 220 rpm. Then filtering is done with the help of Whatman filter paper. The filtrate is stored in a sterilized gray airtight glass bottle and stored in the refrigerator at 4^0C for use. Concentrations namely, 5, 10, 15, 20 and 25% are prepared using destilated water as a solvent from the stock solution. Done as described (Anisah & Sukesi, 2018; Attaullah et al., 2019; Kusariana, 2013; Pavela, 2008; Putu et al., 2016; Yunianti Lapida, 2016).
- *Bioassay*
  The test bottle used is a 250 ml volume glass bottle (surface area of 180 cm^2). Into each glass bottle sprayed 1 ml of bio-pesticides solution according to the concentration of the study (5%, 10%, 15%, 20%, and 25%). So that the insecticide sticks evenly to the entire surface of the bottle and the bottle cap, the glass bottle is rotated and turned over and over again. Furthermore, the bottle is left open for 1 hour so that the bio-pesticides dries completely. The preparation of sample bottles for control was carried out in the same way, but only using distilled water as a bio-pesticides solvent (Brogdon & Chan, n.d.).
  A total of 10 flies derived from rearing were put into an insecticide glass bottle during the specified time contact period (15 minutes, 30 minutes, 60 minutes, and 120 minutes). Experiment with 2 replication. As a control used 10 flies from rearing treated with aquadest. Then, the flies were transferred to the cage (25x25x25 cm^2) and left for 24 hours. Inside the cage is fed 50% sugar water solution or liquid milk, and the cage is placed in a room with a temperature of 27-30°C and humidity 60-80%.
- Analisis Statistik
  Data were analyzed to determine the effect of each and the combined effect of the research variables, namely concentration and contact time. The analysis technique used ANOVA with SAS 9.4 software.

Results

Model

The results of analysis ANOVA found that the value of F = 44.40 and p-value < 0.0001, indicating the significance model. The value of R2 = 97.70%, showed that 97.70% diversity of concentration, contact time and death of *Musca domestica* can be explained by the model.

Death by Concentration

In this study, testing was conducted to determine the effect of the concentration of *Piper betle* leaf extract on the death of *Musca domestica* in six variations of concentration, namely 5%, 10%, 15%, 20%, 25% and 0% (Figure 1).

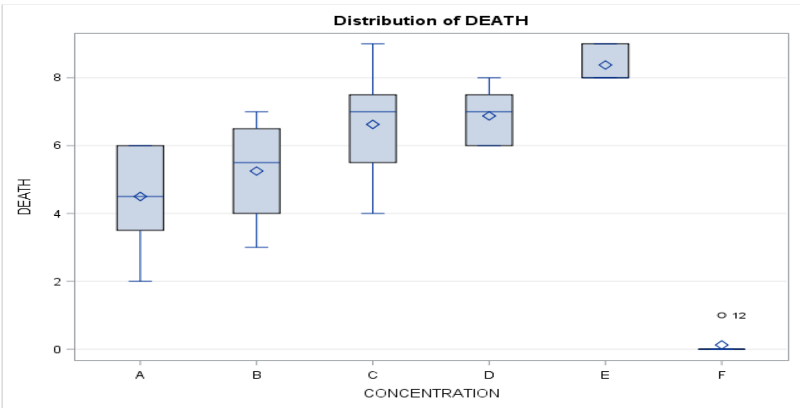

Figure 1. *Musca domestica*'s death by concentration

In the picture (Fig. 1), the highest mortality is at extract concentration of 25%, which is as much as 8.37 individuals (SD = 0.51), and the lowest at concentrations of 5%, as many as 4.50 individuals (SD = 1.5). Whereas in the control, the death rate was 0.12 (SD = 0.35). At a concentration of 15%, there were deaths equal to a concentration of 25%, but a wide data distribution was seen. To determine the effect of concentration on the death of *Musca domestica.* hypothesis testing was conducted as follows:

$H_0 : \alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = \alpha_5 = \alpha_6$
H_0 : At least one of them is different

Statistical analysis results obtained a value of F = 175.42 and p-value 0.0001, proving the difference in the number of deaths based on concentration, or it can be concluded that there is an influence of concentration on the number of *Musca domestica* deaths. In general, differences in mortality occur in all groups, except between 5-10% and 15-20% (Figure 2).

Concentration	5%	10%	15%	20%	25%	0%
Mean	4.500	5.250	6.625	6.875	8.375	0.125

Figure 2. Tukey test results based on concentration

Death by Time Contact

Tests were carried out to determine the effect of contact time on the death of *Musca domestica* in four-time variations; 15, 30, 60, 120 minutes (Figure 1). The results showed that the highest mortality was at a contact time of 120 minutes, which was 6.16 (SD = 3.06), and the lowest at contact time was 15 minutes, as many as 4.08 (SD = 2.71). At 15 minutes contact time, there is a very wide distribution of deaths

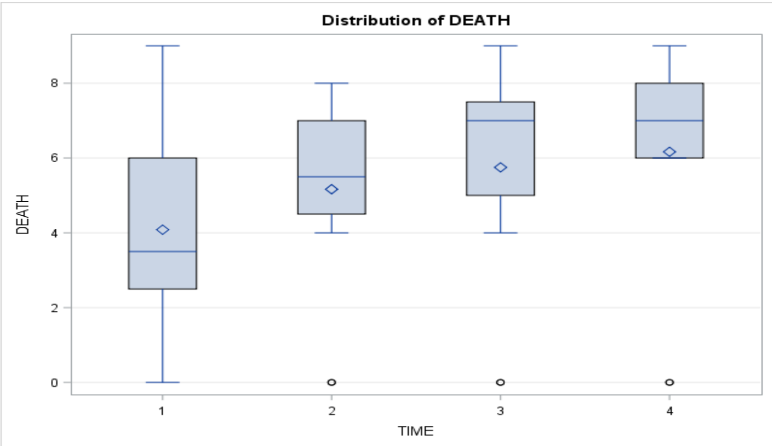

Figure 3. *Musca domestica*'s death by contact time

To find out the effect of contact time on the death of *Musca domestica*, hypothesis testing was conducted as follows:

$H_0 : \tau_1 = \tau_2 = \tau_3 = \tau_4$
H_0 : At least one of them is different

Statistical analysis results obtained a value of F = 26.15 and p-value <0.0001, proving the difference in the number of deaths based on contact time, it can be concluded that there is an influence of contact time on the death of *Musca domestica*. In general, differences in mortality occur in all groups, except between 30-60 minutes and 60-120 minutes (Figure 3).

Time	15	30	60	120
Mean	4.083	5.166	5.750	6.166

Figure 4. Tukey test results based on concentration

Death Based on Concentration and Contact Time

The results obtained the highest mortality at 25% extract concentration in all test contact times, the lowest at 5% concentration with 15 minutes contact time. In control, there were no deaths at 30, 60, and 120 minutes of contact time (Figure 5)

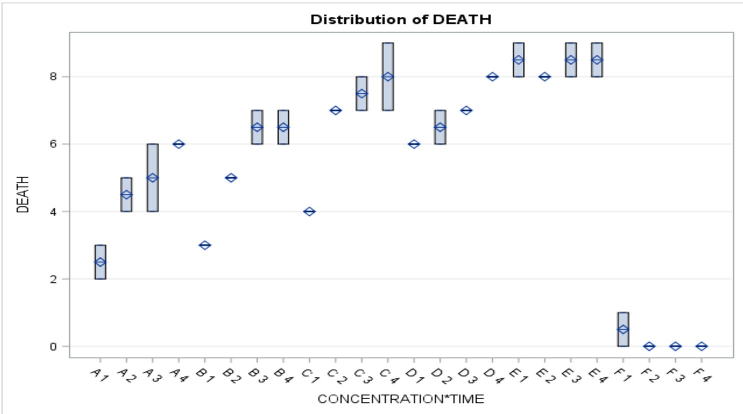

Figure 5. Death of *Musca domestica* based on concentration and contact time

The effect of interaction concentration and contact time on the death of *Musca domestica*, hypothesis testing is as follows:

$H_0 : (\alpha\tau)_1 = (\alpha\tau)_2 = (\alpha\tau)_3 = (\alpha\tau)_4 = (\alpha\tau)_5 = (\alpha\tau)_6$
H_0 : At least one of them is different

Statistical analysis results obtained a value of $F = 4.37$ and p-value <0.0007, proving the difference in the number of deaths based on concentration and contact time. It was concluded that there was an influence of concentration and contact time on the number of *Musca domestica* deaths (Table 1). In this study also calculated the Partial Omega Square value to determine the strength of the relationship (Cohen, 1988). The results show that the relationship between concentration and death of *Musca domestica*, ignoring contact time and interaction interactions and contact time show a strong relationship (>0.14). The relationship between contact time and death of *Musca domestica*, ignoring concentration and interaction and contact time showed a moderate relationship (>0.06). The relationship between concentration interaction and contact time with the death of *Musca domestica*, ignoring concentration and contact time showed a weak relationship (>0.01). Effect Size was also calculated to determine the effect of treatment (Cohen, 1988). The concentration variable gives a large effect size to the model (>0.40). Contact time gives a moderate effect size (>0.25), whereas interaction and contact time give a small effect size (>0.01). The value of the Power of Test of the three variables approaches 1.00, explaining that the probability to get significant results is close to 100% (Table 1).

Table 1
Statistical analysis by ANOVA

	DF	Mean Square	F Value	Pr > F	Omega Square	Effect Size	Power of Test
Model	23	16.648	44.4	<.0001			
Concentration	5	65.783	175.42	<.0001	0.431	0.870	1.000
Contact time	3	9.805	26.15	<.0001	0.061	0.256	1.000
Concentration * Contact time	15	1.638	4.37	0.0007	0.042	0.209	0.996
Error	24	0.375					
R Square	0.9770						

Discussion

The study found that the higher the, the greater the content of the active ingredient in green betel leaf extract which plays a role in causing death in flies (Armianty & Mattulada, 2014; Attaullah et al., 2019; Klauck et al., 2018; Scott, Alefantis, Kaufman, & Rutz, 2000, Yudantari & Sritamin, Made Singarsa, 2015) . *Piperimida* is an active compound that works to attack the nerves of flies, resulting in knockdown and even death. Flies that are inserted into the test bottle will suck, lick and absorb green betel leaf extract, then the extract will enter the fly's body and start working to damage the nervous system of the fly (Boozer, n.d.; Cohen, 1988; Scott et al., 2000). Biological activity of essential oils contained in green betel leaf extract which are contact poisons, respiratory toxins, reduce appetite and inhibit the laying of eggs, and inhibit growth (Armianty & Mattulada, 2014; Attaullah et al., 2019; Pavela, 2008, Ariati & Sukowati, 2011) .

Besides, the essential oil content found in betel leaves has a high pesticide activity. Bioactive compounds contained in extracts of biological materials used as pesticides affect the muscular nervous system, hormonal balance, reproduction, antifeedant and respiratory system OPT, causing death (Dwipayana, Wijaya, & Sritamin, 2017). The higher the concentration of betel leaf extract, the aromatic compounds in betel leaves become more concentrated so that the insect does not like (Armianty & Mattulada, 2014; Attaullah et al., 2019; Klauck et al., 2018; Scott, Alefantis, Kaufman, & Rutz, 2000, Yudantari & Sritamin, Made Singarsa, 2015). Another compound in the green betel leaf that might also be able to kill flies is *tannin* (Adibah & Dharmana, 2017; Anisah & Sukesi, 2018; Attaullah et al., 2019; Dwipayana et al., 2017; Putu et al., 2016; Yunianti Lapida, 2016). This compound functions as a contact poison and stomach poison. Suctioning or licking green betel leaf extract which is exposed on a glass bottle becomes direct contact between the fly and the bio-pesticides, then liquid extract enters the body of the fly through the body's wall, resulting in shrinking of the fly's body tissue so that the flies cannot be well protected and cause death.

The study found the longer contact time would increase mortality. A long-time allows more and more active compounds to be sucked by flies into the body of the fly, causing death (Anisah & Sukesi, 2018; Geden, 2012, Adi Parwata et al., 2009). After the fly is contacted with the *Piper betle* extract, the fly is placed in cages with temperature and humidity that is continuously monitored. The cage used is made in such a way that air remains free to enter the cage which allows the fly to continue to breathe. Increased fly mortality shows that compounds in green betle leaves work to inhibit the respiratory system of flies (Dwipayana et al., 2017; Hastutiek, 2007)

Conclusion

This research proves that green betel leaf extract *(Piper betle)* can be used as an alternative bio-pesticides to house flies *(Musca domestica).* The highest mortality is at 25% concentration and contact time is 60 and 120 minutes. In the future, research is needed to determine the killing power of non-target insects.

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