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The Effectiveness of *Betle Leaf (Piper betle L.)* Extract as a Bio-pesticide for Controlled of Houseflies (*Musca domestica L.*)

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Abstract

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BACKGROUND: The housefly, *Musca domestica L.*, spreads disease by contaminating food. However, chemical insecticides used to combat houseflies can pollute the environment and can harm non-target insects and humans; this demands safer alternatives and pest control options.

AIM: This study aims to evaluate the effectiveness of *Piper betle L.* leaf extract as a bio-pesticide against houseflies.

METHODS: This study using a factorial design with six variations in concentration (0%, 5%, 10%, 15%, 20%, and 25%), four variations in contact time (15, 30, 60, and 120 minutes), and 5-day-old *M. domestica* adults that were bred from residential areas.

RESULTS: results show that mortality was affected by concentration (p-value < 0.000), contact time (p-value < 0.000), and the interaction between concentration and contact time (p-value = 0.0007). Of the three, concentration had the greatest effect.

CONCLUSION: As such, the use of *Piper betle L.* extract is a suitable, cheap, and environmentally safe method for controlling *M. domestica*.

Introduction

Diarrhea is a leading killer of children worldwide, accounting for around 8% of all deaths among children under 5 years of age. Most of these deaths occur in South Asia and sub-Saharan Africa [1], and in low-income countries [2]. Thus, controlling diarrhea is essential if the world desires to achieve sustainable development goals for child health [3]. In Indonesia, diarrhea affects 11% of children under 5 and is the second-largest cause of death [4].

Several interventions have been employed to reduce diarrhea: Improvements in water, sanitation, and hygiene facilities; exclusive breastfeeding, adequate complementary feeding, and continued breastfeeding; Vitamin A supplementation; and a preventative rotavirus vaccine [1], [5], [6], [7]. There is strong evidence that flies are vectors of infectious diseases, especially diarrhea [8], [9]. The efficacy of housefly control as prevention for infectious diarrhea in community settings has long been questioned. Vector control can be accomplished by reducing or eliminating breeding sites, reducing housefly attraction sources, and preventing interaction between flies and food, food utensils, people, or disease-causing organisms [3], [6], [10], [11].

The role of houseflies as mechanical vectors for several diarrhea-causing agents is relatively

well-established [3], [6], [12], [13], [14], [15]. The housefly, *Musca domestica L.* (Diptera: Muscidae), is a vector for over 100 serious pathogens, including typhoid, cholera, salmonellosis, shigellosis, dysentery, anthrax, and parasitic worms [3], [16], [17], [18], [19], [20], [21]. Most infectious diarrhea transmission is fecal-to-oral, although the routes taken from feces to ingestion by a host can vary. The main causes of infectious diarrhea are various types of pathogenic bacteria from human feces [22]. *M. domestica* breeds rapidly and generally settles in human and animal feces, as well as other organic materials such as meat, fruit, and fresh and decayed plant matter [23], [24], [25], [26].

Insecticides are the primary method for controlling houseflies because they are fast, cheap, and convenient. Chemical insecticides such as pyrethroids, organophosphates, and carbamates are used globally. Most of these products no longer exhibit the expected efficacy due to high resistance in pests [16], [17], [18], [27], [28], and the misuse of some insecticides shortens the life of the compounds and pollutes the environment [16], [24], causing harm to non-target insects and humans [23]. Therefore, it is necessary to find plant-based insecticides as an alternative. Bio-insecticides are a group of insecticides derived from plants, such as *Pyrethrum*, pyrethrin, nicotine, rotenone, limonene, and azadirachtin. The use of bio-insecticides for reducing

housefly populations is safe for humans and the environment [17], [18], [23], [24], [29], [30].

There are many types of plants in Indonesia that can be used to make natural pesticides. Over 24,000 plant species in 255 families are reported to contain pesticides. Plants with potential as sources of insecticides contain bioactive compounds such as saponins, flavonoids, alkaloids, tannins, and alkenyl phenols [29], [30], [31]. *Piper betle* L. (*Piperaceae*) is a native Indonesian vine that can reach a height of 15 m; it contains phenol compounds and phenol derivatives from propenyl, eugenol, carvacrol, chavicol, chavibetol, alypirokatekol, cavibetol acetate, alypirokatekol acetate, cineol, estragole, chavibetol methyl ether, p-cymene, caryophyllene, cadin, and cretin. These compounds act as neurotoxins and causes rapid damage and death in insects. These compounds act as neurotoxins and causes rapid damage and death in insects [29], [31], [32], [33]. This study aims to assess the effectiveness of *P. betle* L. leaf extract as a bio-pesticide for *M. domestica* L.

Materials and Methods

Ethical considerations

Ethical considerations were approved by the Health Research Ethics Committee, Tanjungkarang Health Polytechnic, number 162/EC/KEP-TJK.

Study design and setting

This study utilized a factorial design with two replication experiments, six levels of concentration (5%, 10%, 15%, 20%, and 25%, with 0% as a control), and four levels of contact time (15 min, 30 min, 60 min, and 120 min). The research subjects were 5-day-old *M. domestica* L. bred from residential areas, with ten flies per treatment.

M. domestica L.

The study was conducted at the Entomology Laboratory in the Department of Environmental Health, Tanjungkarang Health Polytechnic. Cages that were 45 cm × 47 cm × 47 cm were used for rearing the houseflies used in the study. The cages were covered with mesh gauze and had sleeves on the front and back. A researcher used their forearm to introduce a milk solution, a sugar solution, and an oviposition tray. A cotton pad soaked in a 3% sugar solution was placed in each cage to provide sugar and water. Adult food consisted of 50% glucose and 50% MacConkey broth powder. Sugar solutions and food were provided every day. Cotton pads soaked in fresh milk were provided

to flies for 3 days, in order to increase egg production. After 3 days, the flies were given a solution of milk and sugar. For larval rearing, a sterilized wheat bran mixture (38 g), milk powder (2 g), and 60 ml of water were used as described by Pavela [17], Zahoor *et al.* [30].

The cage for rearing flies was purchased from PD Karya Mitra Usaha, Indonesia. Glucose, and MacConkey broth from MERCK production. Sugar and fresh milk are bought from the market.

P. betle L.

P. betle L. leaves were obtained from traditional markets in Bandar Lampung City and then identified by the Department of Biology at Lampung University. The plants were cleaned using distilled water, and then dried in the shade for 15 days. Crushed, dried plants were placed in a dry-oven at 60°C for 20 min. For extraction, 100 g of the sample was mixed with 300 ml of ethanol, and then the mixture was subjected to a rotary shaker for 24 h at 220 rpm. The mixture was filtered using Whatman filter paper. The filtrate was stored in a sterilized, gray, airtight glass bottle and stored in a refrigerator at 40°C for later use. Concentrations (5%, 10%, 15%, 20%, and 25%) were prepared from the stock solution using distilled water as a solvent. Procedures were similar to those described previously [17], [29], [30], [32].

Waring (Model No. 8010 BU) used to crushed *P. betle* L. leaves, and drying used a Dry-oven (Model No. 01034250001100) from WTB Binder, Germany. Stirring for extraction with a Rotary Shaker (Model No SG-400W-2019A) from Oshiyama, Japan, and evaporated with a water bath (Model No DIN12876-3-K1) produced by Memmert, Germany. All materials were weighed with Analytical balance (Model No. ITA1904375) from Bel Engineering, Italy.

Bioassay

The test bottles used were 250 mL glass bottles with a surface area of 180 cm² (Model No 10121801365, from Duran, Malaysia). Each glass bottle was sprayed with 1 mL of a bio-pesticide solution with the correct concentration (5%, 10%, 15%, 20%, or 25%). The insecticide stuck evenly to the entire surface of the bottle and bottle cap; the glass bottle was rotated multiple times. The bottle was left open for 1 h afterward so that the bio-pesticide could dry completely. The sample bottles for the control were prepared in the same manner, but distilled water was used instead of a bio-pesticide solvent [34], [35].

A total of 10 adult houseflies (aged 5 days) derived from rearing were put into each insecticide glass bottle for a specified amount of time (15 min, 30 min, 60 min, or 120 min). For the control, ten flies were treated with distilled water. After exposure, the houseflies were transferred to the cages (25 cm ×

25 cm × 25 cm) and left for 24 h. The flies were given a 50% sugar water solution or liquid milk; the cage was placed in a room with a temperature of 27–30°C and a humidity of 60–80%.

Statistical analysis

Data were analyzed to determine the individual and combined effects of the research variables (concentration and contact time) using a two-way analysis of variance (ANOVA). Tukey's test determines the individual means, which are significantly different from a set of means. To interpreting the strength association, we used Omega square, following Cohen's guidelines ($\omega^2 = 0.01$ is a small association, $\omega^2 = 0.06$ is medium, and $\omega^2 = 0.14$ is strong). All analyzed using SAS 9.4 software.

Results

Model

The ANOVA test found an F-value of 44.40 and $p < 0.0001$, indicating the significance of the model (Table 1). The R^2 value was 97.70%, meaning that 97.70% of the diversity in the data for concentration, contact time, and *M. domestica* mortality could be explained by the model.

Table 1: Statistical analysis by two-way ANOVA

Source	DF	Mean Square	F-value	Pr > F	Omega Square	Effect Size	Power of Test
Model	23	16.648	44.4	<0.0001			
Concentration	5	65.783	175.42	<0.0001	0.431	0.870	1.000
Contact time	3	9.805	26.15	<0.0001	0.061	0.256	1.000
Concentration × Contact time	15	1.638	4.37	0.0007	0.042	0.209	0.996
R Square		0.9770					

ANOVA: Analysis of variance.

The partial omega squared value was calculated to determine the strength of the relationships among concentration, contact time, and number of deaths [36]. The results show that the relationship between concentration and number of deaths, ignoring contact time and the interaction of concentration and contact time, was strong ($\omega^2 > 0.14$). The relationship between contact time and number of deaths, ignoring concentration and the interaction between contact time and concentration, was moderate ($\omega^2 > 0.06$). The relationship between the number of deaths and the interaction of concentration and contact time, ignoring concentration and contact time, was weak ($\omega^2 > 0.01$).

Effect size was calculated to determine the effect of treatment [36]. Concentration had a significant effect on the model (effect size > 0.40). Contact time had a moderate effect (effect size > 0.25), and the interaction between contact time and concentration had a weak effect (effect size > 0.01). The power of the test for the three variables approached 1.00, meaning that

the probability of getting significant results was close to 100% (Table 1).

Mortality by extract concentration

In this study, testing was conducted to determine the effect of *P. betle* L. leaf extract concentration on the mortality of *M. domestica*. Six concentrations were used: 5%, 10%, 15%, 20%, 25%, and 0% (Figure 1).

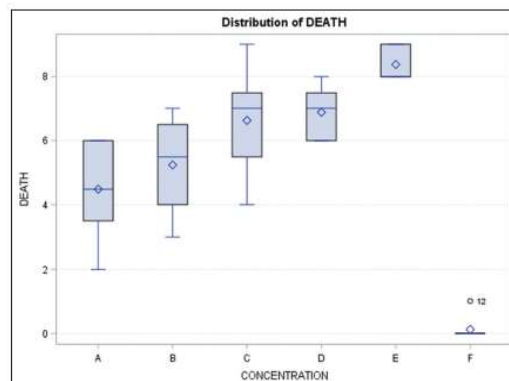


Figure 1: *Musca domestica* mortality by concentration (%)

In Figure 1, the highest mortality was at an extract concentration of 25%, with as many as 8.37 flies dead (SD = 0.51); the lowest mortality was at a concentration of 5%, with as many as 4.50 flies dead (SD = 1.51). In the control, the death rate was 0.12 (SD = 0.35). The number of deaths at a concentration of 15% was equal to the number of deaths at a concentration of 25%, but there was a wider distribution of data.

Statistical analysis found an F-value of 175.42 and $p < 0.0001$. As such, concentration influences the number of *M. domestica* deaths (Table 1). Overall, differences in mortality occurred among all groups, except between 5% and 10% and between 15%, 20% and 20% (Table 2).

Table 2: Turkey test results for concentration

Concentration (%)	5	10	15	20	25
5	—	1.000	0.031*	0.005*	0.000*
10	1.000	—	0.155	0.031*	0.000*
15	0.031*	0.155	—	1.000	0.092
20	0.005*	0.031*	1.000	—	0.411
25	0.000*	0.000*	0.092	0.411	—

*Significant.

Death by contact time

Tests were carried out to determine the effect of contact time on the death of *M. domestica*; there were four durations: 15, 30, 60, and 120 min (Figure 2). The results showed that the highest mortality (6.16 flies, SD = 3.06) was at 20 min of contact, and the lowest (4.08 flies, SD = 2.71) was at 15 min of contact. At 15 min of contact, the deaths were very widely distributed.

Statistical analysis found an F-value of 26.15 and a $p < 0.0001$, demonstrating that the number of

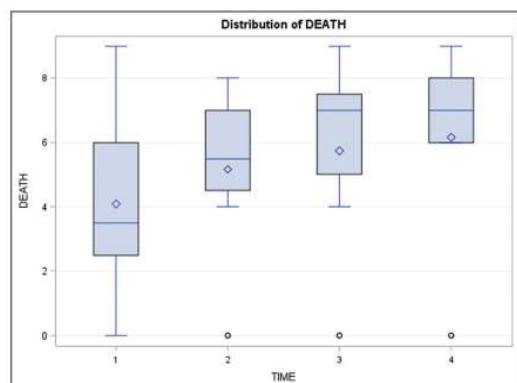


Figure 2: *Musca domestica* mortality by contact time (min)

deaths is influenced by contact time (Table 1). In general, there were no differences in mortality at contact times between 30 and 60 min, and between 60 and 120 min (Table 3).

Table 3: Turkey test results for contact time

Contact Time (min)	15	30	60	120
15	–	0.366	0.021*	0.009*
30	0.366	–	1.000	0.873
60	0.021*	1.000	–	1.000
120	0.009*	0.873	1.000	–

*Significant.

Mortality by concentration and contact time

Statistical analysis found an F-value of 4.37 and a $p < 0.0007$, indicating that the number of deaths is impacted by concentration and contact time (Table 1). For all contact durations, the highest mortality was at a concentration of 25%; the lowest mortality was at a concentration of 5%. In addition, for all concentration, the highest mortality was at a contact time of 120 min; and the lowest mortality was at a concentration of 5 min. In the control, there were no deaths at 30, 60, or 120 min of contact time (Figure 3).

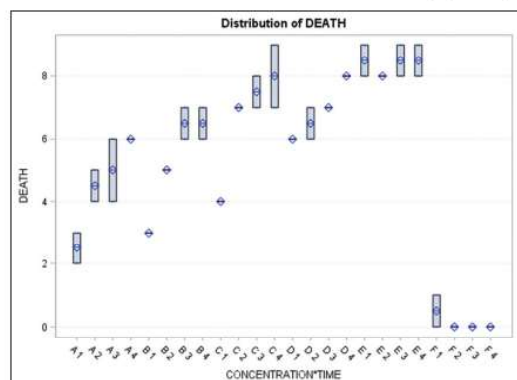


Figure 3: *Musca domestica* mortality by concentration and contact time

Discussion

The previous studies have shown that *P. betle* L. leaf extract has insecticidal properties that could potentially be exploited for pest eradication. Although such research used quantified extracts, the extracts were tested on larvae. Anisah's research [29] has particular relevance because it clearly shows that *P. betle* L. leaf extract mostly contains tannins, flavonoids, and eugenol chavicol, which are effective against *M. domestica*. The results presented here align with previous studies and provide further evidence of the benefits of *P. betle* L. leaf extract for controlling adult *M. domestica*.

There were significant differences between the subject groups and the control (Figure 3). At 60 min of exposure, the 5% extract caused a mortality rate of over 50%. With 15 min of exposure, the 25% extract led to an 80% mortality rate. For each duration of contact, higher concentrations of extract led to higher mortality rates. These results confirm previous research [17], [29], [31].

The most abundant compounds in *P. betle* L. leaves are tannins [29]. Tannins can block muscles' response to skin cell walls and inhibit enzyme and substrate activity, which can cause digestive disorders and damage cell walls; tannins act as a contact poison and stomach poison [29], [30], [31], [32], [37], [38]. Pesticides enter the fly's body, shrinking its body tissue and killing it. Flies' habit of licking, which is related to the shape and structure of their mouths, is a way for tannins to enter the fly's digestion. Flavonoid compounds can affect the respiratory and nervous systems. Flavonoid compounds enter flies' bodies through the respiratory tract and attack the central nervous system, which can cause paralysis and muscle rupture, leading to death [29]. In this experiment, after being exposed, the flies were placed in a cage with continuously monitored temperature and humidity. The cage used was made in such a way that air could enter the cage freely, allowing the flies to breathe. The increase in fly mortality indicates that the compounds in green betle leaves work to inhibit the respiratory system.

Other compounds in *P. betle* L. leaves that can also kill flies are eugenol and chavicol. These two compounds have antiseptic properties but are synergistic as pesticides, especially larvicides [29], [39], [40].

Green *P. betle* L. leaf extract contains contact toxins and respiratory toxins; exposure to the extract can reduce appetite, inhibit egg-laying, and inhibit growth [17], [30], [37]. The bioactive compounds in extracts from biological materials that are used as pesticides can affect the muscular system, nervous system, respiratory system, hormonal balance, reproduction, and antifeedants, leading to death [41]. Studies have shown that prolonged exposure to each concentration increases fly mortality, likely because the

length of exposure allows more active compounds to enter the flies' bodies [23], [29].

Conclusion

Overall, this study supports using *P. betle* L. and its main components (tannins, flavonoids, eugenol, and chavicol) to control housefly populations; *P. betle* L. leaf extract demonstrated remarkable efficacy against adult *M. domestica*. Thus, *P. betle* L. is suitable for use as a cheap and environmentally safe alternative for controlling *M. domestica*. However, further research into the toxicological effects for non-target insects is urgently needed.

Author Contributions

All the authors contributed equally to the preparation, development, and completion of this manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that the other authors have read and approved the manuscript and that there were no ethical issues involved.

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