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The Effectiveness of Solid Waste Extract from Lemongrass (*Cymbopogon citratus*) as a Bioinsecticide for Controlling Whitefly (*Bemisia tabaci*) Pests on Pomelo Plants

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ABSTRACT

This research aims to analyze the effect of lemongrass waste extract on the mortality of whitefly and determine the optimal concentration of lemongrass waste extract to eradicate whiteflies within 24 hours. Lemongrass waste extract (leaves and stems) can be obtained through extraction using maceration. The waste is dried and ground to 60 mesh, then dissolved in 96% ethanol and ethyl acetate solvents at a ratio of 1:8 (w/v). Extraction is carried out over variable times of 2, 4, and 6 days. The optimal results are then used to create concentrations by dissolving 250 ml of distilled water in the mother solution, resulting in test solutions of 0, 200, 400, 800, and 1200 mg/L. Observations on the treatments are conducted after 24 hours, and the number of dead whiteflies is counted. Subsequently, the mortality of the whiteflies is analyzed. Based on the research findings, the highest mortality percentage after 24 hours is observed in the ethanol extract, with a dose of 1200 mg/L at 81.81%. After determining the mortality rates, probit analysis is conducted to determine the LC50 (Lethal Concentration 50) value. The optimal concentration for ethanol extract is found to be 1200 mg/L, with an LC50 of 885.102 mg/L. This indicates that lemongrass waste extract is moderately toxic to whiteflies.

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1. Introduction

Lemongrass (*Cymbopogon citratus*) is a plant commonly found in both highland and lowland areas, characterized by its physical resemblance to wild grass. Lemongrass contains essential oils, with citronella and geraniol being the main chemical compounds. Essential oil is obtained through distillation, yielding approximately 0.5-1.5% essential oil, 8-18% liquid waste (hydrosol), and the remaining 80-90% as solid waste (Adityas et al., 2022). Secondary metabolite content in the distillation residue extract determines total phenol and antioxidant activity (LC50) in phytochemical screening of the residue powder and distillation residue ethanol extract (ERD), indicating the presence of flavonoids, tannins, quinones, phenols, and steroids (Eko Febrianto et al., 2022).
Currently, hydrosol or liquid waste is often processed as floor cleaners or sold to beauty product manufacturers as an additional essence. In contrast, solid lemongrass waste is typically used as

animal feed or discarded into the environment without further utilization. Solid lemongrass waste poses a challenge for lemongrass essential oil producers. The external appearance of solid waste from lemongrass oil distillation still retains the aroma and form of undistilled lemongrass. This demonstrates that lemongrass oil distillation waste still contains essential oils and various other active compounds. The distinctive aroma in the residue storage area, free from fly or insect infestations, supports this claim. Research by Lela et al. (2010) confirms that lemongrass root waste still contains insecticidal compounds, suggesting that lemongrass oil distillation waste, particularly its solid waste, can be utilized as a bioinsecticide.

Therefore, further research is needed to explore the utilization of lemongrass oil distillation waste as a step towards zero waste in bioinsecticide production processes. This includes investigating the effectiveness of solid lemongrass waste extract as a bioinsecticide against whitefly pests in pomelo plants.

2. Methods

2.1. Materials and Equipment Used

The materials used include solid lemongrass waste obtained from PT. Perkebunan Tjengkeh, Selokaton Estate, Pesaren, Sukorejo, Kendal, 96% Ethanol, Ethyl Acetate, distilled water (aquadest), FeCl3, Mg, HCl, and Tween 80. The equipment used in this research includes an analytical balance, 60-mesh sieve, plastic tubes, Whatman filter paper, rotary vacuum evaporator, volumetric flask, cream pot, spatula, stirrer, aluminum foil, Erlenmeyer flask, funnel, measuring glass, plastic spray bottle, and water bath.

2.2. Variables Used

The variables in this study consist of fixed variables, such as maceration times of 2 days, 4 days, and 6 days, and observation times for toxicity testing on test animals, which are 1 hour, 8 hours, 16 hours, and 24 hours. The variables that change are the maceration extraction solvents, which are ethyl acetate and ethanol, and the application doses to test animals, which are 200 mg/L, 400 mg/L, 800 mg/L, and 1200 mg/L.

2.3. Preparation of Materials

The lemongrass waste used consists of its stems and leaves. The waste is cleaned, chopped into small pieces, dried in sunlight for approximately 5-6 hours until the moisture content is <10%. After drying, the leaves and stems are ground into a powder using a blender/copper.

2.4. Maceration Extraction Process

Maceration extraction is conducted using ethyl acetate and 96% ethanol as solvents. The solid lemongrass waste (leaves and stems) is dried under the sun until the moisture content is ≤10%, then ground into powder using a blender. A 25g portion of lemongrass waste powder is macerated with 150 ml of 96% ethanol and ethyl acetate for variable times of 2, 4, and 6 days. After extraction, the solution is filtered using Whatman filter paper. The filtrate is collected and concentrated using a rotary vacuum evaporator at 50℃, with a speed of 60 rpm and pressure of 100 mbar, resulting in ethanol and ethyl acetate extracts.

2.5. Yield Analysis

The concentrated extract obtained from the evaporator is transferred to a pre-weighed cream pot. The yield of ethyl acetate and 96% ethanol extracts is determined by weighing.

2.6. Phytochemical Screening Analysis

2.6.1. Flavonoid Test

Flavonoid testing involves the use of Mg + HCl (p) wilstater powder and a 10% NaOH test. 96% hot ethanol is used to dissolve the ethanol extract, and after that, it is added to two reaction tubes. In tube 1, 2-3 drops of 10% NaOH are added to detect the presence of flavonoid compounds, resulting in an orange/yellow layer. In tube 2, Mg powder and 2-4 drops of HCl (p) are added, causing a color change in the layer to red/yellow, indicating the presence of flavonoid compounds.

2.6.2. Tannin Test

The identification of tannin compounds is performed by dissolving the concentrated ethanol lemongrass waste extract in 96% ethanol. One milliliter of this solution is then added to a reaction tube. A 1% FeCl3 solution is applied with 4-5 drops to the extract mixture. Positive results are indicated by the formation of a greenish-black color in the filtrate.

2.7. Dose Preparation and Toxicity Testing

Toxicity testing aims to detect the toxic effects of a compound, whether natural or chemical, on the biological system of a test animal. The doses of ethanol and ethyl acetate extracts are prepared according to Uni Nur Madinah et al. (2013), where 0.3g of concentrated lemongrass waste extract is dissolved in water, added to a chemical flask with 10 drops of Tween 80 as an emulsifier, and stirred until homogeneous. The solution is then added gradually to a 250 mL volumetric flask, and distilled water is added to the mark, resulting in a mother solution of 1200 mg/L. Test solutions are prepared with concentrations of 0, 200, 400, 800, and 1200 mg/L, derived from the mother solution by taking 0, 12.5, 25, 50, and 100 mL, respectively, and adding distilled water to the mark.

2.8. Test Animal Mortality

Observation data are obtained by calculating the total dead whiteflies in each plastic tube. Mortality is calculated at various time intervals (1 hour, 8 hours, 16 hours, and 24 hours) and recorded in a table. Dead whiteflies are those that do not respond to physical stimuli, such as touch and movement by the tester.Where:\(a =\) Number of dead test animals,\(b =\) Total number of test animals used.

2.9. LC50 Value (Lethal Concentration 50)

To analyze the LC50 value, Probit Analysis is used, which has been simplified in Microsoft Excel by Dr. Alpha Raj .M, MVSc, PhD. The LC50 value is derived from mortality data and is employed to express the toxic effect of a substance/compound based on mortality response to test animals using a spray exposure method.

3. Results and Discussion

3.1. Phytochemical Screening

Compounds with the potential to be botanical insecticides often contain bioactive compounds such as saponins, tannins, alkaloids, flavonoids, and terpenoids (Najmah et al., 2023). In this study, the selection of flavonoid and tannin compounds is based on their known toxicity as insecticides, as supported by research on tigaron leaves by Nur Musyahadah et al. (2015) and a study by Rizky Rahmadi et al. (2022), which states that, in addition to acetogenin, the chemical compounds in soursop leaves that are toxic to pests include tannins and flavonoids. This suggests that these two compounds are primary and stable compounds found in natural plants with toxic abilities, serving as botanical insecticides. The results of phytochemical screening for flavonoid and tannin compounds in ethanol and ethyl acetate extracts are presented in Table 1.

Table 1. Phytochemical screening results of lemongrass waste extract (*Cymbopogon citratus*)

No	Phytochemical Testing	Extracts	Testing Method (SNI)/ Equipment	Conclusion
1	Flavonoid	Etanol	Wilstater	+
2	Tanin	Etanol	FeCl3 1%	+
3	Flavonoid	Etil Asetat	Wilstater	-
4	Tanin	Etil Asetat	FeCl31%	-

(+) Detected Compound, (-) Undetected Compound

In Table 1., ethanol extract showed the presence of flavonoid and tannin compounds, while the use of ethyl acetate solvent did not detect either compound. This proves that the more effective solvent is 96% ethanol, as it is a polar compound capable of extracting the desired toxic compounds, namely flavonoids and tannins.

3.2. The Effect of Lemongrass Waste Solid Extract Application on Whitefly (*Bemisia tabaci*) Mortality

Table 2. Mortality analysis results of 96% ethanol lemongrass waste extract on whiteflies (*Bemisia tabaci*)

No	The concentration (mg/L) of Ethanol	N	% Mortality			
			1 Hour	8 Hour	16 Hour	24 Hour
1	0 (Negative Control)	11	0%	0%	0%	0%
2	200	11	0%	18,18%	18,18%	36,36%
3	400	11	9,09%	27,27%	36,36%	54,54%
4	800	11	18,18%	36,36%	54,54%	72,72%
5	1200	11	27,7%	54,54%	72,72%	81,81%

Table 3. Mortality analysis results of ethyl acetate lemongrass waste extract on whiteflies (*Bemisia tabaci*)

No	Konsentrasi(Mg/L) Etil Asetat	N	% Mortalitas			
			1 Jam	8 Jam	16 Jam	24 Jam
1	0 (Negative Control)	11	0 %	0%	0%	0%
2	200	11	0%	9,09%	18,18%	27,27%
3	400	11	9,09%	18,18%	18,18%	27,27%
4	800	11	9,09%	18,18%	18,18%	36,36%
5	1200	11	9,09%	18,18%	27,27%	36,36%

Negative Control is conducted to determine whether the test animal's death response truly originates from the sample and is not caused by the solvent used. In Tables 2. and 3., the levels of mortality or death of whitefly pests caused by lemongrass waste extract are shown at various concentration levels, except for the control (0%). It can be observed that the most optimal observation time and dose are at 24 hours with a concentration of 1200 mg/L, resulting in a higher mortality rate of 81.81% compared to the mortality of ethyl acetate extract at 36.36%. This is consistent with the absence of toxic compounds, flavonoids, and tannins, as indicated in Table 3.1. The characteristics of animals in the treatment include lethargic body and upward-facing legs, caused by the entry of tannin and flavonoid compounds into the body through the digestive system (Yunita et al., 2009).

(a) (b)

Figure 1. Whitefly Mortality

Observing the mortality process of test animals in stages, initial symptoms shown by the test insects indicate a decrease in movement, as depicted in Figure 1. This is caused by the initial paralysis of the whitefly wings as seen in figure (a), due to the treatment with the spray method. Subsequently, the test animals exhibit a reduction in movement or restricted mobility, impacting their ability to feed and engage in activities. Their bodies become soft and pale, appearing shriveled and shrunken, leading to eventual death, as shown in figure (b). This progressive observation provides insights into the impact of lemongrass waste extract on the physiological responses and mortality of whiteflies.

3.3. Median Lethal Concentration (LC50) of Lemongrass Waste Extract

Table 4. LC50 Analysis Results of Lemongrass Waste Extract

Treatment	Regression Equation	SE	LC50 (mg/L)	Reference Limit for LC50 (mg/L) Lower Bound	Reference Limit for LC50 (mg/L) Upper Bound
96% Ethanol Extract	Y=1,6136x+1,2579	0,081	885,102	613.244	1277.477
Ethyl Acetate Extract	Y=0,6307x+3,4148	0,443	330528.692	44831.004	2436912.122

An extract is considered toxic if the LC50 is below 1000 ppm (1000 mg/L), and above 1000 ppm (mg/L), it is deemed non-toxic (Usman et al., 2020). In Table 4., the LC50 analysis of lemongrass waste ethanol extract is considered effective as the concentration of 885.102 mg/L can cause a 50% mortality rate in whiteflies and does not exceed 1000 mg/L. Conversely, the ethyl acetate extract is deemed ineffective with a concentration of 3305.28 x 102 mg/L as it surpasses 1000 mg/L.

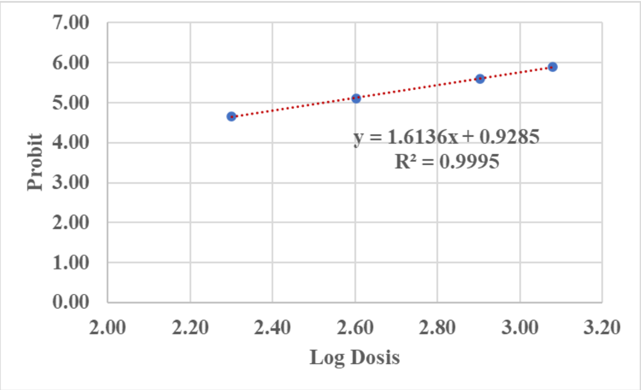

Figure 2. Graph of the Relationship Between Concentration of 96% Ethanol Lemongrass Waste Extract and Whitefly Mortality

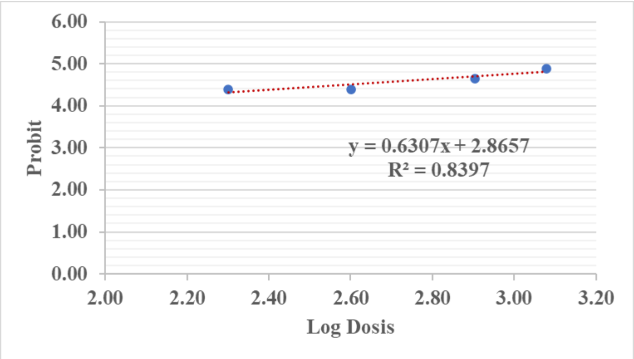

Figure 3. Graph of the Relationship Between Concentration of Ethyl Acetate Lemongrass Waste Extract and Whitefly Mortality

In Figure 2., the graph illustrates that each addition of 1% lemongrass waste ethanol extract to whiteflies causes a mortality rate of 1.6136%. In contrast, Figure 3. shows that each addition of 1% ethyl acetate lemongrass waste extract to whiteflies only causes a mortality rate of 0.6307%. The regression coefficient values for the concentration variable (x) for lemongrass waste ethanol extract and ethyl acetate extract are 1.6136% and 0.6307%, respectively. This indicates that the mortality rate has a positive or direct relationship with the regression line. The relationship pattern between concentration and whitefly mortality in ethanol extract is very strong, as evidenced by the coefficient of determination (R2 = 0.9995) or 99.95%. Thus, at the end of the observation, it is evident that higher concentrations of ethanol extract lead to significantly higher whitefly mortality.

Table 5. Relationship between lc50 and toxicity categories

Category	LD50 and LC50
Super Toxic	≤ 5 mg/L
Extremely Toxic	5 – 50 mg/L
Highly Toxic	50- 500 mg/L
Moderately Toxic	0,5 – 5 gr/L
Slightly Toxic	0,5 -5 gr/L
Practically Non-Toxic	>15 gr/L

The calculated LC50 value for lemongrass waste ethanol extract is 885.102 mg/L. Consequently, referring to Table 5., it can be categorized as moderately toxic. In a study by Nur Musyahadah et al. (2015), the leaves of Tigaron plants yielded an LC50 result of 33.431 g/mL or 33341 x 103 mg/L, suggesting it is not toxic to test animals. Additionally, in a study by Uni Nur Madinah et al. (2013), the bioinsecticidal test of chloroform extract from the stem bark of Bruguiera gymnorrhiza (EKBG) against armyworms resulted in an LC50 value of 4046.19 mg/L, considered high even though it effectively killed test animals on the third day. Lemongrass waste demonstrates effectiveness in inducing mortality in pests, as evidenced by the mortality results on test animals. Despite originating from waste material, it proves to have significant toxicity compared to other natural substances that are not classified as waste.

4. Conclusion

Based on the research findings, it can be concluded that lemongrass waste extract can be utilized as a botanical insecticide and has an impact on the mortality rate of a pest, with an LC50 (Lethal Concentration 50%) value of 885.102 mg/L, categorizing it as moderately toxic. The optimal concentration of lemongrass waste ethanol extract for eradicating or killing whiteflies within 24 hours is at a concentration of 1200 mg/L, capable of causing a mortality rate of 81.81% in whiteflies.

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