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Insecticidal Activity of Kaffir Lime (*Citrus hystrix*) against Rice Weevil (*Sitophylus oryzae*)

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ABSTRACT

The aim of this study is to analyze the potential use of kaffir lime leaves and peel extract as bioinsecticides against rice weevils. The assessment of bioinsecticidal activity was studied through contact activity, repellency, antifeedant, and progeny and growth development tests. Contact activity tests resulted in increased adult mortality with increased extract concentrations from 10 to 50 mg/L after 24 hours. The leaf extract (LC50=18 mg/L) showed a higher mortality rate compared to fruit peel extracts (LC50=54 mg/L), with 100 % and 60 % mortality, respectively. The leaf extracts with a mean repellency of 68 % significantly repelled rice weevils compared to the 42 % repellency of fruit peel extracts. The leaf extract was classified in repellency class IV, while the peel extract was in repellency class II. With the use of Kaffir lime leaf extracts, F1 adults were completely suppressed at 20 mg/L concentration of leaf extract with a 100 % inhibition rate.

Keywords: Insecticidal activity, rice weevil, kaffir lime, kaffir lime leaves, kaffir lime peel.

1. INTRODUCTION

Sitophilus oryzae L., also known as the rice weevil, is one of the most destructive pests that can destroy the stored grain worldwide by directly feeding on the kernel of the grains [1]. This will result in economic losses for the consumers and industry themselves [2]. There are various methods to control the infestation of this rice weevil, such as chemical methods with the use of synthetic insecticides and physical and biological methods. Presently, the method of controlling rice weevils depends on fumigation and residual grain protection by applying synthetic insecticides, which contain dangerous chemicals [3]. Furthermore, the use of chemical insecticides may contribute to side effects on both consumers and the environment.

Thus, the alternative way to control the presence of a rice weevil is by using a biological insecticide. The most common bioinsecticide studied all over the world comes from plant extract. Over the years, aromatic plants have been used as one of the ways to repel insect pests. Plants from the Rutaceae family and in the genus of citrus, such as kaffir lime, usually have an aromatic and strong scent [4]. The insecticidal activity of aromatic plant extract against rice weevils is limited. Several tests on the insecticidal activity using kaffir lime extract have been done on a few types of insect pests, but little has been conducted against weevils. Common tests such as repellency and contact activity were conducted against this insect pest, but little information was obtained on the efficacy of the plant extract on the antifeedant activity and progeny growth test. Thus, in this study, kaffir lime (*Citrus hystrix*) extracts were used as the main component of

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bioinsecticide to assess the insecticidal activity of kaffir lime extract on the repellency, mortality and antifeedant activity as well as progeny growth and development of the rice weevils.

2. MATERIAL AND METHODS

2. 1 Insect Rearing

Rice weevils (*Sitophilus oryzae*) were collected from the infested and stored rice of Jasmine Super Special. The rice weevils were reared in a 600 mL clear plastic container containing milled rice, enclosed with a cloth, and protected with rubber bands. The rice weevils were also reared with eggs laid on rice and at 28±2°C and 75±5% RH. The adult populations were removed using the sieving method, and only unsexed adults were used in the insecticidal activity test.

2. 2 Preparation of Plant Material

Kaffir lime (*Citrus hystrix*) leaves and fruits were purchased from a local market at Kangar, Perlis. Fresh leaves and fruit peel were dried at 50˚C for 24 hours in the oven. The dried samples are then ground and sieved to produce a fine powder.

2. 3 Plant Material Extraction

The extraction of both Kaffir lime leaves and peel was done according to [4] and [5]. About 250 g of kaffir lime leaves and fruit peel powder were dissolved in 1 L of ethanol and placed inside the rotary shaker at 30 ℃ at a speed of 150 rpm for 48 hours. After 48 hours, the mixture was filtered using Whattman filter paper. All the filtrates were concentrated in an incubator after the solvent was removed using a vacuum glass rotary evaporator to yield concentrated kaffir lime. The extracts were stored inside the refrigerator at 4°C until they were used for the insecticidal activity test.

2. 4 Repellency Test

The test was conducted using the method described [1,6] with time modifications. The Whattman No. 1 filter papers were cut into two equal halves. Then, the kaffir lime leaves and peel extract were dissolved in acetone and diluted to 50, 40, 30, 20 and 10 mg/L. These extracts were applied to the first part of Whattman filter paper (treated) using a micropipette, while the other half was applied with 1 ml of acetone and acted as the control.

The treated and control parts were air-dried to remove the solvent completely and placed together with cellophane tape to form a full filter paper disc. The filter papers were placed in a petri dish with 10 unsexed adult rice weevils released in the center. The petri dish was covered and set aside for evaluation. The weevils were exposed to the extracts for 5 hours, and the number of weevils that were being repelled was evaluated. The test was replicated three times in all of the experiments using different concentrations. The number of rice weevils on the control part (NC) and treated (NT) part were recorded every 30 minutes. Percent repellency (PR) values were calculated using Equation 3.1. The mean repellency values were classified using a standard scale [7], as shown in Table 1.

$$PR = \left[\frac{(NC-NT)}{(NC+NT)}\right] \times 100 \quad (1)$$

Where,

PR is the percent repellency (%)
NC is the number of Rice Weevil on the control (untreated part)

NT is the number of Rice Weevil on the treated part

Table 1: Repellency rate class (McDonald et al., 1970).

Class	Repellency Rate (%)
0	> 0.01 to < 0.1
1	0.1 to 20
2	20.1 to 40
3	40.1 to 60
4	60.1 to 80
5	80.1to 100

2. 5 Contact Activity Test

The tests were conducted using the method described by [1,8,9] with some modifications. For this test, the kaffir lime leaves and peel extract were dissolved in acetone and diluted to 50, 40, 30, 20, and 10 mg/L and acetone served as control. Ten adult weevils were released into each petri dish, and the extracts were directly sprayed onto them. The acetone and extracts were evaporated for 10 minutes to remove the solvent completely, and 5 g of rice was placed in a petri dish as the food source. The rice weevils were exposed to the extract of different concentrations for 24 hours with dead rice weevils, whom have no response to the forceps touch, were counted and recorded. The experiments were repeated three times. Insect mortality was recorded and expressed as percentages. The mortality percentages were used to find the lethal concentration of LC_{50} using probit analysis [10].

2. 6 Progeny Growth and Development Test

This test was conducted by using a method by [3] with some modifications. In this test, the kaffir lime leaves and peel extract were dissolved in acetone and diluted to 20, 16, 12, 8 and 4 mg/L. About 20 grams of disinfected rice were put in a glass jar, and twenty rice weevils were released into the jar. The glass jars were then covered with cotton cloth and secured with a rubber band. The rice weevils were cultured, allowed to reproduce, and removed after two weeks. After that, the rice was then mixed with the different concentrations of the extracts. The culture was continued, and the development was monitored until the new weevil's progeny had emerged. The newly emerged progeny were calculated after 49 days. The inhibition rate expressed in percentage or reduction in the number of emerged weevils was calculated by using the method [11] as follows:

$$IR\ (\%) = \left(\frac{Cn-Tn}{Cn}\right) \times 100 \qquad (2)$$

Where,

IR is the inhibition rate expressed in percentage.
Cn is the number of newly emerged insects in the untreated (control) sample.
Tn is the number of insects in the treated sample.

2. 7 Statistical Analysis

The data were collected from the observation of the repellency, contact activity, and progeny growth and development test. Those data were recorded and analyzed. The average and standard deviation of the data were figured out from the statistical analysis using analysis of variance (ANOVA). The percent of mortality data of rice weevils were used to calculate the lethal concentration LC_{50} using probit analysis. Probit-transformed percentage insect mortality was regressed on log10 concentration, and the regression equation was noted.

3. RESULTS AND DISCUSSION

3. 1 Repellency Test

According to Tables 2 and 3, kaffir lime leaves and fruit peel extracts show an increase in repellency effect with the increase in the extract concentration. Kaffir lime leaves extract demonstrated mean repellency of 30.66 % to 68 % compared to kaffir lime fruits peel extract with repellency of 21.33% to 42.67% when the concentration increased from 10 to 50 mg/L after 5 hours of exposure. The repellency class of leaves extracts increased from II to IV and I to II for fruit peel extracts. By comparing both kaffir lime leaves and fruit peel extract, it can be concluded that kaffir lime leaves extract showed the highest repellent activity compared to the fruit peel extract. For this reason, kaffir lime leaves have a better ability to repel rice weevils from infesting the stored rice. The effect of different kaffir lime leaves concentration and time of exposure towards the percentage repellency of rice weevils are statistically significant with $p \leq 0.05$. In contrast, the effect of kaffir lime fruits peel concentrations of extract towards the percent percentage repellency is insignificance, but the time of extract exposure is statistically significant with $p \leq 0.05$. According to the analysis, both the leaves and fruit peel show a significant interaction between the times of exposure and extract concentration.

Table 2: Percent repellency of kaffir lime leaves extract on adult rice weevils in 5 hours of exposure.

Concentration (mg/L)		**10**	**20**	**30**	**40**	**50**
Leaves Extract						
Percent Repellency (%)	1 H[2]	13.33±10.18	33.33±3.85	33.33±3.85	40±6.67	66.67±10.18
	2 H	13.33±10.18	40±6.67	40±6.67	73.33±3.85	60±6.67
	3 H	33.33±3.85	40±6.67	40±6.67	73.33±3.85	60±6.67
	4 H	33.33±3.85	46.67±3.85	66.67±3.85	73.33±3.85	66.67±3.85
	5 H	60±6.67	66.67±10.18	73.33±3.85	73.33±3.85	86.67±3.85
Mean Repellency (%)		30.66±6.95	45.33±6.24	50.67±4.98	66.66±4.41	68±6.24
Class Repellency		II	III	III	IV	IV

[1] Percentage repellency PR(%): [(NC-NT)/(NC+NT)]x100. [2] Treatment hours of exposure.[3] Values are expressed as Mean ± Standard error for three replications of 10 adult's rice weevils each after 5 hours of exposure. [4] Repellency class according to (McDonald et al., 1970).

Table 3: Percent repellency of kaffir lime peel extract on adult's rice weevils in 5 hours of exposure.

Concentration (mg/L)		**10**	**20**	**30**	**40**	**50**
Fruits Peel Extract						
Percent Repellency (%)	1 H[2]	40±13.33	0±6.67	13.33±7.7	40±6.67	26.67±3.85
	2 H	33.33±10.18	26.67±10.18	13.33±7.7	53.33±3.85	40±11.55
	3 H	20±6.67	13.33±3.85	13.33±7.7	46.67±3.85	46.67±7.7
	4 H	6.67±3.85	13.33±3.85	26.67±3.85	53.33±3.85	46.67±7.7
	5 H	6.67±3.85	20±0	33.33±3.85	46.67±3.85	53.33±3.85
Mean Repellency (%)		21.33±7.58	14.67±4.91	20±6.16	48±4.41	42.67±6.93
Class Repellency		I	I	I	II	II

[1] Percentage repellency PR(%): [(NC-NT)/(NC+NT)]x100. [2] Treatment hours of exposure.[3] Values are expressed as Mean ± Standard error for three replications of 10 adult's rice weevils each after 5 hours of exposure. [4] Repellency class according to (McDonald et al., 1970).

Kaffir lime extract can reach 65 % repellency and as high as class repellency of IV using 4 and 8 µL of the extract after 5 hours of exposure [9]. The repellent protection of the ethanolic extract of kaffir lime was found to be higher than kitchen mint and mint weed, and it can reach 65 % of repellency towards rice weevils after 1 hour using 6.4 mg/mL of the extract [3]. It was reported that kaffir lime peel could repel all cockroaches using 50% concentration, and it may be considered one of the most potent botanical insecticides [13]. It was also reported that Kaffir lime fruits extract were found to have the highest repellency activity of complete protection against mosquitoes with only 0.0002g/cm^2 and was a strong repellent for mosquito control [12]. The repellent activity of kaffir lime leaves and fruit peel extract were dependent on the extract concentration itself, where different concentration gives different values of percentage repellency. This statement was supported by a study using black pepper and physic nut against rice weevils, which stated that the repellent activity of plant extracts were dependent on the extraction dosage [1, 6]. Kaffir lime can repel insect due to the major main compound such as citronellal which known as plant-based insect repellent [13]. This is because the sense of smell stimulus that repels the insect and the volatile compounds that present a strong odor could lead to their death by blocking the tracheal respiration. The repellency effect of fruits peel extract were lower because of the amount of citronellal in fruits peel were lower than leaves [14].

3. 2 Contact Activity

The percentage mortality of leaves extracts increased from 23 % to 100 % as the concentration of the extract increased from 10 to 50 mg/L, according to Table 4. Meanwhile, the percentage mortality for fruit peel extract increased from 7 % to 60 % as the concentration of the extract increased from 10 to 50 mg/L after 24 hours of exposure. The effect of different kaffir lime plant parts towards the percentage mortality of rice weevils are statistically significant with $p \leq 0.05$. Furthermore, the effect of extract concentrations towards the percent mortality is also statistically significant with $p \leq 0.05$. It can be concluded that the percentage mortality of the rice weevils depends on the concentration of the extract used, as an increase in concentration caused an increase in percentage mortality. The median lethal concentration of LC_{50} for 50 % mortality is at 18 mg/L for leaves extract and 54 mg/L for fruit peel extract. Kaffir lime leaves extract is more effective as it only takes 18 mg/L of extract compared to fruits peel extract, which requires 54 mg/L concentration to kill 50 % of the rice weevil's population. Both extracts are able to kill the rice weevil that infests stored rice. However, the leaves extract is more effective compared to fruit peel extract.

Table 4: Median lethal concentration LC_{50} of the kaffir lime extract on adult's rice weevils.

Concentration (mg/L)	Percentage Mortality (%)	Empirical Probit	Regression Equation	LC_{50}
Leaves Extract				
0	0			
10	23	4.26		
20	50	5.00	y = 3.3998x + 0.772	18
30	80	5.84		
40	90	6.28		
50	100			
Fruits Peel Extract				
0	0			
10	7	3.52		
20	13	3.87	y = 2.2851x + 1.0366	54
30	17	4.05		
40	37	4.67		
50	60	5.25		

Kaffir lime leaves extract was reported to be the most potent and have the ability to kill the rice weevils based on the LC_{50} value. The LC_{50} of kaffir lime leaves extract has been reported to be 29.254 µL/g at 24 hours and 26.748 µL/g after 48 hours after treatment on *Spodoptera litura [15].* The low value of LC_{50} is desirable because the lower the concentration used to kill, the better, and it indicates the efficacy of the extract. The bioactive compound in kaffir lime can act as an insecticide by interfering with the normal respiratory activity causing asphyxiation and subsequent death. The major compound present in kaffir lime extract is known as β-Citronellal, with a total of 66.85%, followed by β-Citronellol with 6.59%, which might be toxic to the test larvae [3,15]. Others also stated that the major compound found in kaffir lime was citronellal [4,14]. Also in this study, the effect of fruit peel extract is lower than leaves extract because the amount of active compound in fruits peel is lower than leaves. The amount of citronellal in fruits peel (11.7 % to 12.6 %) is lower than in leaves (61.7 % to 71.5%) [14].

3. 3 Progeny Growth and Development Test

The rice weevil's progeny was completely suppressed by 20 mg/L of kaffir lime leaves extract. The inhibition rate, as shown in Figure 4, increases from 68% to 100 % as the concentration of extract increases. The inhibition rate showed positive trends as the concentration of kaffir lime extract increased.

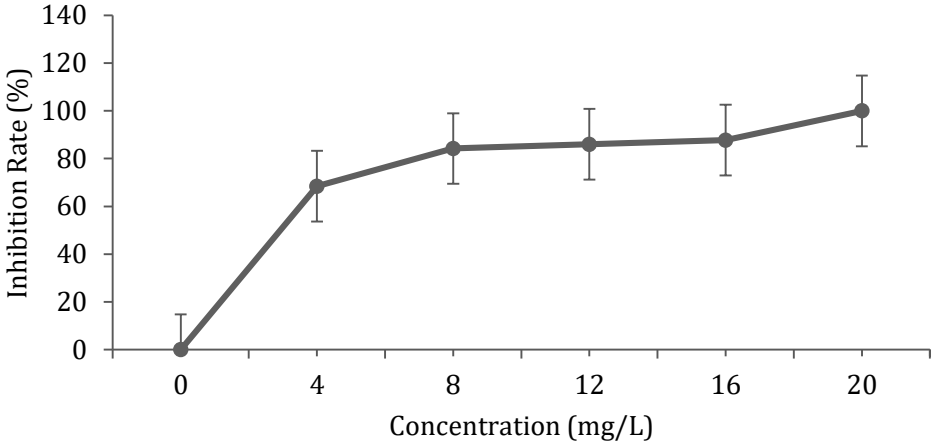

Figure 4: Inhibition Rate of the rice weevils over concentration.

The number of F1 progeny in controlled rice is higher, with an inhibition rate of 0 %, than that in the treated rice because of the emergence of adult's rice weevils and the development of the larvae inside the rice kernel. The effect of concentration on the number of F1 progeny after the feed was soaked with extract with different concentrations is statistically significant with $p \leq 0.05$. A similar study stated that the number of F1 progeny of rice weevils decreased from 14.33 to 8.67 when the concentration of kaffir lime extract increased from 0.8 % to 6.4 % while the percentage of inhibition increased from 65.32 % to 79.0% [3].

4. CONCLUSION

In general, leaves extract was the most effective compared to fruit peel extract in terms of its efficacy. The bioactive compound presence in kaffir lime, such as citronellal and linalool, strongly affects the mortality, repellency and progeny growth development of rice weevils in this study. The aromatic chemical in kaffir lime are capable of disturbing normal breathing activity, causing

asphyxiation and consequent death, and contain irritants and foul-smelling chemicals which strongly repelled the rice weevils [3,13].

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