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Bioinsecticidal Activity of Roots and Leaves Extract of *Cymbopogon citratus* on *Sitophyluz oryzae*

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ABSTRACT

Sitophilus oryzae is a storage pest of serious concern nowadays, which causes various losses by destroying large quantities of grains, legumes, and other food materials. Several methods have been utilized to overcome the infestation of this pest, including synthetic pesticides. However, this method somehow negatively impacted the surroundings, especially the non-targeting organism. Thus, biopesticides safe to nature and ecosystems can be applied as an alternative. The aim of this research was to study the bioinsecticidal activities of Cymbopogon citratus leaves and root extract against Sitophilus oryzae. The bioinsecticidal activity was assessed through repellency tests, contact activity, and phytochemical analysis. Roots and leaves were extracted using solvents such as ethanol, methanol, and chloroform. Extract concentration was varied to 50, 100, 150, and 200 ppm for repellency and contact activity tests. Based on the data obtained, it was best described that ethanolic extract of C. citratus root shows the highest repellency of 100% after 5 h exposure to 200 ppm extract concentration with a mean repellency of 80.7%. Both ethanolic extract of C. citratus root and leaves at 200 ppm show 100% mortality of S. oryzae after 24 h exposure. Based on phytochemical screening, saponin, phenols, and tannins were detected in both the root and leaves of C. citratus, which might be possible to contribute in the bioinsecticidal activity.

Keywords: Bioinsecticide, *Cymbopogon citratus*, Plant extract, Rice weevil.

1. INTRODUCTION

Grain is an important commodity and is persistently threatened by a number of insect pests during storage. Rice weevil, scientifically known as Sitophilus oryzae pests, is the world's largest crop and is commonly infested with rice and other grains and derivatives [1]. It is a major stored product pest that attacks sound, undamaged grain, which causes the grain to become more susceptible to secondary pests [2]. They invade wheat, corn, oats, barley, dried beans, cashew nuts, and even cereal commodities, particularly macaroni. The growth of the seed kernel or any man-made counterpart must be complete through young *Sitophilus oryzae* and larvae. As for the adult females, they consume the seed and make a hole to place the eggs inside. Then, *Sitophilus oryzae* is going to seal it with the glands emerging from the ovipositor. When the larva grows completely, it will hollow out by feeding the egg. The larva is then pupating from the seed [3].

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It is crucial in many countries that stored biological products must be protected from attacks by storage pests. Many synthetic chemical insecticides and fumigants were used to control these species has been focused. Some severe issues, including ecological waste, food crop degradation, pesticide resistance, and non-target toxicity, have been reported [4], contributing to several problems. Currently, phosphine fumigation is used in the management of grain pests in many regions. Even though this method is fast-acting in getting rid of pests, major concerns arise regarding phosphine resistance since no suitable alternative fumigant can be used [2, 5, 6].

Several studies have found potential insecticides from terrestrial plants. A significant source of bioactive metabolites is developed, providing a wide variety of insects with anti-feeding, repellent, and poisonous properties [7, 8]. Vascular plant species can supply alternative sources of natural pesticides and repellents, which may be used to protect a stored product through extracts and essential oils. One of these natural pesticides is *Cymbopogon* roots and leaves, a folk medicinal plant commonly found in Malaysia and several other states. It also contains bioactive compounds responsible as bioinsecticidal agents against several insects [9-11], which can be used to repel or eliminate *Sitophilus oryzae.* The purpose of this study is to research the bioinsecticidal activity of leaf extract of *C. citratus* on *Sitophilus oryzae*. The use of this crop instead of a pesticide material would encourage sustainable development by reducing chemical pollution (environmental pollution) and saving costs. Additionally, this crop can easily be found in Malaysia.

2. MATERIAL AND METHODS

The *sitophilous oryzae* were collected from infested rice from a local market. *S. oryzae* was reared in several glass jars lined with a nylon net, protected with a rubber band to traverse the air at 28±20 °C and 70-80 % relative humidity. Only adult weevil was used in the experiment.

2. 1 Plant Extraction

Fresh lemongrass or *C. citratus* have been purchased from a market in Guar Chempedak. Fresh roots and leaves from the plant have been dried under the shade at 27^{o}C before grinding to fine powder with an electric 5.0 Hp kitchen grinder. The resulting powders were placed in an airtight and kept at 4°C before use.

The *C. citratus* extracts were prepared according to Udobre, Alabi & Uko [12] with modifications. The cold extraction method was used with three different solvents, which are 90% ethanol, 90% methanol, and 90% chloroform, at a ratio of 1:9. The mixture was then placed on the bench store, and the cover was partially open for oil to be removed from the mix. In order to evaporate the solvent (methanol), filtrates were then extracted via a rotary evaporator. The extract was then stored in the refrigerator in an air-resistant jar for 48 hours.

2.2 Repellency Test

Filter papers were symmetrically divided into two sections used as the test areas. The first section of the filter paper has been immersed in the acetone (C_3H_6O) solution of the plant extracts and will be evenly saturated. The second part, which served as control, remained untreated. Both the handled part and the untreated component were air-dried to evaporate the solvent fully. Then, the handled portion was gently taped to the control part with adhesive paper tape to remake the whole disk. Every filter paper was set in a petri dish, and 10 adults of Sitophilus oryzae were released inside the petri dish in the center of each filter paper disk, adequately covered and kept under the same environmental conditions as the rearing. The sample size of *Sitophilus oryzae* present in the untreated part (control, NC) has been recorded for 0 to 300 minutes. The percentage of the repulsion values for the repulsion test have been calculated using the formula in (Eq. 1) below [13]:

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

Where:

PR = the percent repellency (%)
NC = number of *Sitophilus oryzae* on the control (untreated part)
NT = number of *Sitophilus oryzae* on the treated part

2.3 Contact Activity

The leaves and root extract from *C. citratus* have been prepared by dissolving them with acetone (C_3H_6O). Ten sex-free adults of *Sitophilus oryzae* for each concentration have been used. After 24 hours of treatment, mortality rates have been reported. The lethal concentration can be calculated using the formula as in Eq. 2.

$$LC_{50} = \left[(\frac{EC_{50}}{3}) \times W_m \times 10^{-4}\right] \quad (2)$$

Where;

LC = the median lethal concentration
EC_{50} = the effective concentration
W_m = the weight of the sample, g

2.4 Phytochemical Screening

2.1.1 Test for Flavonoids

A few drops of concentrated hydrochloric acid have been applied to a small quantity of plant matter extracts. Immediately, a red color was taken that revealed the existence of flavonoids [14].

2.1.2 Test for Saponin

The saponin test have been done using a frothing test: Exactly 0.5 g of the extract has been dissolved in the distilled water in a test tube. The existence of saponin has been known by the warming that indicated the frothing happened [15].

2.1.3 Test for Terpenes

When 5 g of the extract has been added to 2 ml of chloroform ($CHCl_3$) and 3 ml of H_2SO_4 the formation of a reddish-brown ring indicated the presence of terpenes [16].

2.1.4 Test for Steroids

Liebermann-Burchard reaction: 2 ml of acetic anhydride and 2 ml concentration H_2SO_4, was added to 5 g of the extract. The color change from red-violet to green-bluish confirms the presence of steroids [17].

2.1.5 Test for Tannins

0.5 ml of plant extract, 1 ml of water, and 1-2 drops of ferric chloride solution have been added together in a test tube. The blue color was observed for gallic tannins and green-black for catecholic tannins [18].

3. RESULTS AND DISCUSSION

3.1 Repellent activity

Cymbopogon citratus extract from roots and leaves parts were assessed for their bio-insecticidal activity against the *Sitophilus oryzae*, rice weevil through a repellency test. Tables 1 and 2 show the repellent activity of *S. oryzae* after exposure within the treatment time of 5 hours using leaves and root extracts by different extraction solvents and different concentrations.

Based on the result, both parts of the plant extract tested were strongly repellent to *S. oryzae,* and the concentration-response analyses were significant. The repellent activity of the extracted plant parts from *C. citratus* was significantly influenced by the concentration applied, and the activity increased with the increase in exposure time. *Sitophilus oryzae* was a particularly sensitive ethanolic extract of *C. citratus* roots since 100% repellence was obtained with the concentration of 200 mg/L during 5 h treatment compared to 96.7% repellency by leaves extracted using the same solvent.

By comparing the mean repellency for three different solvents used, both roots and leaves extracted using ethanol show high mean repellency compared to chloroform and methanol. The strong repellent activity of root extract could be due to the presence of bioactive compounds. These results were in agreement with Uwamose, Nmor, Okulogbo & Ake [19], where the toxicity of lemon grass, *C. citratus* powder, and methanol extract against rice weevil *Sitophilus oryzae* showed a proportional increase in mortality *Sitophilus oryzae* with *C. citratus* concentration. The finding of the present study is in agreement with other researchers who had previously reported that the plant powder and extracts show an increase in repellency effect with an increase in concentration [20–22].

Table 1: Repellent activity of plant extract of roots of *C. citratus* against *Sitophilus oryzae* at different exposure times.

Concentration (mg/L)		**50**			**100**			**150**			**200**		
		Roots Extract											
		Chloroform	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**
Percent Repellency PR (%)[1]	1 hr	30.0 ±0.52	40.0 ±0.1	43.3 ±0.47	45.0 ±0.32	53.3 ±0.47	51.7 ±0.24	55.0 ±0.32	55.0 ±0.48	51.7 ±0.24	60.0 ±0.37	65.0 ±0.32	63.3 ±0.30
	2 hrs	53.3 ±0.30	53.3 ±0.47	50.0 ±0.52	55.0 ±0.32	61.7 ±.24	56.7 ±0.30	60.0 ±0.1	66.3 ±0.30	58.3 ±0.24	63.3 ±0.30	66.7 ±0.30	72.7 ±0.24
	3 hrs	53.3 ±0.30	60.0 ±0.37	55.0 ±0.32	60.0 ±0.37	63.3 ±0.30	63.3 ±0.60	63.0 ±0.24	65.0 ±0.32	58.3 ±0.24	66.7 ±0.47	81.7 ±0.43	73.3 ±0.30
	4 hrs	55.0 ±0.32	71.7 ±0.24	61.7 ±0.43	65.0 ±0.32	71.7 ±0.43	65.0 ±0.32	71.7 ±0.24	68.3 ±0.24	65.0 ±0.48	70.0 ±0.37	90.0 ±0.37	81.7 ±0.43
	5 hrs	76.7 ±0.30	76.7 ±0.24	73.3 ±0.30	76.7 ±0.60	81.7 ±0.60	76.7 ±0.30	83.3 ±0.47	81.7 ±0.43	76.7 ±0.47	78.3 ±0.43	100 ±0.1	86.7 ±0.60
Mean Repellency (%)		53.77 ±0.30	60.3 ±0.3	56.7 ±0.41	60.3 ±0.38	66.3 ±0.41	62.7 ±0.35	67.7 ±0.274	66.7 ±0.35	62.0 ±0.33	67.7 ±0.39	80.7 ±0.30	75.3 ±0.37

*Percentage repellency PR (%): [(NC-NT)/(NC+NT)] × 100. Treatment hours of exposure. Values are expressed as Mean ± Standard error for three replications of 10 adult rice weevils each after 5 hours of exposure.

Table 2: Repellent activity of plant extract of leaves of *C. citratus* against *Sitophilus oryzae* at different exposure times.

Concentration (mg/L)		**50**			**100**			**150**			**200**		
		Leaves Extract											
		Chloroform	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**	**Chloroform**	**Ethanol**	**Methanol**
Percent Repellency (%)	1 hr	23.3 ±0.70	33.3 ±0.30	36.7 ±0.30	33.8 ±0.43	46.7 ±0.70	45.0 ±0.32	51.7 ±0.43	51.7 ±0.57	48.3 ±0.24	56.7 ±0.47	61.7 ±0.24	60.0 ±0.1
	2 hrs	46.7 ±0.30	46.7 ±0.60	43.3 ±0.47	48.3 ±0.43	55.0 ±0.48	50.0 ±0.37	56.7 ±0.30	60.0 ±0.52	55.0 ±0.32	60.0 ±0.52	63.3 ±0.30	68.3 ±0.43
	3 hrs	46.7 ±0.30	53.3 ±0.60	48.3 ±0.43	53.3 ±0.47	56.67 ±0.60	56.7 ±0.60	65.0 ±0.32	61.7 ±0.57	55.0 ±0.32	63.3 ±0.47	78.3 ±0.43	70.0 ±0.52
	4 hrs	48.3 ±0.24	65.0 ±0.32	53.3 ±0.79	58.3 ±0.57	65.0 ±0.71	58.3 ±0.43	68.3 ±0.43	65.0 ±0.48	61.7 ±0.57	66.7 ±0.30	86.7 ±0.30	78.3 ±0.67
	5 hrs	70.0 ±0.37	70.0 ±0.52	66.7 ±0.30	70.0 ±0.82	70.0 ±0.89	70.0 ±0.52	80.0 ±0.63	78.3 ±0.43	73.3 ±0.70	75.0 ±0.61	96.7 ±0.30	85.0 ±0.61
Mean Repellency (%)		47.0 ±0.38	53.7 ±0.47	49.7 ±0.46	52.7 ±0.54	58.7 ±0.68	56.0 ±0.45	64.3 ±0.42	63.3 ±0.51	58.7 ±0.43	64.3 ±0.47	77.3 ±0.31	72.23 ±0.47

*Percentage repellency PR (%): [(NC-NT)/(NC+NT)] × 100. Treatment hours of exposure. Values are expressed as Mean ± Standard error for three replications of 10 adult rice weevils each after 5 hours of exposure.

3.2 Contact Activity

The contact activity test was done in order to evaluate the bio-insecticidal activity of plant extracts of *Cymbopogon* towards *Sitophilus oryzae* adults after 24 h exposure. Figure 1 shows the mortality rate of *S. oryzae* to *C. citratus* roots, while the results for leaves are depicted in Figure 2.

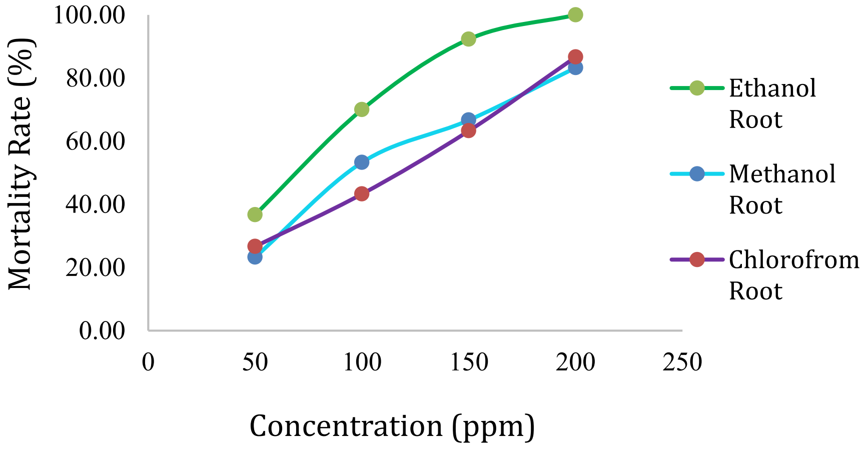

Figure 1: Graph of mortality rate of *Sitophilus oryzae* against concentration for roots part of *C. citratus*.

Based on Figure 1, the mortality rate of *S. oryzae* increases with the increase of root extract concentration for all three different solvent extracts used. Ethanolic extracts of *C. citratus* roots show 36.70% mortality at the lowest concentration (50 ppm) while 100% mortality when using the highest concentration of 200 ppm after 24 hours. For the chloroform extraction, at the lowest concentration of 50 ppm, a mortality rate of 26.70% was recorded, while the highest extract concentration only caused 86.70% mortality. Methanol extraction shows 23.30% mortality at the lowest concentration at 50 ppm, while at the highest concentration at 200 ppm, the mortality rate was 83.30%. Statistical analysis using two-way ANOVA indicates that the result is significant since the P-value is 0.0002, which is lower than 0.05.

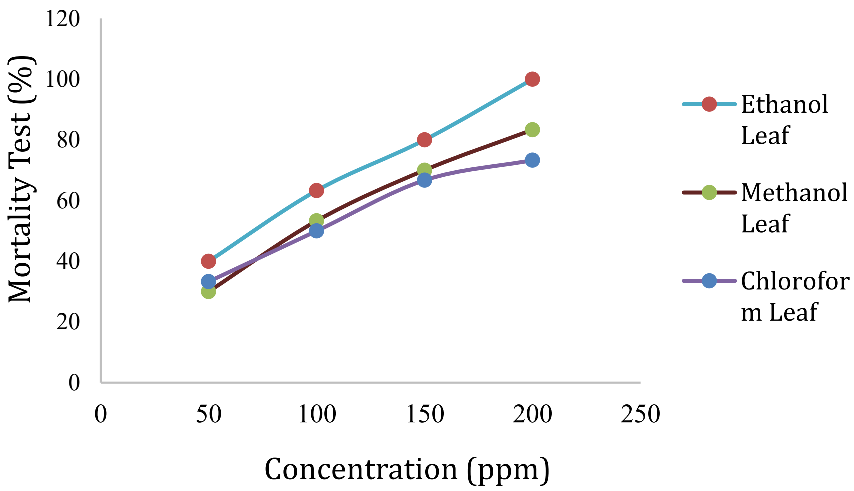

Figure 2: Graph of the mortality rate of *Sitophilus oryzae against* concentration for leaves part of *C. citratus*.

Figure 2 shows the mortality rate of *S. oryzae* after exposure to ethanolic, methanolic and chloroform extracts of *C. citratus* leaves. The higher mortality was recorded by using ethanol as a solvent, with 40% at 50 ppm and 100% mortality at 200 ppm extract concentration. On the contrary, only 30% and 33.3% mortality rates were recorded at 50 ppm for methanol and chloroform extracts while 83.3% and 73.3% mortality were recorded at the highest concentration for methanol and chloroform extracts, respectively. Based on both figures, ethanolic extracts show the highest mortality compared to the other two solvents used. These results are comparable to those of Waliullah et al. [23], who reported that *Clerodendrum viscosum vent.* (Verbenaceae) cause 32-72% mortality at 72 hours and 28 – 60% mortality at 24 hours of exposure with 3.93 and 0.25 mg cm^{-2} doses. It is also comparable to Khani, Awang and Omar [24], who reported that *M.piperita* and *P.nigrum* essential were relatively less toxic to adults of *Sitophilus oryzae* at 370 ppm that cause 97% mortality of *Sitophilus oryzae* adults within 72 hours of exposure. Statistical analysis by using two-way ANOVA indicates that the result is significant since the P-value is 0.0029, which is lower than 0.05.

3.3 Phytochemical Screening

The results of phytochemical screening are presented in Table 3. Ethanolic extracts of *C. citratus* roots and leaves show the existence of chemical constituents such as phenol, tannins, saponin and terpenes with the absence of flavonoids, steroids and sterols.

Table 3: Qualitative phytochemical analysis of plant extract of leaves and flowers of *C. citratus* in different solvent systems [Here: '+' = presence; '-' = absence].

Plant Constituent	Extractive	
	Roots	Leaves
Flavonoids	-	-
Phenols and Tannins	+	+
steroids and sterols	-	-
Saponins	+	+
Terpenes	+	+

This result was in agreement with the preliminary qualitative screening of *C. citratus* confirming the presence of diverse types of phytochemicals like phenol [25]. Phenolic compound is known to constitute a large group of secondary metabolites, which serve as antimicrobial, and antiviral and are also responsible for attracting or repelling insects. Bioactive compounds play a major role in preventing plants from pathogens, toxins, and herbivores based on their anti-microbial, anti-pilzing, anti-parasitary, and anti-feed [26]. Research showed that saponin could be toxic to insects [27]. This toxicity mainly appears in the way that the behavior of feeding interferes. Several saponins are anti-feeding, as are saponins isolated from *Ilex apocea* that inhibit *Limantria dispar* intake.

4. CONCLUSION

This project aims to study the bio-insecticidal activity of roots and leaf extracts of *Cymbopogon citratus* on *Sitophilus oryzae* (rice weevil). Based on the repellency test, both parts of the roots and leaves of C. citratus could repel *Sitophilus oryzae*. By comparing both parts, the roots of the plant showed the highest number of repel of *Sitophilus oryzae* compared to leaves extract. The ethanolic extract from the roots showed the highest repellent activity, achieving 100% repellence at a concentration of 200 mg/L over 5 hours, while the leaves achieved 96.7% repellence at the same concentration. It can be concluded that *S. oryzae* expose to both ethanolic extract shows a higher mortality rate than the other solvents with 100% mortality at 200 ppm concentration while 40% and 36.7% mortality for leaves and root extract at 50 ppm, respectively. The bioactive compound in *C. citratus* may serve as antimicrobial, antiviral and bioinsecticidal against insect pest, which might include toxicity that interferes with the insect's behavior.

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