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Phytochemical Screening and Insecticidal Activity of *Cyperus iria, Fimbristylis globulosa* and *Fimbristylis miliacea* toward *Sitophilus oryzae L.*

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ABSTRACT

The rice weevil, Sitophilus oryzae is one of the famous insect pests that cause the low quality of the rice seeds. Fimbristylis miliacea, Fimbristylis globulosa and Cyperus iria are sedge weeds that are common near swampy areas. These weeds are classified as potential due to their high nutritional value and chemical compound content. Natural compounds from plant extracts prove the potential to develop as a new natural bio-insecticide in terms of agriculture usage. Cyperus iria, Fimbristylis globulosa and Fimbristylis miliacea extracts were screened for their secondary metabolite constituents. All extracts at a concentration of 1% were also evaluated for their feeding and fumigant toxicity against Sitophilus oryzae. Phytochemical analysis showed the presence of terpenoids, saponin, and phenolics in Fimbristylis miliacea extract. Fimbristylis globulosa extract contains flavonoids, terpenoids and phenolic. Phenolic and flavonoid compounds are also included in Cyperus iria extract. However, Fimbristylis miliacea extract showed the strongest feeding toxicity against Sitophilus oryzae at a mortality of 100% at the highest concentration starting after 6 hours, while the other extracts had moderate feeding toxicity with a mortality rate (50-80%) at a low of concentration. Fimbristylis miliacea extract also showed a higher repellency in the fumigant toxicity at (100%) maximum in 5 hours.

Keywords: Phytochemical, insecticidal, saponin, *Sitophilus oryzae*, *Fimbristylis miliacea*

1. INTRODUCTION

Cyperaceae are true ecosystem builders, creating habitats for a variety of other species. *Cyperaceae* are of worldwide economic significance, with about 10% of species used by humans, particularly in the tropics [1]**.** The *Cyperaceae*'s distribution is an ideal model family to study evolutionary biology due to their species richness, global distribution, large discrepancies in lineage diversity [2]**,** broad range of ecological preferences and diverse phenotypes [3]**.** Although many *Cyperaceae* species are considered undesirable weeds, and their presence is particularly important in some regions, sedges have several traditional applications by humans. Edible species are consumed as nutritional supplemental food, others are used for weaving household items, and several species are used for their medicinal properties.

Insects are probably the most difficult pest to control in agriculture worldwide due to their incredible diversity and adaptability. Insect pest management has always been, and will continue to be, a persistent issue for agricultural researchers and producers alike. Controlling insect pest

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infestations will become increasingly challenging as insect resistance to routinely used pesticides grows and more hazardous chemicals are removed from the market. As farmers battle to remain profitable in a highly competitive global economy, they must face the constant issue of producing high-quality, pest-free crops within financial means while protecting the environment and worker safety. Those studies aimed to determine the phytochemical screening activities of *C. iria, F. globulosa* and *F. miliacea.* The second is to compare the insecticidal activities of *C. iria, F. globulosa* and *F. miliacea*. Third is to identify the correlation between the phytochemical screening and insecticidal activities of the *C. iria, F. globulosa* and *F. miliacea* against *S. oryzae*.

2.0 LITERATURE REVIEW

2.1 *Cyperaceae*

The *Cyperaceae* family consists of 100 genera and 5000 species [4]**.** *Cyperaceae* is also known as sedges. Despite the unwanted existence of this species in the rice fields, a few genus of this species were used in traditional folk medicines. *Fimbristylis* species are commonly found in wetlands or near bodies of water; some are used in traditional medicine. The genus *Fimbristylis* (*Cyperaceae*) consists of approximately 200−300 species and is distributed primarily in tropic, subtropic, and temperate zones of the Northern Hemisphere [4]**.** *Fimbristylis* is characterized by the usual presence of leaf blades, leaf sheaths without long silk hairs at the apex, and achene with a deciduous style base [5]**.** *Cyperus* is a large and diverse plant genus in the *Cyperaceae* family, better known as sedges. They can be found worldwide, including tropical and temperate zones, and are frequently associated with wetlands or other moist habitats. Rice-field flat sedges are non-native flat sedges introduced to North America in the 1840s and can be found in moist, disturbed soils in fields and waste areas in Connecticut. It has long, ascending branches with rounded spikelets. Research on various aspects of *Cyperus iria* has been documented by Manandhar et al. [6].

2.2 *Sitophilus oryzae*

S. oryzae is an internal feeding insect that bores into stored grain and is a common and economically significant pest. Adult weevils feed primarily on the endosperm, reducing carbohydrate content, while larvae feed primarily on the grain germ, increasing carbohydrate content and removing a large percentage of the protein and vitamins. Insects that attack the germ selectively will cause a greater loss of germination.

2.3 Insecticidal activity

The methods used in the insecticidal activity are fumigant toxicity and feeding toxicity. The high mortality is caused by the quantity of secondary metabolites, particularly those plant extract ingredients that more actively operate as a food inhibitor than other extracts. The potent substances directly inhibited sensory cells' ability to function, resulting in the starving death of insects. In the meantime, chemoreceptors identified in the mouthpart, which are particular eating repellent nerves, were stimulated by antifeedant. These chemical receptors interfere with the perception of the stimulation to eat by interacting with other chemical receptors [7, 8]. According to previous research, the dose, species assayed, toxicity techniques, and target are a few of the variables that affect insecticidal activity [9].

3. MATERIAL AND METHODS

The first process is rearing the *S. oryzae,* then preparing plant extraction. From the plant extraction process, the phytochemical screening and insecticidal activity will be done. The method of feeding toxicity and fumigant toxicity will be used in this experiment. The method of feeding toxicity directly feeds the *S. oryzae* and fumigant toxicity, in which *S. oryzae* will be treated by the gas that is produced by the extract dilution. Mortality of *S. oryzae* on each method will be recorded. In the experiment, methanol became the negative control while *Quercetine* as the positive control became standard.

3.1 Rearing *S. oryzae*

S. oryzae samples have been collected from the store, and grain continues to be rearing. They were reared on disinfested seeds in the laboratory at 26±1°C.

Figure 1: *S. oryzae.*

3.2 Preparation of plant extract

F. miliaceae, F. globulosa and *C. iria* were found near a paddy field in SERKAM, MALACCA. The samples were washed with running water and dried in a shaded area. Both samples were then oven-dried for 24 hours at 60°C. Using a mechanical blender, the dried samples were ground into powder. 100 grams of *F. miliaceae*, *F. globulosa* and *C. iria* powders were soaked in 500 ml of 99.99% methanol for 72 hours at room temperature. After that, the extracts were filtered through the Whattman No. 1 filter paper. The extracts were then roto-vaped with a rotary evaporator to evaporate the solvent residue. For 4 to 5 hours, set the rotary evaporator to 100rpm and 60°C. The crude extracts were kept in a chiller at 4°C until they were used.

3.3 Phytochemical screening

Phytochemical screening of the extracts of *Cyperaceae* was carried out according to the standard methods described by Harborne (1973) [10] and Evans (2002) [11].

3.3.1 *Test for saponins*

For saponins, 0.5 g of the extract was shaken vigorously with 5 mL distilled water. The test tube was covered with parafilm for 15 minutes. Stable foam indicated the presence of saponins.

3.3.2 *Test for tannins*

For tannins, 0.5 g of the plant extract was stirred with 1 mL of distilled water and filtered, and ferric chloride solution or reagent was added to the filtrate. A blue-black or blue-green precipitate was taken as evidence for the presence of tannins.

3.3.3 *Test for terpenoids*

For terpenoids, 0.5g powdered samples were soaked with chloroform, and the solution was then filtered into a new test tube. 3ml sulfuric acid was added to the solution. The reddish-brown coloration indicated the presence of terpenoids.

3.3.4 *Test for phenolic*

For phenolic, 0.5g powdered sample was soaked in 4 mL distilled water and methanol. The ratio used was 5:5 (distilled water: methanol). The solution was filtered in a new test tube. 2 drops of ferric chloride were added. The coloration of blue/green/purple color indicates the presence of phenolic.

3.3.5 *Test for phenolic*

For flavonoids, 0.5 g of the extract was soaked with ethanol and filtered into a new test tube. A few drops of ferric chloride were added, and a few magnesium strips were mixed with concentrated HCl. A green or blue color indicated the presence of flavonoids.

3.4 Feeding toxicity

The contact/feeding toxicity of *Cyperaceae* extracts at 1% concentration will be tested on adult ants. A 10 ml aliquot of each extract will be put into a petri dish leaf disc. Methanol will be used as the only treatment for the controls. After the solvent from the treated leaf discs has evaporated, ten unsexed adults of rice weevil will be placed into the petri plates. The petri dishes will be shut down. Insect mortality will be recorded every hour for up to 24 hours. Adults will be considered dead if they are probed with sharp objects and show no signs of life. Each sample has been replicated in 3 replications and becomes the sample size.

3.5 Fumigant toxicity

Cyperaceae extracts at 1% concentration will be tested against adult rice weevils for fumigant toxicity. A plastic jar with a screw cover will be applied as a fumigation chamber. Each extract will be applied to a 5 cm diameter filter paper disc. Adhesive tape will be used to secure the treated filter paper to the underside of the lid. Ten unsexed adults will be transferred to a 10 ml vial and wrapped in a fine cloth. Three vials containing the insects will be placed in the fumigant chamber as replications. To create an airtight environment in the chamber, the lid will be closed and secured with adhesive tape. Each of the samples has been replicated in 3 replications and becomes the sample size.

Table 1: Extract of dilution.

Sample	Concentration	Dilution of extracts
T_0	0 ppm	Methanol
T_1	100 ppm	10 ml Methanol + 1 mg of *F. miliacea/ F. globulosa/ C.iria/ Quercetine*
T_2	500 ppm	10 ml Methanol + 5 mg of *F. miliacea/ F. globulosa/ C. iria/ Quercetine*
T_3	10,000 ppm	10 ml Methanol + 100 mg of *F. miliacea/ F. globulosa/ C. iria/ Quercetine*
T_4	30,000 ppm	10 ml methanol + 300 mg of *F. miliacea/ F. globulosa/ C. iria/ Quercetine*

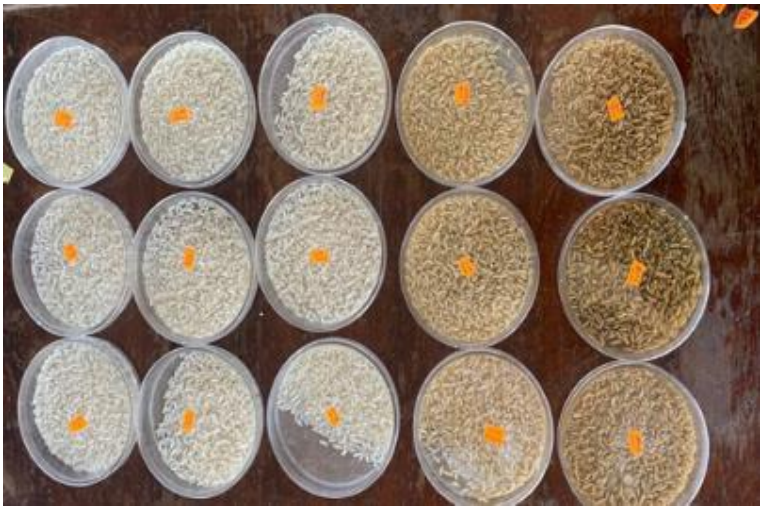

Figure 2: The setup for feeding toxicity test towards *S. oryzae.*

Figure 3: The setup for fumigant toxicity test towards *S. oryzae.*

4.0 RESULTS AND DISCUSSION

In this study, the insecticidal activity of plant extract varied according to the development stages, the species and the application method. The number of hours of fumigant or contact toxicity was observed with the plant extract of *F. miliacea, F. globulosA* and *C. iria*. Repellency on *S. oryzae* by using the method of feeding toxicity and fumigant toxicity was recorded. The activity of feeding toxicity was observed for about 10 hours, while the activity of fumigant toxicity was observed for 5 hours due to the mortality of the *S. oryzae*. Syahputra (2008) [12] claims that the presence of chemicals (active compounds) that are inhaled or touched by taste organs causes signals that promote appetite to be disrupted, which subsequently has an impact on eating behaviors. Based on the previous study, the genera *Cyperaceae* and *Fimbristylis* are useful in insecticidal activity. This finding was similar to Ilzamunnabil et al. [13].

4.1 Phytochemical screening

Every phytochemical molecule has the potential to be beneficial. The result of phytochemical screening above in the study showed that the phenolic and flavonoid were presented in the *C. iria*. However, the compound of saponin, terpenoid and tannin were absent in the *C. iria*. Saponin, terpenoid, and phenolic compounds were detected in *F. miliacea*; the flavonoid and tannin compounds were absent in the *F. miliacea* extract. On *F. globulosa*, the phytochemical compounds of terpenoid, phenolic, and flavonoid except saponin and tannin were present. There have been

several studies on the interactions between plants and phytophagous insects, particularly those on the toxicity of specific compounds to insects. Insecticidal activity of saponins can be interference with the feeding behavior. Some saponins have antifeeding activity, as is the case of saponins extracted [14].

Table 2: Phytochemical compounds represent.

Phytochemical compound	*C. iria*	*F. miliacea*	*F. globulosa*
Saponin	-	++	-
Terpenoid	-	+	+
Phenolic	+	+	++
Flavonoid	+	-	+
Tannin	-	-	-

(+) weakly positive reaction, (++) positive reaction, (-) absent

4.2 Feeding Toxicity

The concentration starts with 100 ppm, 500 ppm, 10,000 ppm and 30,000 ppm. Methanol had become the control for screening the metabolite's presence for this insecticidal activity; meanwhile, *Quercetine* became the standard. According to the usage of methanol without any weed extract, no insecticidal activity occurred. This shows that the *S. oryzae* does not react to the methanol only. However, all the weed extracts with methanol show the insecticidal reaction on *S. oryzae*.

Quercetine, as a standard, became the higher of the insecticidal reaction seems on 2 hours, the *S. oryzae* started to react on the feeding toxicity, and *S. oryzae* full mortality was recorded even at the smallest concentration at 100 ppm. *F. miliacea* becomes the best plant extract that shows the fast reaction of mortality on *S. oryzae* compared to the plant extracts of *C. iria* and *F. globulosa*. Based on the phytochemical screening, there was a saponin compound representing which saponin compound has antifeeding activity as is the case of saponins extracted. This class compound has an intriguing pesticide potential. Based on observation [15], saponin contributes to its insecticidal activity against *S. oryzae* adults. Saponins can impair cell viability by enhancing membrane permeability, allowing insects to get through.

Figure 4: Mortality rate of feeding toxicity against *S. oryzae.*

Extract	Concentration (PPM)	Mean ± SD									
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours	9 hours	10 hours
METHANOL	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	10,000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	30,000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
QUERCETINE	100	0.00±0.00	1.00±0.00	2.33±0.58	4.00±0.00	5.33±0.58	6.67±0.58	7.67±0.58	9.00±0.00	10.00±0.00	10.00±0.00
	500	0.00±0.00	1.00±0.00	2.33±0.58	3.33±0.58	4.67±0.58	6.00±1.00	9.00±1.00	9.67±0.58	10.00±0.00	10.00±0.00
	10,000	0.00±0.00	1.33±0.58	3.00±0.00	5.33±1.15	7.00±1.00	9.67±0.58	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
	30,000	0.00±0.00	2.00±1.00	3.67±1.15	7.00±1.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
C. IRIA	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	2.00±0.00	4.33±0.58	5.33±0.58	6.67±0.58	8.33±0.58
	500	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.58	1.67±1.15	3.00±1.00	4.33±0.58	5.6±1.15	7.66±0.57	8.67±0.58
	10,000	0.00±0.00	0.00±0.00	1.67±0.58	3.33±1.15	6.33±2.08	7.33±2.08	9.00±1.00	9.67±0.58	10.00±0.00	10.00±0.00
	30,000	0.00±0.00	1.00±0.00	2.67±0.58	5.00±1.00	6.00±3.46	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
F. GLOBULOSA	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±1.00	2.67±0.58	4.33±0.58	5.33±0.58	6.33±0.58
	500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±0.58	2.33±1.53	3.33±2.08	4.00±2.65	5.33±2.89
	10,000	0.00±0.00	0.00±0.00	0.67±0.58	2.00±1.00	3.67±0.58	5.33±1.52	6.67±1.53	7.67±1.53	9.33 ±1.15	10.00±0.00
	30,000	0.00±0.00	1.33±0.58	3.33±0.58	5.67±1.15	7.33±1.15	9.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
F. MILIACEA	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.33±0.58	4.00±1.00	5.67±0.58	7.67±0.58	8.67±0.58	9.33±0.58
	500	0.00±0.00	0.00±0.00	0.00±0.00	2.33±0.58	3.67±0.58	6.33±0.58	8.33±0.58	9.33±0.58	10.00±0.00	10.00±0.00
	10,000	0.00±0.00	0.00±0.00	1.67±1.53	3.67±0.58	5.33±0.58	7.33±0.58	8.67±0.58	10.00±0.00	10.00±0.00	10.00±0.00
	30,000	0.00±0.00	2.33±0.58	3.67±0.58	5.33±0.58	7.33±0.58	8.67±0.58	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00

4.3 Fumigant Toxicity

The strongest fumigant toxicity of plant extract is by *F. miliacea*, which contains the saponin compound. It shows that on concentration 500 ppm, the *S. oryzae* already showed the reaction of full mortality compared to the *F. globulosa* and *C. iria* shows the weak of fumigant toxicity against *S. oryzae*. *S. oryzae* mortality breathed through their trachea and spiracle. These spiracles may have been prevented from suffocating as a result. The respiratory system is slowed down by the natural plant extract's obstruction of the spiracles.

Figure 5: Mortality rate of fumigant toxicity against *S. oryzae.*

Extract	Concentration (PPM)	Mean ± SD				
		1 hour	2 hours	3 hours	4 hours	5 hours
METHANOL	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	10,000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	30,000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
QUERCETINE	100	2.33±0.58	3.67±0.58	5.67±0.58	7.33±0.58	10.00±0.00
	500	3.67±0.58	5.33±0.58	7.00±1.00	8.67±0.58	10.00±0.00
	10,000	4.00±1.00	5.00±1.00	8.00±1.00	10.00±0.00	10.00±0.00
	30,000	5.00±1.00	7.67±1.53	10.00±0.00	10.00±0.00	10.00±0.00
F. GLOBULOSA	100	0.00±0.00	0.00±0.00	2.33±0.58	4.33±0.58	6.33±0.58
	500	0.00±0.00	1.67±1.53	4.00±1.00	6.33±0.58	8.33±0.58
	10,000	3.33±0.58	6.33±0.58	8.67±1.15	10.00±0.00	10.00±0.00
	30,000	3.67±0.58	7.33±0.58	10.00±0.00	10.00±0.00	10.00±0.00
C.IRIA	100	0.00±0.00	0.00±0.00	2.67±0.58	5.00±1.00	7.67±0.58
	500	0.00±0.00	0.00±0.00	3.33±0.58	6.00±1.00	8.67±0.58
	10,000	0.00±0.00	3.33±0.58	6.33±0.58	8.33±0.58	10.00±0.00
	30,000	0.00±0.00	3.67±0.58	7.33±0.58	10.00±0.00	10.00±0.00
F. MILIACEA	100	0.00±0.00	3.00±1.00	4.33±0.58	5.67±0.58	7.33±0.58
	500	0.00±0.00	4.00±1.00	6.33±0.58	8.33±0.58	10.00±0.00
	10,000	4.67±0.58	7.33±0.58	9.67±0.58	10.00±0.00	10.00±0.00
	30,000	5.67±0.58	9.33±1.15	10.00±0.00	10.00±0.00	10.00±0.00

5.0 CONCLUSION

In conclusion, natural insecticides are alternatives to synthetic ones, aiming to reduce negative impacts like residues, resistance, and environmental harm. In a study, invasive weeds like *F. miliacea, F. globulosa* and *C. iria* were investigated for insecticidal properties due to their significant phytochemical content. Different *Cyperaceae* species possessed different biological activities such as insecticidal, antimicrobial and antioxidant activity. This may be due to the difference in secondary metabolite content in the plant itself. Phytochemical testing revealed compounds in the weeds, contributing to protective functions. *F. miliacea* extracts demonstrated higher insecticidal efficacy and more phytochemical compounds compared to *F. globulosa* and *C. iria*. The saponin content of *F. miliacea*, which is toxic to insects, has great potential for future investigation in order to develop into a natural agricultural product. However, the weed extract's response to the insecticidal reaction on the *S. oryzae* cannot generate better results than insecticides.

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