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Bioassay of Leaves Extract of Gamal (*Gliricidia sepium*) Against Papaya Mealybugs *Paracoccus marginatus* (Hemiptera: Pseudococcidae)

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Original Research Article

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Abstract: Since the use of chemical pesticides is known to have an impact on the environment and health, the search for plant-based insecticides is a necessity. This study is aimed to find out whether the leaf eaxtract of *Gliricidia sepium* is also potent to be used in contolling papaya mealybugs, *Paracoccus marginatus*. Bioassay was done by feeding mealybugs with young fruits of papaya that previously soaked in aqueous and methanolic extracts of gamal for 10 minutes. Five levels of extract concentration are applied namely 0% (control), 0.05%, 0.10%, 0.15%, and 0.2%. Mortality of the insects were examined and assessed at the12th, 24th, 48th and 72th hour. The results showed both types of gamal leaves extract, the aqueous and methanolic, are lethal to the papaya mealybug *P. marginatus* with LD_{50} values of 0.063% and 0.09% respectively. But when both are compared, it appears that the methanol extract is slightly more toxic than the water extract. In conclusion, leaves extract of gamal is potent to use as a bioinsecticide in controling papaya mealybugs.
Keywords: gamal, *Gliricidia sepium*, papaya mealybug, *Paracoccus marginatus*, bioinsecticide.

INTRODUCTION

Indonesia is among the top three papaya producing countries in the world after India and Brazil. In the production period of 2008-2010 India produces 38.61%, Brazil produces 17.5%, and Indonesia contributes 6.89% of the world's papaya [1].

In the last decade, papaya farmers in India and Indonesia have been challenged by the emergence of a very destructive new papaya pest, the papaya mealybugs, *Paracoccus marginatus* (Hemiptera: Pseudococcidae). This polyphagous pest is actually recorded for the first time in Asian Region, especially Indonseia (Java) and India (Tamil Nadu), in 2008 [2].

Because of its polyphagous nature, these pests not only found to infest papaya, but many other types of agricultural crops. In Sri Lanka, the papaya mealybug found to heavily infest more than 40 plant species including papaw, the major host, and several horticultural and floricultural crops like *Plumeria*, manioc, bread fruit, *Alstonia macrophylla* and *Jatropha* spp [3]. From Israel, *Paracoccus marginatus* is reported to develop large populations on annona, hibiscus, mulberry and papaya [4]. Losses of papaya farmers mainly due to the pest infestation reduce the fruit quality and market value. Papaya mealybug infestation covers all parts of the plant including stem, leaves and fruit. The infested fruits become blackish color, wrinkles, rot and more easily fall [5].

So far, laboratory test results show that the most effective way to control papaya mealybug is by using chemical pesticides such as thiamethoxam (94.44% mortality), profenophos (92.30%) and acephate (89.30%) [6]. Given the high risk of chemical pesticides on the environment and health, it is necessary to consider the use of plant-based insecticides. In Indonesia alone, efforts to control papaya pests using botanical insecticides have been initiated. Sifa *et al.*, for instance found that third-instar nymphs of *Paracoccus marginatus* effectively controlled using leaf extract *Tephrosia vogelii*, seed extract of *Annonasquamosa*, leaf essential oil of *Cinnamomum multiforum* and their mixtures [7].

In Lampung Province of Indonesia, Nukmal *et al.*, in their search for botanical insecticides to control coffee pests, found that leaf extract of gamal (*Gliricida sepium*) effectively kills *Planococcus citri* Risso [8]. Current study is aimed to find out whether the leaf eaxtract of *Gliricidia sepium* is also potent to be used in contolling papaya mealybugs, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae).

METHODS

Plant Samples and Extraction

Leaves of gamal (*Gliricidia sepium*) used in this study were collected from district of Pringsewu, Lampung province, Indonesia. For taxonomic verification, the plant sample was brought to the Botany Laboratory, Faculty of Mathematics and Sciences, University of Lampung, Indonesia. The leaves were air-dried at room temperature for 10 days and ground into fine powder using disk-mill machine. The dry leaves powder then prepared to be aqueous and methanolic extract. For aqueous extract, the powder (500g) were consecutively soaked in n-hexane dichloromethane, methanol and water. Each maceration step runs for 24 h with three replication. For methanolic extract, the symplicia were macerated consecutively using n-hexane, dichlorometthane and methanol solvents with the same duration and replication as water extraction. After being evaporated using a rotary evaporator, both water and ethanol extracts were diluted in series according to the desired concentration namely 0% (control), 0.05%, 0.10%, 0.15%, and 0.2%.

Bioassay Procedures

Bioassay conducted by giving mealybugs with young fruits of papaya that previously soaked in the gamal extracts for 10 minutes. After air dried, each fruit was put into a beaker glass. Into each beaker then 10 imago mealybugs were placed and allowed to feed on the papaya fruit. Each treatment repeated three times. Mortality of the insects were examined and assessed at the12th, 24th, 48th and 72th hour.

Study Parameters and Data Analysis

Mortality rate of test insects was expressed as percentage, whereas effectiveness of extract in killing the insect was expressed as LD_{50} (lethal dose 50%). Two-way Anova was applied to compare mortality rate between level of extract concentration and duration of exposure. The LD_{50} was determined using Probit EXE Analysis Program.

RESULTS

Mean percentage of mortality rate of papaya mealybugs fed on aqueous and methanol extracts of gamal plan leaves of different concentration by duration of exposure (hours) are presented in Table-1. Results of mean diference analysis of the data using two-way Anova are tabulated in Table-2. Based on the F- and P-values of each source of variation it can be inferred as follows. Extract types, the aqueous and methanolic, resulted in different levels of mortality on the papaya mealybugs. Both concentration levels of extract as well as duration of exposure, either in separately or in a combination, showed significant effects on the insect mortality. However, there is no significant interaction effect between extract type and time of exposure, also no interation effect shown by types of extract and concentration levels and duration of exposure on the mortality rate of mealybugs.

Table-1: Effects of on aqueous and methanolic extracts of gamal plant leaves on mortality rate of *P. marginatus*

Time (hours)	Concentration	Mortality by extracts (%)	
		Aqueous	Methanolic
12	0	0	0
	0.05	0	0
	0.1	0	0
	0.15	0	0
	0.2	0	0
24	0	0	0
	0.05	0	3.33
	0.1	6.67	6.67
	0.15	0	0
	0.2	0	6.67
48	0	0	10
	0.05	10	20
	0.1	33.33	43.33
	0.15	23.33	56.67
	0.2	33.33	53.33
72	0	6.67	10
	0.05	53.33	66.67
	0.1	73.33	76.67
	0.15	66.67	73.33
	0.2	63.33	66.67

Table-2: Two-way ANOVA of mortality rate of mealybugs (*P. marginatus)* by aqueous and methanolic extract of gamal

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	893.325[a]	39	22.906	47.391	.000
Intercept	564.377	1	564.377	1168000	.000
Extract	19.417	2	9.708	20.086	.000
Concenration	112.900	4	28.225	58.397	.000
Time	303.700	3	101.233	209.448	.000
Extract*Concentration	3.833	8	.479	.991	**.449**
Extract*Time	4.417	2	2.208	4.569	.013
Concentration*Time	76.800	12	6.400	13.241	.000
Extract*Concentration*Time	4.167	8	.521	1.078	**.387**
Error	38.667	80	.483		
Total	1491.000	120			
Corrected Total	931.992	119			
a. R Squared = .959 (Adjusted R Squared =.938)					

Results of probit analysis for analyzing the dose-response data are presented in Table 3. It is clear from the data that both methanolic and water extracts of the gamal leaves are all lethal to the papaya mealybug *P. marginatus* with LD_{50} values of 0.063% and 0.09% respectively. But when both are compared, it appears that for the papaya mealybugs the methanol extract is slightly more toxic than the water extract.

Tabel-3: LC_{50} of aqueous and methanolic extract against mealybugs *P. marginatus* at 48 and 72 hour of exposure

Time (hours)	LC_{50} by Extracts		Difference
	Aqueous	Methanolic	
48	0.213	0.124	0.089
72	0.090	0.063	0.027

DISCUSSION

The results of this study confirm some previous research results on the insecticidal effects of gamal plant (*Gliricidia sepium*) extract. From Pakistan it reported that ethanol extract of *G. sepium* leaves showed repellent activity against *Aedes aegypti* mosquito [9]. On Anopheles mosquitoes, *G. sepium* leaf extracts revealed to have larvicidal effects [10]. Next, tests against *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) the ethanolic extracts from G. sepium showed acaricidal effects and evidenced by maximum mortality (100%) when used at a concentration of 20% [11]. Additionally, a study on the antifeedant activity against third instar larvae of *Helicoverpa armigera* revealed that *G. sepium* leaf extracts exhibited significant antifeedant activity at LC50 [12].

What bioactive compounds are actually that make gamal leaf extracts have toxic effects on living organism? Phytochemical analysis performed by Kumar and Simon (2016) in their efforts to evaluate the bactericidal effect of gamal leaf extracts revealed the presence of glycosides, alkaloids, essential oils, sapponins and flavonoids [13]. On cattle nematode *Cooperia punctata*, von Son de Fernex et al. (2016) found that extract of *G. sepium* showed ovicidal activity by fully inhibited egg hatching. Chemical analysis showed that the extract exhibited content of polyethylene glycol and polyphenols, though the latter were not the main bioactive compounds [14].

Concerning flavonoids, among bioactive compounds that also found in gamal leaf extracts, many studies have shown the toxic effects of these substances in various species of organisms. Flavonoid compouds that are known to be toxic to insects are biochanin and pinocembrin. Biochanin was suggested as promising phytochemical with ability to reduce fecundity in primary reproductives of the termite [15]. Meanwhile, pinocembrin was known to show anti-feeding and mortality effects in the larvae of butterfly *Spodoptera frugiperda* [16]. Biochanin and pinocembrin, in fact, are also theflavonoids commonly found in leguminous plants such as *Dalbergia odorifera* [17]. Very likely the lethal activity of the flavonoid-rich fractions of the aqueous and methanolic extracts of gamal leaves was related to the high-specific content of such isoflavonoids. Given *G. sepium* is belonging to pea plants family, the Fabaceae (Leguminosae), it is quite natural that the aqueous and methanolic extracts of the plant effectively kill mealybugs.

CONCLUSION

Aqueous and methanolic leaf extracts of gamal *Gliricidia sepium* exhibit lethal effect against papaya mealybugs *Paracoccus marginatus.* However, when both are compared based on the LC_{50}, methanolic extract seems little more toxic than that of aqueous.

Overall, leaves extract of gamal is potent to be used as a bioinsecticide in controlling papaya mealybugs.

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