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Insecticidal Effect of Leaf Extract of Gamal (Gliricidia sepium) from Different Cultivars on Papaya Mealybugs (Paracoccus marginatus, Hemiptera: Pseudococcidae)

Nismah Nukmal, Gina Dania Pratami, Emantis Rosa, Aprilia Sari, Mohammd Kanedi*

Department of Biology, Faculty of Mathematics and Sciences,University of Lampung, Bandar Lampung, Indonesia
Corresponding Author: Mohammd Kanedi

Abstract: *Polar extract of gamal leaves (Gliricidia sepium) from various cultivars in Lampung, Indonesia, is revealed to show insecticidal effect on various types of mealy bugs, but its effect on papaya meally bugs (Planacoccus marginatus) is not yet known. This study aims to determine the insecticidal effects of water extract formulation of gamal leaf powder from four different cultivars, namely Bandar Lampung (BL) cultivars, Pringsewu (PR), North Lampung (LU) and West Lampung (LB) against papaya mealy bugs. There were five formulation preapared namely Formula 1, 2, 3, 4 and 5. Each formula is differentiated based on the LC_{50} ratio of each cultivar extract (BL:PR:LU:LB) consecutively are 1:1:1:1, 2:1:1:1, 1:2:1:1, 1:1:2:1 and 1:1:1:2. Mortality data were analyzed by probit analysis to determine LC50, then continued with Anara test and LSD test with SPSS 16.0 to determine the effectiveness of the formulation. The results showed that crude leaf extracts of gamal from LU, PR, LB, and BL cultivars can kill papaya mealy bugs with the $LC_{50\text{-}72}$ values of 0.033%, 0.090%, 0.184% and 1.818% respectively. The bioassay test of the fromulation showed a a mortality rate up 86.7%. Formula 1 is more effective than the other four formulas. Thus it can be concluded that the extract of gamal leaf water has the potential to be used as a natural insecticide.*

Keywords*:mealybug, papaya mealybug, Paracoccus marginatus, gamal, Gliricidia sepium*

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I. Introduction

India, Brazil and Indonesia are the top three papaya producing countries in the world. In the period of 2008-2010 these three countries contributed up 63% of world's papaya [1]. Unfortunately, since 2008 papaya farmers in India and Indonesia face challenges that are not easily overcome, the presence of a new pest, papaya mealybug named *Paracoccus marginatus* (Hemiptera: Pseudococcidae). This highly destructive polyphagous pest is recorded for the first time in Java, Indonseia and Tamil Nadu, India [2]. In Indonesia alone, infestation of mealy bugs on papaya plants that occured in 2009, causing a decrease in papaya production in Bogor Regency by 58% and economic losses reaching 84% [3].

Lately, there have been many studies testing the polar extract (methanol, ethanol and water) of gamal leaves on a laboratory scale against mealy bugs. The gamal plant extracts from different cultivars namely Bandar Lampung (BL), West Lampung (LB), North Lampung (LU), and Pringsewu (PR) cultivars. Among the results reported are presented in Table 1.

Current study is aimed to find out whether formulation prepared from leaf extracts of *Gliricidia sepium* from four cultivars mentioned above is potent to be used in controlling papaya mealybugs, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae).

Table 1. Results of previous tests used extracts of gamal from different cultivars against mealybugs

Type of Extract	Cultivar	Insects	$LC_{50\ (\%)}$	References
Water and ethanol	BL	*Paracoccus marginatus*	0.40 - 2.26	[4]
Water and methanol	PR	*Paracoccus minor*	0.047 - 0.054	[5]
Water and methanol	LB	*Paracoccus cryptus*	0.061 - 0.096	[6]
Water and methanol	LU	*Paracoccus citri*	0.033 - 0.039	[7]

II. Material and Methods

Plant Samples and Extraction

Leaves of gamal plant (*Gliricidia sepium*) used in this study were collected from four different districts in Lampung province namely Bandar Lampung (BL cultivar), Pringsewu (PR cultivar), West Lampung (LB cultivar) and North Lampung (LU cultivar). 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 (500 g) then prepared to be aqueous extract using maceration technique for 24 h with three replication. After being evaporated using a rotary evaporator, extracts were diluted in series according to the desired concentration. The extract concentration levels of each gamal cultivar prepared to be used in bioassay were based on LC_{50} values found in previous researchs shown in Table 1, which is as follows (Table 2).

Table 2. Concentration series of gamal plant extract prepared from four different cultivars

Cultivar	Extract concentration series (%)				
	1	2	3	4	5
BL	0	0.7	1.4	2.1	2.8
PR	0	0.05	0.1	0.16	0.20
LB	0	0.06	0.12	0.18	0.24
LU	0	0.02	0.04	0.06	0.08

Bioassay test

Bioassay carried out in this experiment was a residual test by soaking young papaya fruits in leaves extract of gamal of 5 different concentration levels as shown in Table 2 for 10 minutes. Insect mortalities were observed at 24, 48 and 72 hours after treatment. Each experimental treatment was repeated three times. Test solution with an LC_{50} value of less than 5% considered as an effective ingredient.

Formulation test

There were five formulation preapared namely Formula 1, 2, 3, 4 and 5. Each formula is differentiated based on the LC_{50} ratio of each cultivar extract (BL:PR:LU:LB) consecutively are 1:1:1:1, 2:1:1:1, 1:2:1:1, 1:1:2:1 and 1:1:1:2. Then the formula is tested on insects in the same way as the bioasai test described above. Observations on insect mortality were carried out at 24, 48 and 72 hours and repeated three times.

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 LC_{50} (lethal concentration 50%). Two-way Anova was applied to compare mortality rate between levels of extract concentration and duration of exposure. The LC_{50} was determined using Probit EXE Analysis Program.

III. Results

Mean mortality of papaya mealybugs fed on aqueous leaf extracts of gamal cultivars by level of concentration and duration of exposure (hours) are presented in **Table 3**. Results of mean diference analysis of the data using two-way Anova are tabulated in **Table 4**. Based on the F- and P-values of each source of variation it can be inferred as follows. Both plant cultivar (BL, PR, LB and LU) and time of exposure (24, 48 and 72 hours) resulted in different levels of mortality on the papaya mealybugs. The interaction between cultivar and time of exposure is also show a significant effect on the mortality of mealy bugs ($p < 0.05$).

Table 3. Mortality rate of papaya mealy bugs after treatment with leaves extract of gamal from different cultivars by duration of exposure

Cultivar	Concentration (%)	Mortality (%)		
		24 hours	48 hours	72 hours
BL	0.00	0	0	10
	0.70	20	53	83
	1.40	30	33	60
	2.10	20	27	47
	2.80	30	37	47
PR	0.00	0	0	7
	0.05	0	10	53
	0.10	7	33	73
	0.15	0	23	67
	0.20	0	33	63
LB	0.00	0	0	10

	0.06	17	27	37
	0.12	7	20	47
	0.18	20	40	53
	0.24	30	57	57
	0.00	0	0	17
	0.02	7	17	50
LU	0.04	20	20	53
	0.06	23	23	70
	0.08	17	27	87

Results of probit analysis for analyzing the dose-response data are presented in **Table 5.** The data suggest that all leaf extracts of gamal cultivars (BL, PR, LB and LU) are toxic to the *P. marginatus* with LC_{50} values of 1.818%; 0.091%; 0.184%; 0.033% respectively. However, when the four cultivars are compared, it appears that for the papaya mealybugs, gamal extract of LU cultivar is the most toxic, whereas extract of BL cultivars is somewhat less toxic.

Graphical presentation in Fig.1 shows the effect of five formula made from leaf extracts of gamal from different cultivars on mortality of papaya mealybugs (*P. marginatus*) after 72 hours of exposure. Statistical analysis using Anova and a post hoc test (LSD) resulted in the values tabulated in Table 6.

Table 4. Two-way ANOVA of mortality rate of mealybugs (*P. marginatus)* by gamal cultivars and duration of exposure

Source of variation	df	Seq SS	Adj MS	F	Sig
Cultivar	5	131.704	26.341	15.80	0.000
Time of exposure	2	171.370	85.685	51.41	0.000
Cultivar*Time of exposure	10	38.407	3.841	2.30	0.033
Error	36	60.000	1.667		
Total	53	401.481			

Table 5. LC_{50} values of crude water extract of gamal from different cultivars after 72 hours of treatment

Cultivar	LC_{50} (%)
BL	1,818
PR	0,091
LB	0,184
LU	0,033

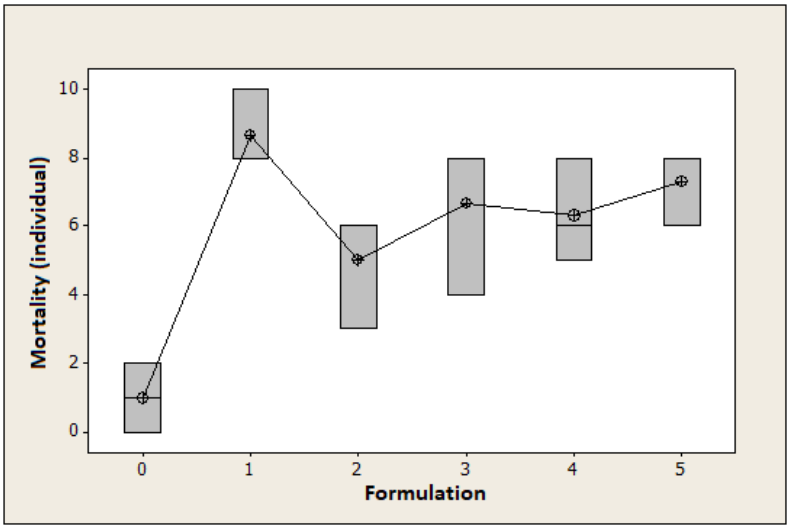

Fig. 1. Boxplot for Formula 1, 2, 3, 4 and 5 after 72 hours of treatment. The concentration ratio (BL:PR:LU:LB) used in each formula consecutively are 1:1:1:1, 2:1:1:1; , 1:2:1:1, 1:1:2:1 and 1:1:1:2.

Table 6. Mortality of papaya mealy bugs treated with 5 different formulation of leaf extracts of gamal at different hours of exposure

Hours of Exposure	Mortality of insect (mean ± SD)					
	Control	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
24	0.00 ± 0.00^{a}	1.33 ± 0.58^{ab}	2.00 ± 1.00^{bc}	1.00 ± 0.00^{ab}	1.33 ± 1.53^{ab}	3.33 ± 2.31^{c}
48	0.00 ± 0.00^{a}	4.67 ± 1.53^{c}	3.67 ± 1.53^{bc}	2.67 ± 0.58^{b}	3.67 ± 0.5^{bc}	4.67 ± 1.15^{c}
72	1.00 ± 1.00^{a}	8.67 ± 1.15^{c}	5.00 ± 1.73^{b}	6.67 ± 2.31^{bc}	6.33 ± 1.53^{bc}	7.33 ± 1.15^{bc}

Values in the same row followed by the same superscript are not different at α=0.05 by LSD test

IV. Discussion

The results of this study confirm some previous research results on the insecticidal effects of gamal plant (*Gliricidia sepium*) extract. From Tamil Nadu,India, it reported that ethanol extract of *G. sepium* leaves showed repellent activity against *Aedes aegypti* mosquito [8, 9]. It has been also reported that ethanolic extracts from G. sepium showed acaricidal effects against *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) [10]. In addition, *G. sepium* extract revealed to be effective for controling insect pest (*Godasa sidae*) of *Mansonia altissima* (A.Chev) seedlings [11].

Bioactive components that have been identified from plant extracts of G. sepium which have anti-insecticide effects consist of Alkaloids, Terpenoids, Phenolics, Coumarin, Tannins, Saponins, Flavonoids, Quinones, Proteins, and Sterols [12]. Tannin, saponins, free fatty acids and alkaloids are among bioactive found in flower extract *Calotropis gigantea* that were revealed to have insecticidal activity (90-95%) at 2000 ppm within 24 hours against papaya mealy bug infestation in *Ailanthus excelsa* [13].

Concerning flavonoids, many study reports have shown the toxic effects of these substances in various species of organisms. Among flavonoids that are known to be toxic to insects are biochanin and pinocembrin. Biochanin was revealed to reduce to reduce fecundity in primary reproductives of the termite [14]. Wheareas, pinocembrin was known to show anti-feeding and mortality effects in the larvae of butterfly *Spodoptera frugiperda* [15].

This study successfully demonstrated that gamal extract from different cultivars had a deadly toxic effect on papaya mealybugs. However, the toxic levels of each cultivar extract is somewhat different. This is something natural because differences in cultivars cause differences in plant secondary metabolites [16]. In Tithonia diversifolia, for instance, the metabolites in the inflorescence and root parts were mainly affected by variation of some soil nutrients such as Ca, Mg, P, K and Cu [17].

V. Conclusion

All leaves extract of gamal cultivars from different districts of Lampung Province, Indonesia, either separately or in combintation (formulation) have lethal effect on papaya mealy bugs Paracoccus marginatus. Thus, plant leaves extract of gamal (Gliricidia sepium) is potential to be use as bioinsecticide for papaya mealy bugs.

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