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(Research Article)

Assays of physical stability and insecticidal activity of leaf extract of *Gliricidia sepium* on *Pseudococcus longispinus*—mealybugs infecting rose apple

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Abstract

This experiment is a follow-up study of our efforts to develop gamal plant (*Gliricidia sepium*) as natural insecticides. The purpose of this study was to determine the stability of the physical properties and the toxic activity of the extract that had been stored for up to three years. There were two types of extract prepared, namely water and methanol. Physical profile of the extract was assessed using organoleptic and pH of the extract. Meanwhile, the stability of the toxic properties was tested using residual effect techniques and probit analysis to determine the LC50 value of the extract. An overview of the study results obtained is as follows. The organoleptic properties and pH value of the gamal leaf extract remained stable for two years of storage. While the toxicity of the extract remained unchanged for 3 years of storage. Thus, it can be concluded that the leaf extract of the gamal plant has the potential to be developed into a plant-derived insecticide.

Keywords: Kutu Putih; Mealybug; *Psedococcus longispinus*; Gamal; *Gliricidia sepium*; Extract Stability

1. Introduction

Several studies that we have conducted in the last five years have revealed that plant extract of *Gliricidiasepium (*Syn*. G. maculata)* actually has insecticidal properties against mealy bugs (Hemiptera: *Pseudococcidae***)**. Among the mealybugsspecies that are known to be killed using leaf extract of *G. maculata*, a plant named gamal in Indonesia, are coffee mealybugs (*Planococcus citri* Risso) and papaya mealy bugs (*Paracoccu smarginatus*) [1, 2].

The question is whether gamal leaf extract is feasible to be developed into an economical insecticide?To answer this question, it is important to do physical and chemical stability tests as well as the efficacy of the ingredients.It is through stability testing that the efficacy and safety of the use of these pesticide materials can be monitored and maintained [3, 4].

Our initial study of the impact of storage time on the toxic effects of gamal plant extract on mealybugs revealed that storage periods of up to 24 months did not show significant changes. From the initial experiment, it was known that the toxicity stability of the *G. maculata* leaves extract was influenced by the type of the plant cultivar and the type of solvent used in the extraction [5].

To find out more about the impact of the long storage time of gamal leaf extract on physical stability and its toxic activity against mealybugs, we conducted a follow-up study, the results of which are presented in this article.

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2. Material and methods

2.1. Plant samples and extraction

Gamal leaves used in this experiment were collected from Pringsewu District, Lampung Province, Indonesia. The plant leaves were finely chopped, dried, and then ground into a fine powder. The dried powder was put in two different maceration bottles of 250 mg each. Each plant leaf sample powder then macerated using two different types of solvent namely distilled water and methanol. Both extract were evaporated using a rotary evaporator to obtain a crude extract paste. The extraction was carried out on different occasions and then stored for a specified time to observe its stability (0 years, 1 year, and 2 years) at a later date.

2.2. Physical stability assays

There are three parameters of physical stability tested (observed) namely organoleptic, acidity (pH), and temperature cycle tests. The organoleptic test was carried out by adopting the method of Elya et.al. (2013) [6], by observing changes in color, odor, and texture of the plant leaf extract of gamal that have been stored for 0, 2 and 3 years. The pH stability measurement of the extract was carried out by dissolving 0.3 g of the extract into 30 ml of distilled water. The mixture was measured using a digital pH meter with three repetitions. Furthermore, the temperature cycle test was carried out by adopting the procedure used by Cannell (1985) [7] as follows. The plant leaf extract of gamal (3 g) was placed in a tightly closed Petri dish and stored at 4°C for 24 hours then transferred to a place at 40°C for 24 hours (one cycle). This test was carried out for 6 cycles in 12 days.

2.3. Toxicity assays

To test the toxic stability of the gamal leaf powder extract on the mealy bug, we use rose apple mealybug (*Pseudococcus longispinus*) as the target insect. Five concentration levels of leaf extract of gamal were prepared namely 0,00%, 0,041%, 0,061%, 0,081%, 0,101%.The bioassay was carried out using residual effect method by rearing adult female insects (10 insects each) in plastic container contained fresh leaves of rose apple (*Syzygiumaqueum*) that have been dipped for 10 minutes in gamal leaf extract of desired concentration. The insect mortality was recorded at 24, 48, and 72 hours after treatment.

2.4. Data analysis

In the data analysis, both descriptive and inferential statistical methods were applied. Organoleptic test result was analyzed descriptively, while pH and temperature cycle as well as toxic stability parameters were analyzed inferentially. Toxicity effect of the extracts assessed using Probit Analysis based on LC_{50} values at the level of significance of less than ≤ 5%.

3. Results and discussion

3.1. Physical characteristic cof the extracts

Organoleptic and pH profile of gamal leaf extract stored for different durations are presented in consecutively in Table 1 and 2. The data in Table 1 and 2 showed that both methanol and water extracts of gamal leaves are remains stable for two years of storage. All physical parameters of the extract i.e. texture, color and odor as well as the pH of the extract only change after three years.

Table 1 Texture, color and odor of the gamal leaf extracts by storage time

Type of solvent	Organoleptic parameters	Storage time (years)		
		0	2	3
Distilled water	Texture	lumpy paste	lumpy paste	loose paste
	Color	brownish-green	brownish-green	dark green
	Odor	highly pleasant	pleasant	unpleasant
Methanol	Texture	highly thick paste	thick paste	less thick paste
	Color	dark green	dark green	pale green
	Odor	very distinctive	moderate distinctive	less distinctive

Table 2 pH of gamal leaf extracts by storage time

Type of solvent	Storage time (years)		
	0	2	3
Distilled water	4.79±0.032[a]	5.12±0.070[a]	6.00±0.049[b]
Metanol	4.51±0.015[a]	3.84±0.085[a]	5.76±0.045[b]

pH values are Mean ± SD. Values on the same row followed by the same superscript are not statistically different at α = 5%

Following temperature cycle test, the organoleptic profile and pH values of the extracts were measured again, the results of which are presented consecutively in Table 3 and 4. Based on the physical parameters (organoleptic and pH) of leaf extract of gamal after temperature cycle test shown in the two mentioned tables (Table 3 and 4), it can be assumed that both water and methanol extracts of the gamal leaves relatively stable for 2 years of storage.

Table 3 Profile of texture, color and odor of the gamal leaf extracts by storage time after temperature cycle test

Type of solvent	Organoleptic parameters	Physical changes		
		0	2	3
Distilled water	Texture	no changes	no changes	a bit lumpy
	Color	a little faded	no changes	No change
	Odor	no changes	no changes	no changes
Methanol	Texture	no changes	no changes	a little change
	Color	a little darker	no changes	no changes
	Odor	no changes	no changes	no changes

Table 4 pH of gamal leaf extracts by storage time after temperature cycle test

Type of solvent	pH values of the extract		
	0	2	3
Distilled water	4.70±0.032[a]	5.08±0.015[a]	5.75±0.011[b]
Metanol	4.10±0.025[a]	3.78±0.026[a]	5.31±0.026[b]

pH values are Mean± SD. Values on the same row followed by the same superscript are not statistically different at α = 5%.

3.2. Toxicity profile of the extracts

Toxicity test of leaf extract of gamal plant (*G. sepium*) against mealybugs resulted in mortality rate data (Table 5), LC_{50} of extract before temperature cycle test (Table 6), and LC_{50} of extract after temperature cycle test (Table 7).

Table 5 Mortality rate of mealybugs (n =10) after treated with leaf extract of gamal for 72 h by storage duration

Type of solvent	Mortality rate of mealybugs		
	0 year	2 years	3 years
Distilled water	0.97±0.865[a]	1.17±1.028[a]	0.84±0.672[b]
Metanol	0.97±0.865[a]	1.17±1.028[a]	0.84±0.672[b]

Mortality rate are Mean± SD. Values on the same row followed by the same superscript are not statistically different at α = 5% based on Tukey's post hoc test

In line with the changes in the physical properties of the extract after the temperature cycle test shown in Tables 3 and 4, the toxicity of the leaf extract of the G. sepium plant also persisted for two years and only changed significantly after 3 years of storage. The mortality rate of the tested insects was significantly lower when given the extract that had been stored for 3 years (Table 5).

Table 6 LC_{50} of leaf extract of gamal plant on mealybugs *P. longispinus* by storage time of the extracts

Type of solvent	Treatment time (h)	Mean values of LC_{50}		
		0 year	2 years	3 years
Distilled water	24	0.177 ± 0.049	0.148 ± 0.029	0.214 ± 0.091
	48	0.146 ± 0.027	0.038 ± 0.026	0.148 ± 0.028
	72	0.135 ± 0.023	0.120 ± 0.018	0.144 ± 0.027
Metanol	24	0.211 ± 0.080	0.298 ± 0.190	0.289 ± 0.181
	48	0.191 ± 0.060	0.182 ± 0.056	0.199 ± 0.068
	72	0.211 ± 0.080	0.298 ± 0.190	0.189 ± 0.061

Values are representing Mean ± SE.

Table 7 LC_{50} of leaf extract of gamal plant on mealybugs *P. longispinus* by storage time of the extracts after temperature cycle test

Type of solvent	Treatment time (h)	Mean values of LC_{50}		
		0 year	2 years	3 years
Distilled water	24	0.263 ± 0.142	0.193 ± 0.063	0.168 ± 0.042
	48	0.201 ± 0.074	0.152 ± 0.034	0.153 ± 0.032
	72	0.155 ± 0.038	0.140 ± 0.028	0.143 ± 0.027
Metanol	24	0.307 ± 0.205	0.241 ± 0.113	0.274 ± 0.163
	48	0.214 ± 0.086	0.165 ± 0.041	0.199 ± 0.068
	72	0.162 ± 0.043	0.129 ± 0.020	0.180 ± 0.054

Values are representing Mean ± SE

Regarding LC_{50} values of the extracts against tested mealybugsbefore and after temperature cycle test (Table 6 and 7) both water and methanol leaf extract of the gamal plant shows no statistical difference in toxicity.

In summary, referring to the results obtained in this study, it can be emphasized that the insecticidal properties of the gamal plant leaf extract that had been stored for more than two years did not change significantly. The stability of the toxic properties of the leaf extract of this plant against mealybugs can be explained as follows.

There have been many research reports revealed that the gamal plant, Gliricidia sp. belongs to Fabaceae family, rich in bioactive compounds such as glycosides phytosterols, alkaloids, saponins, phenols, polyphenols, tannins, and flavonoids [8,9]. Among the these phytochemicals, namely alkaloids, glycosides, and tannins along with other secondary metabolites such as proteins, oxalic acid and oxalates, coumarins, furocoumarins, lectins, solanines and chaconine are known as plant-derived toxin [10].

The toxic effects of bioactive compounds extracted from *Gliridia* sp. plants such as alkaloids, flavonoids, phenols, tannins and saponins were not only seen in insects but also in mite *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) [11].

In addition to its toxic properties, several active compounds of the gamal plant are also known to have strong antioxidant properties, such as phenolic acids, saponins, alkaloids and flavonoids. [12, 13]. Among the active ingredients contained in the leaf extract of the gamal plant, flavonoids are one of the bioactive compounds that have high stability both at low temperatures (4^{o}C) and at high temperatures (30 ^{o}C) [14].

Although the toxicity of the extract of the gamal plant was relatively stable in storage for more than two years, the physical properties of the extract of this plant leaf powder (organoleptic and pH) significantly changed. The change in physical stability was probably caused by imperfect extract preparation techniques. According to Kostelansk et al. (2022) the physico-chemical stability of medicinal substances is influenced by the technique of preparation. Solid and dry preparations, in the form of tablets, for example, are the best [15].

4. Conclusion

Gliricida sepium leaf extract, which was stored for more than two years, experienced a decrease in physical stability (organoleptic and pH) but relatively did not experience changes in toxic properties (insecticide activity). Thus the extract of the gamal plant can be developed into an economical insecticide.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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