The Effect of Storage Time on the Raw Material of Insecticide Candidate from Gamal Leaves (*Gliricidia maculata*) on The Stability of Toxicity to Control Mealy Bugs

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Abstract. Mealybugs are pests that cause economic losses to cultivated crops such as papaya and cocoa. Mealybug pests cause productivity and fruit quality to decrease. Control using botanical insecticides that are more environmentally friendly can be done, one of which is using gamal leaves (Gliricidia maculata). Research on the potential of gamal leaves as a vegetable insecticide has been carried out in the last 10 years. To develop it into a botanical insecticide formula, a basic study is neededlevels of flavonoids and their toxicity to storage time. This study aims to determine the toxicity and levels of active compounds in the form of flavonoids on the storage time of gamal leaf powder. The test used raw materials for gamal leaves from 3 different origin of plant, namely North Lampung, West Lampung, and Pringsewu in the form of a powder which is then extracted using water and methanol as a solvent. The toxicity test used bioassay with the residual effect of two extracts, namely water extract and methanol extract against mealybugs. Mortality data were analyzed using probit analysis to determine LC_{50}. The correlation between storage time and toxicity was analyzed using correlation analysis with the SPSS application.. The yield of methanol and water extract was decrease 1.05% – 9.41% and 0.34% - 34.70% this indicate that extract methanol of G. *maculata* more stable than water extract.The LC_{50} value from the probit analysis tended to increase between 0.011% - 0.077% with the storage time of powder raw materials, this indicated a decrease in the effectiveness of the toxicity between 28.2 - 78.1% depending on the type of origin of plant gamal leaf. The results show that the storage time of 6 - 24 months does not have significant effect on the stability of the toxicity and yield of the insecticide raw material powder from gamal leaves, but the longer the storage time, the lower the toxicity.

1. Introduction

Mealybugs are pests that are harmful to crop cultivation in the world. This pest causing damage and economic losses These pests attack many cultivated crops such as cocoa, papaya and coffee [1]. One of the plants that could potentially be developed for plant-based insecticides in controlling mealybugs is gamal (Gliricidia maculata) [2].

The leaf extract of the gamal plant (Gliricidia maculata) is known to have the ability to act as an anti-fungal, rodentisia, and natural insecticide. This is because gamal leaves contain secondary metabolites in the form of flavonoids. Based on the results of research over the last 10 years it was found that water and methanol extract of gamal leaf powder taken from 4 cultivars (Bandar Lampung, West Lampung, North Lampung, and Pringsewu) was effective in killing 4 species of white lice pests (on papaya.*Paracoccus marginatus*, in Pseudococcus cryptus soursop, in Planococcus citri coffee, and in cocoa *Planococcus minor)* [1].

Plant-based insectides have many advantages, especially for the preservation of nature because they are free of residues, do not cause resistance to pests, do not cause poisoning to plants, and can be combined with other control measures. In addition, the manufacture is not difficult and the price is relatively cheap and available in nature, can make it easier for farmers to apply it. However, behind these advantages, vegetable insecticides have disadvantages, including if the insecticide storage time is long it will experience a decrease in effectiveness. This is because the active compounds degrade and decompose easily when used in the environment [3].

Therefore, research is needed to find out the stability of the raw material and the stability of the toxic power due to the storage time of the insecticide candidate raw material originating from the leaves of the gamal used.

2. **Experimental**

1.1. Material

The plant material of *Gliricidia maculata* levaes were collected from three different places in Lampung, there were North Lampung, Pringsewu, West Lampung. The leaves were stored as powder in freezer for 6 – 24 months. Aquades and methanol for extraction. Mealybug as animal treatment.

1.2. Extraction

1.2.1. Methanol extract

Gamal leaf powder ± 250 grams is put into a maceration bottle, then 1 liter of methanol is added. Gamal leaves are macerated for 2x24 hours. Then the filter residue is macerated again with the same steps. The results of the methanol extract were then evaporated using a rotary vacuum evaporator to obtain a crude extract and freeze dried an then weighed.

1.2.2. Water extract

Gamal leaf powder as much as ± 250 grams is put into a maceration bottle, then added with distilled water as much as 1.5 liters. Gamal leaves are macerated for 2x24 hours. Then the filter residue is macerated again with the same steps. The aqueous extract was then evaporated using a rotary vacuum evaporator to obtain a crude extract and freeze dried an then weighed.

The maserate obtained was then calculated the yield of the thick extract.

$$\%\ yield\ = \frac{\text{weigh of extract (g)}}{\text{weigh of simplisia (g)}} x\ 100\%$$

1.3. Toxicity Test

The toxicity stability test was carried out by bioassay on mealybugs using water extract and methanol extract from 3 cultivars of plant-based insecticide raw materials.

The bioassay is a mortality test with residual effect following the Nukmal and Andriyani method [4]. The residue test was carried out by immersing the test media with 5 concentration levels for 10 minutes, 10 adult female mealybugs that had been acclimatized 1 day before treatment, namely placed on the test medium and maintained in the test container.

The concentration used for bioassay on the tested insects was 0,00%, 0,05%, 0,10%, 0,15%, 0,20%. Observation of the mortality of the tested insects was carried out at 24, 48, and 72 hours after treatment. The percentage of mortality for each extract was analyzed using a probit analysis program to determine the relationship between concentration and insect mortality. Each treatment was repeated 3 times. The mortality data obtained were analyzed using probit analysis to determine the LC50 value.

3. Result and discussion

3.1. Effect of time storage on yield extract

The results of the extraction of 250 gr of three origin of plant of gamal leaf powder from West Lampung, Pringsewu and North Lampung, which have been stored in the freezer, obtained methanol extract and water extract with the yield percentage in Table 1.

Table 1. Effect of time storage on yield methanol extract

Origin of Plant	Time Storage (month)	Yield methanol extract of G. *maculata* (%, w/dw)		Difference
		Early	End	
North Lampung	24	15.75 ± 0.5	14.7 ± 0.4	1.05
West Lampung	12	21.22 ± 0.2	17.6 ± 0.6	3.62
Pringsewu	12	19.09 ± 0.4	10.5 ± 1.2	9.41

w: weight; dw: dry weight

Table 2. Effect of time storage on yield water extract

Origin of Plant	Time Storage (month)	Yield water extract of G. *maculata* (%, w/dw)		Difference
		Early	End	
North Lampung	24	39.28 ± 0.2	38.94 ± 0.5	0.34
West Lampung	12	35.93 ± 0.9	26.44 ± 0.9	9.49
Pringsewu	12	59.50 ± 1.2	23.80 ± 0.3	35.70

w: weight; dw: dry weight

The percentage yield of extract produced was different for each origin of plant and the solvent used (Tables 1 and 2). The difference of yield from different origin of plant may be due to differences of environmental factor in origin of plant such as light, soil, temperature, and salinity. Response of plant to biotic stress can contribute to this difference of yield metabolite secunder of the plant [5]. According to Suryani et al. [6], the nature of the solvent affects the differences in compounds obtained in an extract, according to the principle of like dissolves, a solvent will tend to dissolve compounds that have the same degree of polarity.

As can be seen from result in Tables 1 and 2, a smallest difference of yield extract methanol and water was obtained from North Lampung with value 1.05 and 0.34. The reduced yield of the extract after storage can indicate a decrease in the active compound contained, in this case, namely flavonoids. The difference in the percent yield of the methanol and water extract can be caused by the interactions that occur between the solvent and flavonoid compounds [7]. Based on the result, extract from North Lampung have good stability than others. Furthermore, the reduction in yield of the methanol extract more stable than water extract, as can be seen from the differences before and after storage.

3.2. Effect of time storage on toxicity to control mealy bug

Bioassay on the effect of powder G. *maculata* storage time on the percentage reduction in the effectiveness level of methanol and water extract using the residual effect method on mealybugs is presented in Tables 3 and 4.

Table 3. Effect of powder storage time on the percentage reduction in the effectiveness of methanol extract on mealybug mortality.

Origin of Plant	Time storage (month)	LC_{50} value (%)		Difference (%)	Reduction Effectiveness (%)
		Early	End		
North Lampung	24	0.039	0.050	0.011	28.2
West Lampung	12	0.061	0.107	0.046	75.4
Pringsewu	12	0.054	0.090	0.036	66.6

Table 4. Effect of powder storage time on the percentage reduction in the effectiveness of water extract on mealybug mortality.

Origin of Plant	Time storage (month)	LC_{50} value (%)		Difference (%)	Reduction Effectiveness (%)
		Early	End		
North Lampung	24	0.033	0.011	0.077	28.2
West Lampung	12	0.096	0.171	0.075	78.1
Pringsewu	12	0.063	0.106	0.043	68.3

The LC_{50} value of gamal leaf water extract experienced a greater decrease in effectiveness than the methanol extract of gamal leaf (Table 2 and 3). This shows that the crude methanol extract of gamal leaf powder is more stable and effective than the water extract of gamal leaf powder. As can we seen from the result we found that powder from North Lampung has smallest reduction of effectiveness to control mealybug with the same value for methanol and water extract 28.2%. Based on this result, powder from North Lampung was the most stable than others. Futhermore based on LC_{50} value, powder from North Lampung has the smallest value, this was indicate that powder from North Lampung was the most effective than others.

Table 5. The correlation between storage time on the percent yield and toxicity of extract G. *maculata*

	Significance (p-value)		Correlation coefficient (r)	
	Water	Methanol	Water	Methanol
Yield	0.506	0.473	-0,700	-0,737
Toxicity	0.314	0.112	-0.686	-0,985

Based on the results of the correlation analysis (Table 5), the significance value was > 0.05 so that the correlation between storage time on percent yield and toxicity is not significant, so it can be said that storage time does not significantly affect the toxicity of extracts against mealybugs. From these results it can be concluded that gamal leaf powder has good stability. The minus results on the correlation coefficient value indicate that the correlation between storage time with toxicity and yield is that the longer the storage time, the lower the percentage of yield and toxicity.

4. Conclusion

Based on the results of the research that has been done, it can be concluded that the storage time of candidate raw material insecticide from G. maculata leaves for 6 -24 months does not have significant effect on the stability of the toxicity and yield. The correlation between storage time with toxicity and yield is that the longer the storage time, the lower the percentage of yield and toxicity.

Acknowlwdgement

The authors are grateful to DIPA Unila who has funded this research. This research is part of the 2020/2021 budget Postgraduate Research Contract Number: 1513 / UN26.21 / PN / 2020.

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