The effect of storage time on the raw material of insecticide candidate from gamal leaves (Gliricidia maculata) on the toxicities stability to control mealybug

The effect of storage time on the raw material of insecticide candidate from gamal leaves (*Gliricidia maculata*) on the toxicities stability to control mealybug

K U F Murad[1], N Nukmal[1*], and E Setyaningrum[1]

Faculty of Mathematic and Natural Sciences, University of Lampung
Jalan Prof. Dr. Ir. Sumantri Brojonegoro No. 1, Gedong Meneng, Bandar Lampung, Lampung 35141

E-mail: nnukmal@yahoo.com

Abstract. The purpose of this study to evaluate the effect of storage time on the persentage of yield extract and toxicity stability of Gamal leaves (*Gliricidia maculata*) powder to control mealybugs. The gamal leaves were colected form three different cultivars (North Lampung, West Lampung, and Pringsewu). The leaves powder was stored in the freezer (-4 ^{o}C) during 12 and 24 months. The powder storaged was macerated with methanol and aqudest, then concentrated by rotary vacuum evaporator to yield the crute exstract (paste), the paste was used for bioassay to mealybugs with residual effect methode. The result shows that the storage time of the powder raw material of insecticide candidate from *G. maculata* leaves for 12 -24 months does not significantly affect to the stability of the toxicity and persentage paste yield. But the long time of storege culd be decrese the toxicyty effectiveness and persentage paste yield gained

1. Introduction

Pesticides have become a necessity for farmers to control pest attact in agriculture. The benefits of using pesticides might increasing the quantity and quality of agricultural product, and without pesticides will result the low quality of product [1]. There are two kinds of pesticides i.e. natural pesticide and synthetic pesticide. Currently, the pesticides that widely using are synthetic pesticides because of their easy and fast used. Beside the benefit, there are many risks arising from using synthetic pesticide, such as species resistency, enviromental and non-target species toxicity [2], water, air, and soil system pollution from residual of pesticides [3] the high risks for people especially farmer population [4], damaging public health [5]. Therefore, many study are developed about natural pesticides that have lower risk than synthetic pesticides.

Gamal (*Gliricidia maculata*) is a plant that have potential to be developing as bioinsecticide to control mealybugs. Because gamal leaves contain flavonoid as secondary metabolites products. Based on the research was done in last years, knowing that the water and methanol extracts of gamal leaves powder from four different cultivars (Bandar Lampung, West Lampung, North Lampung, and Pringsewu) were toxic to four species of mealybugs i.e. *Paracoccus marginatus*, *Pseudococcus cryptus*, *Planococcus citri*, and *Planococcus minor* [6]. *Gliricidia* leaves also used as antimicrobial [7], antioxidants [8], anti-imflamantory, larvisidal, nitrogen fertilizer [9].

Raw material of gamal leaves as a candidate of bioinsecticide can be stored as powder. During storage, the natural bioactive compound of secondary metabolite product some could be labile, necessiating to use the methodes of storage that do not alter the content and activity of compounds.

Previous study has shown that the bioactive compound [10] and biological activity [11] of secondary metabolite product can be changed during storage time.

Based on the description abave, this study aims to evaluate the effect of storage time on stability of the yield extract and toxicity of gamal leaves pouder (*Gliricida maculata*) to control mealybug. The parameters observed in this study i.e. the total amount of extract yied of gamal leaves pouder that was stroraged and biological activity of this extract as insecticide to kills the mealybugs.

2. Methods

2.1. Materials

The material used in this study are the plant material of *Gliricidia maculata* levaes were collected from three different places in Lampung, Indonesia, there were North Lampung, Pringsewu, West Lampung. The leaves were stored as powder in freezer for 12 – 24 months. Aquades and methanol for extraction. Mealybug as animal treatment. Equipment used are vacuum rotary evaporator, glass beaker, maseration bootle, freezer, analitycal scales, spatula, oven, beaker glass.

2.2. Procedurs

2.2.1. Raw material preparation. Gamal leaves were cleaned dried during 7-10 days in room temperature. The dried leaves were milled into powder. The powder stored in freezer (-4^{o}C) during 12-24 months.

2.2.2. Extraction.. Gamal leaves powder was put in two different maceration bottles, Each maceration bottle contain 250 gram pouder of gamal leaves, and then one bottles added 1 liter of methanol and anather 1 liter water. Gamal leaves was macerated for 2x24 hours. Then the filter residue was macerated again with the same steps. The results of the methanol and water extract were evaporated using a rotary vacuum evaporator to obtain a crude extract (paste) and then weighed.

The persenatage of the extract (paste) yield was calculated by formula:

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

2.2.3. Toxicity Test . The stability of the toxicity of water extract and methanol extract from three different cultivars of gamal leaves pouder were tested to mealybugs. The bioassay with residual effect was carried out by immersing the media testing with 5 concentration levels (0,00%, 0,05%, 0,10%, 0,15%, 0,20%) of the extracts for 10 minutes, 10 adult female mealybugs, had been acclimatized 1 day before treatment, introduced to this media testing and rearing in plastic container. Each treatment was repeated 3 times.

The mortality data of the tested insects was recorded for 24, 48, and 72 hours after treatment.

2.2.4. Data analysis. The mortality data were annalyzed using probit analysis to determine the LC_{50} value. Correlation between storage time with yield of extract and LC_{50} values was analyzed with correlation analysis with SPSS programe.

3. Result and discussion

3.1. The Effect of time storage on extract yield

The presentage of yield methanol and water exstract from the extraction of 250 gr of gamal leaves powder from West Lampung, Pringsewu and North Lampung, which have been stored in the freezer for 12 -24 months, could be seen in Table 1.

The percentage yield of the extract gained was different for cultivar and the solvent used (Tables 1. and 2). As can be seen from result in Tables 1 and 2, there are a smalest decrease of pesentage yield gained from methanol (1.05%) and water (0.34%) extracts from North Lampung cultivar before and after storege compare with two other cultivars. The difference of yield from different cultivar 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 [12]. These difference also be influenced by internal factor such as plant age and external factors such as soil nutritions, geographical climate, season, and processing method.

According to Muraina *et al*., climatic condition of the difference origin of plant have an effect on the result of the yield extract where the cold area (lower room temparature) produced a higher yield [13].

Table 1. The effect of time storage on persentage yield of methanol extract

Cultivar	Time Storage (month)	Yield methanol extract of *G. maculata* (%, w/dw)		Difference
		before	after	
North Lampung	24	15.75	14.70	1.05
West Lampung	12	21.22	17.60	3.62
Pringsewu	12	19.09	10.50	9.41

w: wet weight; dw: dry weight

Table 2. The effect of time storage on persentsge yield of water extract

Cultivar	Time Storage (month)	Yield water extract of *G. maculata* (%, w/dw)		Difference
		Before	After	
North Lampung	24	39.28	38.94	0.34
West Lampung	12	35.93	26.44	9.49
Pringsewu	12	35.50	23.80	11.70

w: wet weight; dw: dry weight

The reducing presentage yield of the extract after storage may be indicate a decrease in the compound containing, namely flavonoids. According to Muhamad *et al.* [14], aqueous solvents are more widely used for the extraction flavonoid than non-polar solvents. The aqueous solvent has a higher polarity than methanol solvent that increases the hydrolyzed compound and extraction yield. Solvent has the most influence on the extraction yield because the differences in polarity and dependding on the extracted compound. Therefore, flavonoid more effective when dissolved in polar solvent because water can increase the diffusion of phenolic compounds in plant [15].

3.2. The Effect of time storage on toxicity stability to mealy bugs

The percentage reduction of the effectiveness level of methanol and water extract of gamal leaves powder that have been stroraged in freezer using the residual effect method on mealy bugs is presented in Tables 3 and 4.

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

Cultivar	Time storage (month)	LC_{50} value (%)		Difference (%)	Reduction Effectiveness (%)
		Before	After		
North Lampung	24	0.038	0.049	0.011	28.2
West Lampung	12	0.060	0.106	0.046	75.4
Pringsewu	12	0.053	0.089	0.036	66.6

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

Cultivar	Time storage (month)	LC_{50} value (%)		Difference (%)	Reduction Effectiveness (%)
		Before	After		
North Lampung	24	0.034	0.012	0.077	28.2
West Lampung	12	0.097	0.172	0.075	78.1
Pringsewu	12	0.062	0.105	0.043	68.3

The LC_{50} value of gamal leaves water extract shows a greater decrease in effectiveness than the methanol extract of gamal leaves (Table 2 and 3). This indicated that the crude methanol extract of gamal leaf powder is more stable and effective than the water extract of gamal leaves powder. Previous study was reported that the methanol extract of *O. stamineus* have siginificantly different in active compound and activity of antiocidant than the water extract [16].

As could be seen from the result in Tabel 3 and 4, both methanol and water extracts from North Lampung cultivar have smallest reduction (28.2%) of effectiveness to kill mealybug. This result indicated, powder from North Lampung cultivar was the most stable than others. Futhermore based on LC_{50} value, cultivar from North Lampung have the LC_{50} value smallest than others, this indicated cultivar from North Lampung was the most effective than others. The stability of effectiveness levels influence by origin of plant, because of the rate of secondary metabolite production in plants as a result of interactions between abiotic and biotic factors of the plant [17]. The reducing of effectiveness of insectisides increase gradually before and after storage. The stability of toxicity influenced by condition of storage (heat, temperature) and type of active compound of the insectiside [18].

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

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

The results of the correlation analysis (Table 5), show that the significance value ware > 0.05, so that the correlation between storage time on percent yield and toxicity is not significant, it could be said that storage time does not significantly affect the toxicity of extracts against mealybugs. In other words can be concluded that gamal powder has good stability. The minus marks on the correlation coefficient value indicated that the negative correlation between storage time with toxicity and persentage of exstract yield. The longer of the storage time could decreased the percentage of yield exstract and toxicity effectivnees.

4. Conclusion

It can be concluded that the storage time of raw material of insecticide candidate from *G. maculata* leaves for 12-24 months does not have a significant effect on the stability of the toxicity and persentage of extract yield. The correlation between storage time with toxicity and yield is negative correlation, the longer of the storage time, would be lowering the presentage of yield extract and toxicity effectiveness.

Acknowledgement

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

References

[1] Damalas C A 2009 Understanding Benefits and Risks of Pesticide Use *Acad. Journals* **4** 945–9
[2] Aktar M W, Sengupta D, Chowdhury A 2009 Impact of pesticides use in agriculture: their benefits and hazards *Interdiciplinary Toxicol.* **2** 1–12
[3] Gill H K and Garg H 2014 Pesticides: Environmental Impacts and Management Strategies *Pesticides - Toxic Aspect* (InTech) pp 187–228
[4] Arbuckle T E, Lin Z, Mery L S 2001 An Exploratory Analysis of the Effect of Pesticide Exposure on the Risk of Spontaneous Abortion in an Ontario Farm Population *Child. Heal. Artic.* **109** 851–7
[5] Sarwar M 2015 The Dangers of Pesticides Associated with Public Health and Preventing of the Risks *Interbational J. Bioinforma. Biomed. Eng.* **1** 130–6
[6] Apriliyani, Nukmal N, Rosa E (University of L 2016 Development of Botanical Insecticide from Flavonoid of Compound Leaf Extract Gliricidia maculata to Control Coffee Mealybug Planacoccus citri *International Wildlife Symposium* ed S Nurcahyani, Endang Gs (Bandar Lampung: Research and Development Center of Environment, Institute for Research and Community Service, University of Lampung) pp 197–204
[7] Cherian T and Thambi M 2019 Phytochemical investigation of the leaves of Gliricidia sepium and its antimicrobial properties *Parma Innov. J.* **8** 194–596
[8] Eskandarighadikolaii S, Cruz T E, Bungihan M 2015 Antioxidant Properties of Fungal Endophytes Associated with the Three Medicinal Plants Gliricidia sepium, Canna indica and Gardenia jasminoides *J. Sci. Res. Reports* **6** 217–26
[9] Jasmine T, Sundaram R M, Poojitha M, Swarnalatha G, Padmaja J, Kumar M R, Reddy K B 2017 Medicinal Properties of Gliricidia sepium: A Review *Int. J. Curr. Pharm. Clin. Res.* **7** 35–9
[10] Djaoudene O and Hayette L 2016 Impact of Storage Conditions on the Bioactive Compounds and Antioxidant Capacity of Commercial Orange Jam *J. Anal. Bioanal. Sep. Tech.* **1** 8–11
[11] Laher F, Aremu A O, Van Staden J, Finnie J F 2013 Evaluating the effect of storage on the biological activity and chemical composition of three South African medicinal plants *South African J. Bot.* **88** 414–8
[12] Mohiuddin A K (World U of B 2019 Environmental Factors on Secondary Metabolism of Medicinal Plants *MAT Journals* **1** 1–23
[13] Muraina I A, Adaudi A O, Mamman M, Kazeem H M, Eloff J N 2008 Effects of geographical location on the yield and bioactivity of Anoigeissus leiocarpus *J. Pharm. Bioresour.* **5** 68–72
[14] Norlia M, Muhmed S A, Yusoff M M, Gimbun J 2014 Influence of Solvent Polarity and Conditions on Extraction of Antioxidant, Flavonoids and Phenolic Content from Averrhoa bilimbi *J. Food Sci. Eng.* **4** 255–60
[15] Elboughdiri N 2018 Effect of Time, Solvent-Solid Ratio, Ethanol Concentration and Temperature on Extraction Yield of Phenolic Compounds From Olive Leaves *Eng. Technol. Appl. Sci. Res.* **8** 2805–8
[16] Hashim N, Shaari A R, Mamat A S, Ahmad S 2016 Effect of Differences Methanol Concentration and Extraction Time on the Antioxidant Capacity, Phenolics Content and Bioactive Constituents of Orthosiphon Stamineus Extracts *IConGDM* (EDP Sciences) pp 1–7
[17] Ncube B, Finnie J F, Van Staden J 2012 Quality from the field: The impact of environmental factors as quality determinants in medicinal plants *South African J. Bot.* **82** 11–20
[18] Hala M I, Hasan S T, Naglaa K Y 2016 Effect of Storage on The Stability and Biological Effectiveness Of Some Insecticides *J Biol. Chem. Envirorontal Sci.* **11** 256–182