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Comparative Study of Bioinsecticide Quality of Bintaro Leaf (*Cerbera odollam* G.) between Laboratory and Pilot Scale

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ABSTRACT

Bioinsecticide constitutes a sustainable solution to mitigate the deleterious consequences of excessive reliance on synthetic insecticides. Bintaro (*Cerbera odollam* Gaertn.) is a promising candidate for bioinsecticide production due to its efficacy. However, it is imperative to note that the scale of production significantly impacts the process conditions and the quality of the resulting products. To this end, a comparative study was conducted on the manufacturing of bintaro leaf bio-insecticide on a pilot scale.

The objective of this study is to ascertain the discrepancy in saponin content, water content, and toxicity of bintaro extract between laboratory and pilot scale. This study uses the reference of previous research where the best treatment of material to solvent ratio 1:10 (w/v) was obtained, namely using 15 grams of bintaro leaf material and 150 ml of solvent with 7 cycles of socletation. In the pilot scale study there was an increase in the amount of material used to 2 kg of dried bintaro leaves and 30 liters of methanol solvent. The results showed that the crude extract of bintaro leaves had a yield of 27%, saponin content of 0.63%, water content of 19.35%, and toxicity level of 45.33%.

Keyword: *Bintaro, Bioinsecticide, Quality, Pilot Scale*

ABSTRAK

Bioinsektisida merupakan salah satu solusi ramah lingkungan dalam rangka menekan dampak negatif penggunaan insektisida sintetik berlebihan. Salah satu tanaman yang dapat dijadikan bio-insektisida adalah bintaro (*Cerbera odollam* Gaertn.*)*. Praktik produksi dalam skala yang lebih besar akan mempengaruhi kondisi proses dan kualitas produk yang dihasilkan. Oleh karena itu dilakukan penelitian studi komparasi pembuatan bio-insektisida daun bintaro pada skala pilot. Penelitian ini bertujuan untuk mengetahui perbedaan kadar saponin, kadar air, dan toksisitas dari ekstrak bintaro antara skala laboratorium dan skala pilot. Penelitian ini menggunakan acuan penelitian terdahulu dimana didapatkan perlakuan terbaik rasio bahan banding pelarut 1:10 (w/v) yaitu menggunakan bahan daun bintaro sebanyak 15 gram dan pelarut 150 ml dengan 7 siklus sokletasi. Pada penelitian skala ganda terdapat peningkatan jumlah bahan yang digunakan menjadi 2 kg daun bintaro kering dan 30 liter pelarut metanol. Hasil penelitian menunjukkan jika crude extract daun bintaro memiliki rendemen 27%, kadar saponin 0,63%, kadar air 19,35%, dan tingkat toksisitas 45,33%.

Keyword: *Bintaro, Bioinsektisida, Kualitas, Skala Pilot*

1. Introduction

According to Djunaedy (2009), the Indonesian agricultural sector has suffered significant losses amounting to billions of rupiah due to infestations and diseases. These pests and diseases have led to a decline in agricultural

productivity by as much as 20%. The majority of Indonesian farmers continue to rely on synthetic pesticides as a solution to these challenges. The utilization of synthetic pesticides for the management of Plant Disturbing Organisms (PDOs) has been a prevalent practice for an extended period, demonstrating a consistent upward trend in accordance with the growing demands of the agricultural sector.

The utilization of synthetic pesticides has been effective in preserving agricultural products from destruction by pest organisms; however, it has also exerted deleterious effects on the natural world, the environment, and human health.The persistent application of synthetic insecticides can lead to environmental contamination, the emergence of insect pests that are resistant to these chemicals, a resurgence in pest populations, and a subsequent explosion in secondary pest populations (Prasetyani, 2010). This problem is intricate and challenging to resolve, necessitating the exploration of alternative insecticide sources that are less detrimental to the environment and more selective in their targeting of target pests.

A variety of plant species have been identified as possessing the capacity to function as bio-insecticides, a property attributable to their inherent bioactive compounds, which encompass saponins, tannins, alkaloids, alkenyl phenols, flavonoids, and terpenoids (Kristiana *et al*., 2015). A subset of these plants has been demonstrated to exert a lethal effect on insects, thereby justifying their utilization as bio-insecticides. A notable example is the bintaro plant (*Cerbera odollam* Gaertn.), which, according to Sa'diyah *et al*. (2013), exhibits properties that make it a promising bio-insecticide. The genus Cerbera has been identified as having potential applications in various fields, including antifungal, insecticidal, antioxidative, and antitumor properties, as referenced in Yan *et al*. (2011).

Bintaro is a tree belonging to the Apocynaceae family, which are known to be poisonous. The geographical distribution of the Cerbera plant is centred in Southeast Asia, Oceania, and the region surrounding the Indian Ocean, with India being a notable example. Two distinct types of cerbera plants have been identified*: Cerbera manghas* and *Cerbera odollam*, which can be distinguished by the colour of the fruit (*Cheenpracha*). It is noteworthy that this tree is included in the 50% of poisonous trees that cause 10% of cases of poisoning in Kerala, India (Gaillard *et al*., 2004).

Sa'diyah *et al*. (2013) posited that extracts from bintaro leaves could be utilised as bioinsecticides for the management of armyworm pests (*Spodoptera litura* F.) in chilli plants. This assertion is based on the premise that saponin compounds present in the extract bind sterol in the food channel, thereby reducing the rate of sterol hemolymph in Spodoptera litura F., leading to the gradual demise of the caterpillars. The findings of this study corroborate earlier research, thus validating the use of bintaro leaves as a bioinsecticide. This investigation utilised the leaves of the bintaro plant, in addition to the saponin compounds it contains, and also due to the plentiful supply of these compounds when compared to other parts of the plant, such as fruit or bark.

In accordance with the findings of laboratory-scale research conducted by Suherman (2016), it was determined that the optimal material-to-solvent ratio for achieving the desired outcome was 1:10 (w/v). This was achieved by utilising 15 grams of bintaro leaf material and 150 millilitres of solvent, employing seven cycles of sedimentation.The results of this study served as a foundation for the subsequent execution of pilot-scale research. The resulting bioinsecticide's yield value, water content, saponin content and toxicity level were then compared.

2. Method

2.1 Material

The raw materials in the manufacture of this bioinsecticide are bintaro leaves and 96% technical methanol. The leaves of Bintaro that are utilised in this study are characterised by a brownish-yellow pigmentation and a propensity to desiccate and descend to the ground. Materials for the test of saponin content are methanol pro analysis, petroleum ether, ethyl acetate, n-butanol, and diethyl ether. Materials for toxicity analysis are mustard and distilled water.

2.2 Extraction Procedure

The processing of bintaro leaf bioinsecticide on a double scale is achieved by increasing the volume of raw materials and auxiliary materials, and by adjusting the tools and machines used based on a predetermined capacity. In this study, the doubling of raw materials to 2 kg of dried bintaro leaves and 30 litres of methanol has been adjusted to the capacity of the available tools. The leaf drying process was conducted using a tunnel

dryer, while the extraction method employed socletation and the evaporation method utilised rotary vacuum evaporator. The research was conducted in a single stage, as illustrated in Figure 1.

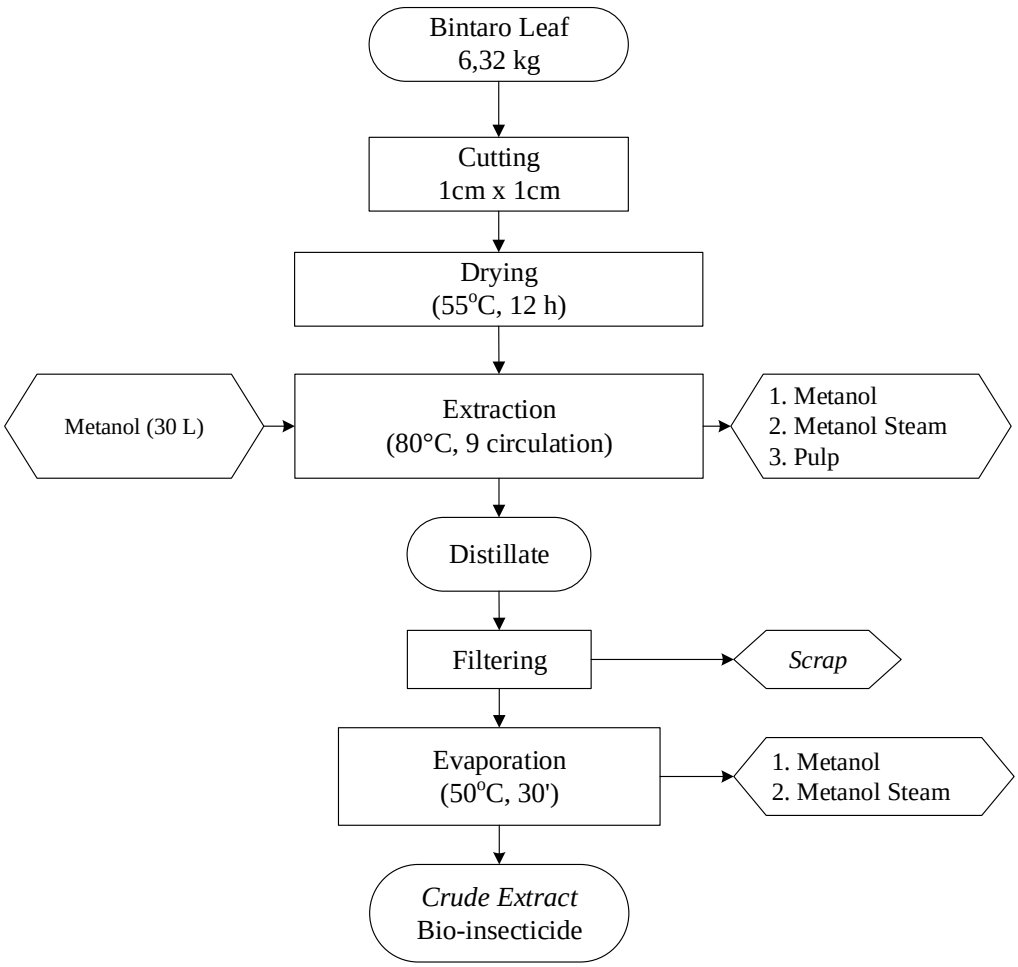

Figure 1. Flowchart of Bintaro Leaf Extraction Process at Pilot Scale

2.3 Determination of Yield

Yield (%) = $\frac{\text{BKA}}{\text{BKB}} \times 100\%$ .....(1) (Tarmadi, 2013)

Where:

BKA = Dry weight of bintaro leaves used (g)

BKB = Weight of extract produced (g)

2.4 Water Content

a. The sample was weighed in a Petri dish with a known weight, with a maximum variation of 3 grams.
b. The sample was dried in an oven at a temperature of 100-105°C for a duration of 3-5 hours. It was then allowed to cool in a desiccator and weighed.
c. The sample was heated in the oven for a duration of ± 30 minutes. It was then cooled in a desiccator and weighed. This process was repeated until a constant weight was achieved, i.e. until the successive weighing difference was 0.2 mg.
d. Calculation of water content with formula (2).

Water content (%) = $\frac{Initial\ weight\ (a) - Final\ weight\ (b)}{Initial\ weight\ (\text{a})} \times 100\%$ .....(2) (Sudarmadji & Bambang, 1997)

2.5 Saponin Content

The steps for determining saponin content are as follows:

a. The material was extracted with methanol in order to obtain saponin compounds.
b. Subsequently, the extract was purified with 25 ml of petroleum ether until a precipitate formed.

c. The precipitate, which still contains non-polar compounds, is then dissolved again with 25 ml of ethyl acetate solvent until the precipitate is pure from non-polar chemical compounds.
d. The precipitate formed was dissolved with 25 ml n-butanol, and then separated between the solvent and saponin compounds with a rotary vacuum evaporator until a thick extract is obtained.
e. The thick saponin extract was dissolved with 5 ml of methanol and then decanted with diethyl ether to form saponin clumps.
f. Saponin clumps were then separated from the solvent using filter paper.
g. The weight of the filter paper was measured, and the lump was oven-dried until constant weight was achieved. The saponin content was then calculated.
h. Calculation of saponin content was done by formula (3).

As posited by Mien *et al*. (2015), the calculation of saponin content can be performed using the following formula:

$$\text{Saponin Content} = \frac{X2-X1}{A} \times 100\% \quad \text{..... (3)}$$

Where:
X_1 = Filter paper weight (g)
X_2 = Weight of filter paper + saponin precipitate (g)
A = Weight of leaf extract obtained (g)

2.6 Toxicity Test

2.6.1 Preparation of Bioinsecticide Solution

The stages of making the insecticide solution are as follows:
a. Prepared bintaro leaf extract with a concentration of 1% (0.5 ml).
b. Prepared distilled water as much as 49.5 ml.
c. Each is put into 3 spray bottles, then shaken gently until evenly distributed.

2.6.2 Application of Bioinsecticide Solution to Test Larvae

The stages of the process of applying bioinsecticide to *Spodoptera litura* F. larvae are as follows:
a. 25 instar *Spodoptera litura* F. larvae were prepared.
b. Put in a jar that has been covered with tissue.
c. The larvae were left without food for 5 hours.
d. Prepared 10 grams of fresh mustard leaves.
e. The mustard leaves were sprayed with the bio-insecticide solution, then air dried for 5 minutes.
f. Each 10 grams of mustard leaves were put into a jar (as feed).
g. The application test feed was replaced after 48 hours of bio-insecticide application with fresh test feed.
h. Insecticidal observations were made 5 days later.
i. Calculate the percentage of larval mortality using formula (5).

$$\text{Larval Mortality (\%)} = \frac{Dead\ larvae}{Total\ larvae} \times 100\% \quad \text{.....(3) (Utami, 2010)}$$

Table 2. Classification of insecticidal extract activity

Activity	Mortality
Strong	m ≥ 95%
Somewhat strong	75% < m < 95%
Moderately strong	60% < m < 75%
Moderate	40% < m < 60%
Somewhat weak	25% < m < 40%
Weak	5% < m < 25%
Inactive	m < 5%

Source: Prijono, 1998

3. Result and Discussion

3.1 Bioinsecticide Extract Yield

The discrepancy in the quantity of ingredients utilised and the capacity of the equipment employed exerts a significant influence on the requisite process conditions, encompassing the extraction time and the energy source employed. In the context of pilot scale research, the extraction time is prolonged to 7 hours and 2 minutes, whereas in the laboratory scale, it is reduced to 5 hours. The variation in the volume of raw material input has a direct impact on the quantity of product yielded. Specifically, the laboratory-scale research yielded 3,013 mL of crude extract, whereas the pilot scale configuration produced 540 mL. The visual difference in the extract produced can be observed in Figure 2.

(a) (b)

Figure 2. Bioinsecticide produced by: (a) laboratorium scale (b) pilot scale

Table 2. Comparison of quality test results

Parameter	Laboratorium Scale (%)	Pilot Scale (%)	Quality Requirements (%)
Yield		27	-
Water Content	19,6	19,35	< 10
Saponin Content	0,37	0,63	-
Toxicity	44,67	45,33	-

3.2 Moisture Content

Based on Table 2, it is known that the moisture content of laboratory scale bioinsecticide has a slightly higher percentage than the pilot scale. The moisture content of the laboratory scale bioinsecticide was 19.6% while the pilot scale was 19.35%. However, both moisture content does not meet the quality requirements for pesticide product moisture content because the value is above 10%. According to Soetarno and Soediro (1997), the moisture content in the extract should not be more than 10%. This aims to avoid the rapid growth of mold in the extract. The value of water content that exceeds the standard indicates that the crude extract of bintaro leaves is still prone to damage to mold growth.

3.3 Saponin Content

Saponin content of bintaro leaf extract was obtained by gravimetric method. In the gravimetric method, extract purification was first carried out to obtain pure saponins. This process involves chemicals such as petroleum ether, ethyl acetate, n-butanol, methanol, and diethyl ether (Mien *et al*., 2015). Petroleum ether serves to dissolve non-polar compounds in the extract, so that polar compounds are separated from non-polar compounds. Ethyl acetate serves to dissolve non-polar compounds that are still present in the extract. The nature of ethyl acetate is more non-polar than petroleum ether, so that non-polar compounds that have not

dissolved in petroleum ether can be dissolved in ethyl acetate. n-Butanol serves to dissolve saponin compounds in the extract. Methanol serves to dissolve polar compounds (saponins) that thicken in the evaporator flask. Diethyl ether serves to wash the remaining non-polar compounds that are still present in the methanolic solution. Then drying the saponins to a fixed weight is carried out.

Based on Table 2, it is known that the saponin content of the pilot scale bioinsecticide product has a greater percentage than the laboratory scale. The saponin content of the laboratory scale bioinsecticide is 0.37% while the pilot scale is 0.63%, it is suspected that the difference is influenced by the temperature and time of the extraction process. The longer the extraction process causes the longer the material is in direct contact with the solvent, so that the opportunity for saponins to diffuse from the material is increasing. According to Yosephine (2011), the yield and quality of saponins are influenced by extraction temperature and extraction time. The higher the extraction temperature causes the solvent to dissolve the saponin more easily and bring it out from the inside/surface of the solid, thus increasing saponin yield. Research by Ma *et al*. (2015), saponin yield increased at 40-80 ^{o}C, but when the temperature was increased to 90 ^{o}C saponin yield decreased. This is due to the decomposition of saponins and the denaturation of proteins in the sample.

3.4 Toxicity

Preliminary toxicity tests on Spodoftera litura F. instar 2 larvae have indicated that bioinsecticides demonstrate moderate insecticidal activity, with a recorded mortality rate of 45.33%. Research by Utami (2010) reported that at concentrations of bintaro leaf insecticide of 0.125%, 0.25%, 0.5%, and 1%, there was a corresponding death rate of 26.67%, 33.33%, 33.33%, and 50% in Spodoftera litura F. larvae, respectively. At the same concentration (1%), the mortality percentage of the pilot scale bioinsecticide was lower than the reference but higher than the laboratorium scale and still showed the same insecticidal activity, namely the medium category.

(a) (b)

Figure 3. Larval condition of *Spodoptera litura* F. : (a) Normal (b) Dead after treatment

The symptoms of *Spodoptera litura* F. death are as follows: the larvae that die from poisoning will dry out, stiffen, and their bodies will be blackish brown and shrink. Normal larvae are fresh, clear, green, while larvae that have been exposed to bioinsecticides will turn yellow and gradually become dark brown and then blacken and die.The appearance of normal and dead Spodoptera litura F. larvae after treatment can be seen in Figure 4.5. This phenomenon is believed to be attributable to the active compounds present in bintaro leaf extract, which are toxic to the larval body. These toxic compounds enter the larval body either through the pores or by ingestion, thereby disrupting its digestive and nervous systems. As posited by Turk (2006), the efficacious constituents of bintaro leaves, namely saponins, possess inhibitory properties that culminate in the induction of muscle spasms and paralysis. These effects are accompanied by an escalation in the permeability of the larval body, a consequence of the impairment to the cell membrane. This, in turn, results in the influx of numerous toxic compounds into the larval body, thereby engendering a state of paralysis and impediment of feeding that ultimately leads to the demise of the larvae.

4. Conclusion

It has been demonstrated that, in general, the quality of bioinsecticides produced at the pilot scale is superior to that produced at the laboratory scale. This is influenced by several factors, including the quantity in one larger process and different operational conditions.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper. It is imperative to note that all research activities, data collection and analysis were conducted independently. This was done in order to ensure that no financial, professional or personal relationships that could influence the results or interpretation of this research were present.

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