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Biopesticide Effectiveness Test from Bioethanol Made from Jackfruit Bark and Tobacco Leaf Waste against *Crocidolomia* sp.

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Abstrak: Penelitian ini didasari akibat pencemaran lingkungan yang disebabkan penggunaan pestisida kimia yang berlebihan. Selain itu, masalah sampah organik juga menjadi penyebab pencemaran lingkungan. Salah satu contoh sampah organik yaitu limbah kulit nangka yang dapat dimanfaatkan menjadi bioetanol. Sehingga peneliti memanfaatkan bioetanol dari limbah kulit nangka dan daun tembakau menjadi biopestisida. Tujuan penelitian ini untuk menguji keefektifan biopestisida berbahan bioetanol. Pembuatan bioetanol menggunakan *Saccharomyces cerevisiae* dalam proses fermentasi. Bioetanol dengan kandungan tertinggi dari setiap variasi menjadi bahan baku dalam pembuatan biopestisida. Dengan variabel yang diukur berupa kadar etanol, mortalitas, rata-rata waktu kematian dan persentase pupa dan imago yang cacat dengan pengamatan selama 1 minggu. Data mortalitas akan dianalisis secara statistik. Bioetanol yang dibuat mendapatkan kadar etanol tertinggi sebesar 30% dan 8% pada masing-masing variasinya. Mortalitas yang terjadi pada larva memiliki rata-rata yang berbeda pada setiap variasinya yaitu 73.5% dan 60%. Serta rata-rata waktu kematian tercepat terjadi pada hari ke dua dan kematian tertinggi terjadi pada hari ke tiga sebesar 16.29. Persentase kecacatan yang terjadi pada pupa dan imago sebesar 5.66% dan 14.2%. Berdasarkan penelitian biopestisida menunjukkan keefektivan dalam mortalitas serta kecacatan pada pupa dan imago.
Kata kunci: Biopestisida; Bioetanol; Kulit Nangka; Daun Tembakau; *Crocidolomia* sp.

Abstract: This study is based on environmental pollution caused by excessive use of chemical pesticides and the accumulation of organic waste. Jackfruit peel waste, which can be converted into bioethanol, was utilized together with tobacco leaf waste to produce biopesticides. This study aimed to test the effectiveness of biopesticides made from bioethanol. Bioethanol was produced using *Saccharomyces cerevisiae* during fermentation. The bioethanol with the highest ethanol content from each variation was used as a raw material for biopesticide production. The measured variables were ethanol content, larval

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mortality, average time of death, and the percentage of pupa and imago defects, with observations conducted for one week. Mortality data were statistically analyzed. The highest ethanol contents obtained were 30% and 8% for each variation. Larval mortality showed different averages among variations, namely 73.5% and 60%. The fastest average death occurred on the second day, while the highest mortality occurred on the third day, reaching 16.29. The percentages of pupa and imago defects were 5.66% and 14.2%, respectively. The results showed that the biopesticides were effective in causing larval mortality and defects in pupae and imago.
Keyword: Biopesticides; Bioethanol; Jackfruit Skin; Tobacco Leaves; *Crocidolomia* sp.

1. Introduction

Croridolomia sp. is one of the pests on cabbage plants [1] which causes 10-90 percent of crop failure in cabbage [2]. In overcoming these pest problems, farmers use pesticides which are chemical compounds used to poison to control plant pests. Farmers usually do not pay much attention to the quality standards of their use, so they use it excessively to get maximum results. Excessive use of pesticides will have bad consequences for the environment, health and other non-target microorganisms [3]. Apart from the consequences of excessive use of pesticides, environmental pollution can also occur due to waste that accumulates, both organic and inorganic waste. Non-organic waste can be reused by recycling while organic waste needs special treatment in its use.

One way to overcome existing environmental problems is to utilize organic waste into bioethanol, which is then tested into pesticides as an alternative to the use of chemical-based pesticides. Bioethanol is a compound produced from the fermentation process of sugar content in carbohydrates, a natural ingredient to obtain ethanol [4]. In its manufacture the materials used must have starch, cellulose, and hemicellulose, which will be fermented and converted into ethanol with the help of microorganisms [5]. One example of waste material that can be used is jackfruit peel because it has the ingredients needed in making bioethanol. In overcoming environmental problems, the use of biopesticides can be a substitute for chemical-based pesticides that can pollute the environment. Biopesticides contain natural compounds that will not pollute the environment [6].

Biopesticide is a solution used to eradicate pests [7]. Biopesticides have the advantage of being more environmentally friendly, not polluting the environment, and not having an impact on health when consumed for a long period of time and other benefits [8]. In the manufacture of biopesticides, the materials used must be *Toxic* or toxic to pests. One example of a material that can be used in the manufacture of biopesticides is tobacco leaves. The nicotine content in tobacco has effects on the nervous system for insects [9] so that it can eradicate harmful plant pests [10]. In previous research, there has been no use of bioethanol and nicotine content to eradicate pests.

The potential and benefits of bioethanol and biopesticides in overcoming environmental pollution problems to overcoming pest problems, this study was conducted to test the effectiveness of bioethanol and biopesticides in overcoming pest control *Crocidolomia* sp. In this study, the variables observed were in the form of ethanol bioethanol levels, larval mortality, larval death time and the percentage of defective pupae and imago as a benchmark for the effectiveness of the biopesticides made.

2. Research Methods

a. Tools and Materials

The tools used in the manufacture of bioethanol are knives, hydrolysis containers, stoves, analytical balances, filters, pH meters, alcohol meters and distillation tools. The materials used are jackfruit peel, tobacco leaves, salt, NPK, urea, fermented yeast, aquadest and HCL 0.4 M.

The tools used in the research on biopesticide manufacturing are measuring cups, funnels, spray bottles and test insect containers. Meanwhile, the materials used are in the form of bioethanol with the highest content in each variation, aquadest and test insect. As well as other supporting tools such as laptops to record the data obtained, cameras to document and others.

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b. Experimental Methodology

Sample Preparation

The materials used in the manufacture of bioethanol and biopesticides are in the form of jackfruit bark waste and jackfruit straw, which were previously cut into small pieces and soaked using salt water to remove the sap, and dried for 3 to 7 days until the jackfruit skin is dry and brownish. As for the tobacco leaves, the used are dried leaves that have been cut into small sizes.

Bioethanol Manufacturing

In the manufacture of bioethanol, the method used is fermentation with the help of fermentation yeast microorganisms, NPK, and urea. The raw materials used during fermentation for 10 days use a variety of ingredients: 90% jackfruit peel + 10% tobacco leaves and 95% jackfruit peel + 5% tobacco leaves. Jackfruit peel and tobacco leaf waste are hydrolyzed to break down lignin to make it easier to ferment to produce ethanol. In the hydrolysis process, it uses the help of a catalyst HCl 0.4 M as a compound that helps the reaction rate. After being hydrolyzed and left to room temperature, the solution is added microorganism compounds that help the fermentation process according to their respective variations. After that, the solution is fermented for 10 days in a closed state. The fermented solution is filtered and measured pH using a pH meter with the best pH of 4.5 – 6 [10] and then distillation is carried out with the rotary method for 1.5 hours. Working principle of the method *Rotary Evaporator* This is by utilizing two different boiling points of the solution to separate the two solutions into one [11]. After distillation, bioethanol is measured in pH and alcohol content using a pH meter and an alcohol meter. From the results of the measurements, bioethanol that has the highest alcohol content in each variety of materials is used and used as a raw material in the manufacture of biopesticides.

Biopesticide Manufacturing

The manufacture of biopesticides uses bioethanol which contains the highest alcohol content of each variety of materials used as well as negative control using water. The variations used in the manufacture of biopesticides are as shown in the following table.

Table 1. Variations of biopesticide manufacturing

Variations	Code
100% bioethanol (Urea)(95% jackfruit peel + 5% tobacco leaves)	A1
75% bioethanol + 25% aquadest (Urea)(95% jackfruit peel + 5% tobacco leaves)	A2
50% bioethanol + 50% aquadest (Urea)(95% jackfruit peel + 5% tobacco leaves)	A3
25% bioethanol + 75% aquadest (Urea)(95% jackfruit peel + 5% tobacco leaves)	A4
100% bioethanol (NPK+Urea)(90% jackfruit peel + 10% tobacco leaves)	B1
75% bioethanol + 25% aquadest (NPK+Urea)(90% jackfruit peel + 10% tobacco leaves)	B2
50% bioethanol + 50% aquadest (NPK+Urea)(90% jackfruit peel + 10% tobacco leaves)	B3
25% bioethanol + 75% aquadest (NPK+Urea)(90% jackfruit peel + 10% tobacco leaves)	B4
Negative control (Water)	C1

The bioethanol used is measured using a measuring cup according to the variations made and added with aquades as a mixture. After being measured, the solution is put into a spray bottle and ready to be tested.

Test Insect Preparation

The test insects used were *Crocidolomia* sp. which was taken from a cabbage plantation in the Simpang Nangka area, Selupu Rejang District, Rejang Lebong Regency. The insects taken will be bred until they reach the 2nd generation and instar 3 to be used as test insects

Biopesticide Effectiveness Test

The biopesticide effectiveness test will be carried out with 3 repetitions with 5 larvae in each repetition. The test will be in the form of *Crocidolomia* sp. *larvae* from the 2nd generation Instar 3 to see the effects of pesticide use. Spraying was carried out 2 times every day in the morning and evening for 1 week. 1 spraying was sprayed 3 times on the larvae tested. The observed variables were mortality (death), average time of death, and percentage of defects that occurred in the pupa and imago. With the formula:

a. Insect mortality

$$Mortality = \frac{number\ of\ dead\ larvae}{total\ number\ of\ larvae} \times 100\%$$

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b. Average time of death (ATD)

$$ATD = \frac{number\ of\ dead\ larvae\ on\ the\ day}{number\ of\ repetitions}$$

c. Percentage of pupae and imago defects (Pp/Pi)

$$Pp/Pi = \frac{number\ of\ deformed\ pupae/imago}{total\ number\ pupae/imago} \times 100\%$$

In addition, data analysis was carried out with *the Anova one-way* RAL model test to see the significance of the effectiveness of biopesticides according to existing variations.

3. Results and Discussion

a. Bioethanol Analysis

In the manufacturing process, several stages are carried out, namely hydrolysis, fermentation and distillation. Hydrolysis is an event where chemical reactions and water can create new compounds and cause a solution to decompose using water. In the hydrolysis process, a catalyst will be added as a substance that helps in accelerating the reaction rate. With the following hydrolysis reaction equation [13].

$$(C_6H_{10}O_5)n_{(s)} + nH_2O_{(i)} \xrightarrow[HCl]{} nC_6H_{12}O_{6(aq)} \text{ .....(1)}$$

Next is fermentation. In the fermentation process, time greatly affects the ethanol yield obtained, too long will cause microorganisms to die caused by higher alcohol levels so that the amount of nutrients decreases which results in death in microorganisms that carry out the fermentation process [14]. Equation of chemical reactions in the process of converting glucose into ethanol [13].

$$C_6H_{12}O_{6(s)} \longrightarrow 2C_2H_6O_{(i)} + 2CO_{2(g)} \text{ …….(2)}$$

Table 2. Analysis of pH and ethanol levels from bioethanol from jackfruit peel and tobacco leaf waste

HCl Concentration	Variations	Additional Compounds	Fermentation Time	Initial pH	Final pH	Ethanol Rate (%)
0.4 m	Jackfruit peel 95% + tobacco leaves 5%	100% yeast	10 Days	2.97	3.32	4%
		100% NPK		3.27	4.69	3%
		100% Urea		3.49	4.00	30%
		50% NPK + 50% Urea		3.23	3.16	4%
	Jackfruit peel 90% + tobacco leaves 10%	100% yeast		2.91	7.47	5%
		100% NPK		3.32	3.44	4%
		100% Urea		5.32	9.17	6%
		50% NPK + 50% Urea		3.56	8.42	8%

The material variation of 95% jackfruit peel + 5% tobacco leaves with a fermentation time of 10 days gets the highest alcohol content of 30% with additional compounds used during fermentation of 100% urea. Meanwhile, in the variety of ingredients of 90% jackfruit peel + 10% tobacco leaves, it was found that the highest alcohol content was 8% by using 50% NPK + 50% Urea as an additional fermentation compound.

Making bioethanol from jackfruit husk straw using the fermentation method using *Saccharomyces cerevisiae* have been done before, with the highest alcohol content was obtained in the manufacturing variation by hydrolysis using HCl 0.4 M and the fermentation time was 24 our and the alcohol content obtained was 1.27%. Meanwhile, in the results of the study with fermentation time was 10 day are the highest alcohol yield was 30% in the variety of ingredients, 95% jackfruit peel + 5% tobacco leaves with an additional compound of 100% urea. This difference is obtained because of the content of the materials used and the time for fermentation. Previous research used jackfruit husks only in the straw and without the addition of other ingredients. Meanwhile, this study uses all parts of jackfruit skin and there is additional tobacco in the manufacturing process. This is what can make a difference in the alcohol content obtained [15].

The two bioethanols with the highest alcohol content of each variation are used as raw materials in the manufacture of biopesticides to test their effectiveness in pest control.

b. Analysis of *Crocidolomia* sp. larval mortality

The analysis carried out in the biopesticide trial was in the form of mortality, which in this study used *Crocidolomia sp.* As a test insect. The purpose of mortality observations is to see how effectively biopesticides kill pests.



Figure 1. Larva *Crocidolomia* sp. Instar 3

Table 3. Average mortality of *Crocidolomia sp larvae*

Variations in the use of bioethanol as a biopesticide	The number of insects killed on each repetition			Quantity	Average	Percentage (%)
	1	2	3			
A1	3	4	2	9	0.60	60%
A2	4	2	4	10	0.67	67%
A3	5	4	3	12	0.80	80%
A4	5	4	4	13	0.87	87%
B1	2	4	3	9	0.60	60%
B2	4	5	3	12	0.80	80%
B3	5	4	1	10	0.67	67%
B4	3	0	2	5	0.33	33%
C1	0	2	0	2	0.13	13%

From the application carried out, there was a difference in the average death of *Crocidolomia* sp. larvae in each variation of biopesticide made. The highest mortality rate is found in the A4 variation of 25% Bioethanol (Urea)(Tobacco 5% + Jackfruit Skin 95%)). Meanwhile, the lowest larval mortality rate occurred in the C1 variation, namely negative control. This average figure shows mortality that occurs to assess the effectiveness and ability of biopesticides in killing pests. In variation A (bioethanol compound additive

urea) the larvae had an average mortality of 73.5%, while in variation B (bioethanol compound additive NPK + Urea) the average mortality was 60%.

The highest larval mortality occurs in the A4 variety, this is due to the nicotine content in bioethanol, nicotine can protonate with aquadest so that it can be distributed freely. Nicotine that is freely distributed will evaporate easily so that it can easily attack the nervous system of the larvae [16]. The ethanol content and pH of the solution also affect the distribution of nicotine [17], neutral pH to alkaline can make the distribution of nicotine free. Apart from nicotine content, death in larvae is also caused by lesions or tissue damage caused by high ethanol content [18]. High levels of ethanol can damage the exoskeleton or cuticle of insects [19]. Damage causes discoloration in the larvae as shown in figure 2a.



(a) (b) (c)

Figure 2. (a) larvae that die from biopesticide spraying, (b) larvae that die from biopesticide spraying are observed through stereo magnification microscope 2, (c) healthy larvae

Based on results *A one-way street* RAL model, that F calculates < F table 1% with a value of 1.359073 < 4.03 which means that there is no significant difference between the variations in mortality data. All variations have their own level of effectiveness, but the use of a variation in the concentration of 25% bioethanol + 75% aquades in the manufacture of biopesticides is the most economically optimal variation. In the use of nicotine content with too high concentrations it can cause toxicity, because nicotine content is a natural content that has high toxicity [20].

In the variation of making bioethanol as a material for making biopesticides, tobacco is contained in it. Tobacco contains active compounds in the form of nicotine that are toxic to insects. The content of nicotine alkaloids,

nicotine sulfates and other contents in nicotine is as a nerve agent, contact poison and even stomach poison for insects [21]. The use of organic active compounds can reduce residues contained in chemical pesticides that are harmful to living things and can pollute the environment [22].

From previous research related to the effectiveness of tobacco waste against natural insecticides on pests *P. xylostella* It was found that tobacco waste can be good in killing pests *P. xylostella*. The same results were also obtained from this study, namely the biopesticides developed have the ability to kill larvae *Crocidolomia* sp. [23] .

c. Time of death of *Crocidolomia* sp. *larvae*

The fastest Time of Death in the larvae occurred on the second day of biopesticide application, with an average mortality of 1,66. The third day was the day with the highest average death rate of 16,29. With the following death trends.

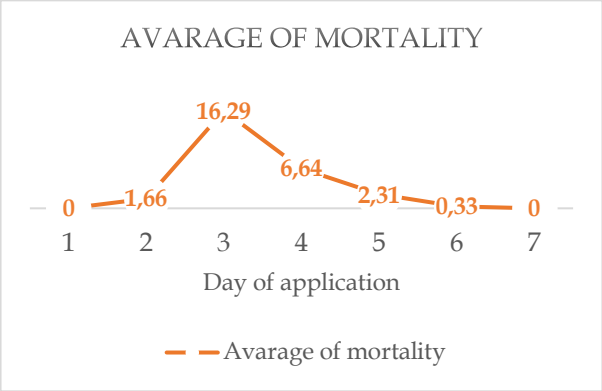


Figure 3. Average larval mortality during one week of application

If described in the form of a table, the larval mortality of each variation will be as follows.

Table 4. Analysis of the average time of death of *Crocidolomia sp* larvae According to the treatment given

Treatment	The average number of larvae die on the day						
	1	2	3	4	5	6	7
A1	0,00	1,33	1,33	0,33	0,00	0,00	0,00
A2	0,00	0,00	1,66	1,00	0,66	0,00	0,00
A3	0,00	0,00	1,66	1,66	0,66	0,00	0,00
A4	0,00	0,33	2,00	1,00	0,66	0,33	0,00
B1	0,00	0,00	2,33	0,66	0,00	0,00	0,00
B2	0,00	0,00	2,33	1,33	0,33	0,00	0,00
B3	0,00	0,00	2,66	0,66	0,00	0,00	0,00
B4	0,00	0,00	1,66	0,00	0,00	0,00	0,00
C1	0,00	0,00	0,66	0,00	0,00	0,00	0,00

The application of tobacco extract can cause death in larvae at different times, This is in line with the results obtained from research related to the effect of tobacco extract use on corn caterpillar mortality. The biggest factor that affects the speed of larval death is the concentration used in its application [21]. Death in larvae is caused by nicotine content which is poisonous to nerves and reacts quickly as a contact poison for insects. Other effects caused by nicotine are in the form of inhibiting the development of eggs, larvae and even pupae, as well as the reproductive process of female insects and disrupting the hormonal system in insects [24].

d. The percentage of defects in the pupa and imago *Crocidolomia sp.*

Pupae are part of the life journey of larvae *Crocidolomia sp* before becoming an imago (adult) [25]. The application of biopesticides has other impacts besides death in the form of defects in the larval development process. A deformed pupa will usually be small in shape and do not experience movements that indicate death in the pupa [26]. As for the imago that is defective, it has characteristics such as its crooked wings [27].

Table 5. Number of pupae and imago that survived and were disabled after treatment and survival percentage

Treatment	Number of larvae that manage to become pupae			Survival percentage (%)	Number of cocoons that have succeeded in becoming imago			Survival percentage (%)	Number of deformed pupae	Number of deformed imago
	1	2	3		1	2	3			
A1	2	1	3	40%	1	0	0	16%	0	1
A2	1	3	1	33%	1	0	0	20%	0	0
A3	0	1	2	20%	0	1	0	33%	0	0
A4	0	1	1	13%	0	0	0	0%	0	0
B1	3	1	2	40%	0	0	0	0%	0	0
B2	1	0	2	20%	0	0	0	0%	1	0
B3	0	1	4	33%	0	1	0	20%	0	0
B4	2	5	3	66%	1	2	1	40%	2	2
C1	5	3	5	86%	5	3	5	100%	0	0

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Of the total number of pupae and imago that managed to survive after treatment, there were several pupae and imago that were deformed due to treatment with the application of biopesticides.



(a) (b) (c)

Figure 4. *Crocidolomia* sp. *pupa*, (a) healthy cocoon, (b) dead cocoon (c) deformed and dead cocoon



(a) (b)

Figure 5. imago *Crocidolomia* sp., (a) healthy imago, (b) defective imago

Table 6. Percentage of defects in the pupa and imago from all treatments

Phase	Defect Percentage (%)
Pupal	5,66
Imago	14,2

Tobacco leaf extract can cause defects in insects, ranging from the larval period to the imago. Defects in these larvae and imago can be caused due to active ingredients or compounds that affect the development of insects.

From the proportion test carried out to see the difference between defects arising from the application of biopesticides compared to control treatments, data was obtained that sig.count < sig. The reference is 0.031 < 0.05 which indicates that there is a significant difference between the treatment of biopesticide application and control in making defects in the pupa and imago.

The active compounds in tobacco play a major role in killing and deformizing the larvae, pupae and imago *Crocidolomia* sp. So that biopesticides from bioethanol made from jackfruit bark and tobacco leaf waste can be a solution in the development of pest control science with the findings of effectiveness in larval mortality and defects in pupa and imago *Crocidolomia* sp.

So that it can overcome the problem of organic waste and dependence on chemical pesticides that can damage the environment [21].

4. Conclusion

From the research conducted, it was concluded that Biopesticides developed from bioethanol showed effectiveness against mortality and defects in pupa and imago *Crocidolomia* sp. with an average mortality of 73.5% and 60% and the percentage of defects in pupa 5.66% and imago 14.2%. The effectiveness of this biopesticide is due to *the toxic* content of tobacco leaf nicotine as the active ingredient used.

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