JARES, Vol.6 No.1 March ; p-ISSN: 2502-826X; e-ISSN: 2503-1163

https://ejournal.unisbablitar.ac.id/index.php/jares

JARES (Journal of Academic Research and Sciences), (6)1, hlm.40-54.

Analysis of Batopang (Bioinsecticide of Brotowali Stem Extract and Ketapang Leaves) Based on SNI 02-3128-1992 and Effectiveness Test against Wood Grasshopper (Valanga nigriconis) with the Method Lethal Concentration 50

Muhamad Aditya Hidayah[1]
[1]UIN Sunan Kalijaga, Jl. Laksada Adisucipto, Depok-Yogyakarta, (0274) 512474
e-mail: madityahidayah@gmail.com

ABSTRACT

This research is an experimental study where the variables in this study are divided into three categories, independent, dependent, and confounding variables. From this research, qualitative data and quantitative data were obtained. The qualitative data includes the results of the phytochemical screening test. The quantitative data includes pH test, bioinsecticide effectiveness test, and preservative effectiveness test. Based on the results of the phytochemical analysis, it shows that ketapang and brotowali leaf extracts contain secondary metabolites in the form of saponins, flavonoids, alkaloids, and tannins. The bioinsecticide with the best formulation is the bioinsecticide with a formula of 15% extract because it has an LC50 value that is close to the LC50 value of the probit regression, which is 13.05%. Based on the results, it shows that the bioinsecticide of ketapang leaves and brotowali stems for all formulas has met the quality qualifications according to the requirements in SNI 06-0475-1996.

Keywords: *Bioinsecticide, Brotowali, Ketapang, Lethal Concentration 50, Grasshopper Wood*

1. INTRODUCTION

Indonesia is an agricultural country with very large agricultural land and abundant natural resources. Therefore, his livelihood as a farmer is the main profession in Indonesia. This is supported by the statement of the Indonesian Central Statistics Agency (BPSI) in February 2019, where the Indonesian population whose livelihoods are in the agricultural/plantation sector is 38,109,196 people out of a total of 129,366,192 people.

The income of a farmer is determined by the quality of the crop. According to Amelia (2007) in Mubaroq (2013), the conditions that affect the quality of agricultural products are rainfall, temperature, season, and control of pests and diseases. Controlling pests and diseases of agricultural commodities is very necessary because if these pests and diseases are not controlled, it will certainly reduce the quantity and quality of crops. One of the pests that attack agricultural commodities is the wood grasshopper (Valanga nigricornis) which destroys agricultural commodities by eating the leaves so that the photosynthesis process is disrupted and causes inhibition of the growth of agricultural commodities.

The solution to dealing with pest problems can be done by using insecticides. According to the 2018 Center for Seed and Forestry and Plantation Pilot Development (BP3KP), insecticides are materials used to kill pests and

diseases in plants. Insecticides according to their active ingredients are divided into two, namely synthetic insecticides and botanical insecticides (bioinsecticides). Synthetic insecticides are insecticides derived from a mixture of synthetic chemicals. The use of synthetic insecticides has a negative impact, namely causing toxicity to plants, killing natural enemies of pests, and causing environmental pollution because they are difficult to degrade. Meanwhile, vegetable insecticides (bioinsecticides) are insecticides whose basic ingredients come from plants. Vegetable insecticides are classified as biochemical insecticides because they contain biotoxins. Biochemical insecticides can control pests with a non-toxic mechanism so that their use does not cause negative effects. Plants contain many chemicals which are secondary metabolite compounds as a means of defense against disruptors. Plants that have potential as bioinsecticides are brotowali and ketapang.

Brotowali plants contain soft resin, starch, glycosides, picroretocytes, bitter substances pikroretin, harsh, alkaloids, and palmatin (Setiawan, 2008: 11 in Tutik, 2012). Brotowali stem contains flavonoids, alkaloids, and saponins. So that it has the potential to be used as a basic material for making bioinsecticides (Sri and Jhony, 1991: 569 in Tutik, 2012), as well as ketapang plants which contain alkaloid compounds, tannins, flavonoids, and phenolic compounds (Arif, 2013).

Therefore, bioinsecticide based on brotowali stem and ketapang leaves is a potential alternative that can be developed as bioinsecticide which is safe and environmentally friendly and can act as an insecticide. The research was conducted on the formulation of bioinsecticide using brotowali stems and ketapang leaves as insecticides. The formulation is done to get the best bioinsecticide formula so that it can be produced for daily needs. That way, this research can also be implemented to the public by providing socialization about the manufacture of bioinsecticides from extracts of brotowali stems and ketapang leaves.

2. METHODOLOGY

This research is an experimental study where the variables in this study are divided into three categories, namely, independent variables, dependent variables, and confounding variables.

1. The Independent Variable

   The Independent variable is a variable that is varied by the research subject. This variable affects or causes changes in the dependent variable. The independent variable in this study was the difference in the concentration of the extract of brotowali stems and ketapang leaves, with a concentration of 10%, 15%, 30%, and 50%.

2. The Dependent Variable

   The dependent variable is the variable that is influenced by the independent variable (Sugiono, 2011). The dependent variable in this study was the percent mortality of wood grasshoppers and the percentage of microbial reduction obtained from each treatment.

3. The Confounding Variable

   The confounding variable in this study were the condition of the

ecosystem around the cage and feeding during the adaptation stage, as well as the aseptic level in the preservative effectiveness test.

From this research, qualitative data and quantitative data were obtained. The qualitative data includes the results of the phytochemical screening test. The resulting quantitative data includes pH test, bioinsecticide effectiveness test, and preservative effectiveness test.

a. Preparation of Samples

Brotowali stems and fresh ketapang leaves that have been cleaned are then dried using an oven at 70°C. Brotowali stems and dried ketapang leaves are mashed using a blender then sieved with a 100 mesh sieve. Brotowali stems and smooth ketapang leaves are ready to be extracted.

b. Extraction of Active Ingredients.

The extract was made by the maceration method. The samples were macerated in 70% ethanol for 24 hours. The extract was separated through filtering and the resulting filtrate was concentrated by a *rotary evaporator* at a temperature of 50 ° C. then proceed with evaporation on a bath and produce a thick crude extract. The extract was tested for ethanol-free by adding acetic acid and concentrated sulfuric acid, then heated. The ethanol-free indicator is carried out by the sense of smell if there is no ethyl acetate odor, the thick extract will no longer contain ethanol.

c. Making Biopesticides

As much as 10 mL, 30 mL, and 50 mL of the active ingredient extract were put into a vial and diluted to 100 mL extract volume, then the solution was homogenized and packaged.

d. Preliminary Analysis

Preliminary analysis is in the form of qualitative phytochemical analysis. This analysis was conducted to determine the active compounds contained in the brotowali stem and ketapang leaves. phytochemical analysis was carried out based on the method of Harborne (1987). The compounds identified were alkaloids, flavonoids, saponins, and tannins.

Identification of flavonoids was carried out using the cyanidin test. A total of 0.1 g of the extracted sample was added with 5 mL of 30% methanol and then heated for 5 minutes. The filtrate is added with a few drops of concentrated HCl and magnesium powder. The formation of red color indicates the presence of flavonoid compounds.

Alkaloid identification was carried out using the method Culvenor-Fitzgerald. A total of 4 g of the extracted sample added with chloroform solvent and added 10 mL of 0.05 N ammonia. Then filtered into a test tube. Added H_2SO_4 2N and shaken vigorously, the filtrate was allowed to stand until two layers were formed (chloroform layer and sulfuric acid layer). The two layers were separated using a dropper. The sulfuric acid layer was then used to identify the alkaloids by adding Mayer's reagent. If when the addition of Mayer's reagent forms a white precipitate or the solution becomes cloudy, then there is a positive alkaloid compound.

Saponin identification was carried out by alkaline test. Brotowali stem and ketapang leaf extract samples were put into a test tube and distilled water was

added. Then heated at a temperature of 100 ° C for 3 minutes and cooled. Once cool, shake vigorously for 5 minutes. A foam that is not <1 cm high and remains stable after being left for 15 minutes indicates the presence of saponins.

Identification of tannins, as much as 0.1 g of extract was added with 5 mL of distilled water in a test tube. Then boil it for 5 minutes. After boiling, the residue and filtrate are separated by filtering.$FeCl_3$ 1% solustion was added to the resulting filtrate. If dark blue or black leaves are formed, it shows positive tannins.

e. Product Analysis (SNI 02-3128-1992)

Insoluble Acetone

As much as 10 g of sample (W) is put into the Erlenmeyer Flask then added 150 mL of acetone, put in an upright cooler, and heat until the sample dissolves. Then it is followed by a funnel whose fixed weight is known (A). The rest of the filter is washed with acetone for three washes. Dried at 110 ° C for 30 minutes, cooled, and weighed to constant weight (B).

$$Insoluble\ Part\ in\ Acetone\ \left(\%\frac{b}{b}\right) = \frac{(BA)}{W} x100$$

Note:
B = Residual Weight + Filter Paper
A = Weight of Empty Filter Paper
W = Weight of Sample

pH Test

A total of 5 g of sample is put into a beaker containing 100 mL of distilled water. And the pH of the solution was measured with a pH meter that has been calibrated to Buffer pH 4, 7, and 9.

Effectiveness of the Bioinsecticide with the Method *Lethal Concentration* 50

The bioinsecticide effectiveness test was carried out using the LC50 method with treatment (brotowali stems and ketapang leaves) consisting of three concentrations (10%, 30%, and 50%) active ingredients, plus two treatments, wood grasshopper as a negative control without being treated and synthetic insecticide solution as a positive control. First, the wood grasshopper is given an adaptation period of 3 days and is given routine feeding in the form of reeds three times a day. Each treatment was tested with 10 wood grasshoppers. Observations were made to determine the effect of the ability of the bioinsecticide concentration on the mortality of 50% of the wood grasshopper pest population for 6 hours.

The Effectiveness of Preservatives for Determination of Shelflife

A total of 25 mL of bioinsecticide for all 10%, 15%, 30%, and 50% formulas were piped into an Erlenmeyer containing 225 mL BPW solvent, then homogenized as a 10^{-1} dilution. (work is done aseptically and is done in *Laminar Air Flow*). Then, pipette 1 mL into a test tube containing 9 mL BPW solvent and homogenize it as a 10^{-2} dilution.

From each serial dilution, 1 mL pipette into a petri dish, then 15 mL of PCA media are poured into it, homogeneous and wait until it solidifies. Incubated at 36 ± 1 ° C for 48 hours, then the bioinsecticide was stored again at room temperature 25 ° C for further re-testing on days 7, 14, 21, and 28.

3. RESULT AND DISCUSSION

Results

1. Yield Extraction Results

Table 1. Result of Yield Extraction

Sample	Simplicia Weight	The Crude Extracts Weight	% Yield
Brotowali 1	128.805	41.1024	31.91
Ketapang 1	140.6860	34.7558	24.70
Brotowali 2	212.1557	67.8262	31 97
Ketapang 2	242.1784	59.9876	24.77

Note:
1 = The extraction process for testing
2 = The extraction process for the manufacture of products

2. Test *Screening* Phytochemical

Table 2. Results of *Screening* Phytochemicals

Extract	ScreeningPhytochemicals			
	Flavonoids	alkaloids	Saponins	Tannins
Ketapang	+++	+++	+ ++	+++
Brotowali	+++	+++	+++	+++

Description:
+ = Positive Weak
++ = Positive Strong
+++ = Positive Very Strong

3. Insoluble Acetone Test

Table 3. Test Results Insoluble Acetone Test

Formula	Sample Weight (g)	Weight Filter Papper (g)	Weight FP + Residue (g)	Residue Weight (g)	Insoluble Acetone (%)
10%	10,0000	0,8900	1,2045	0,3145	3,145
15 %	10,0000	0,8921	1,2085	0,3164	3,164
30%	10,0000	0,8867	1,2233	0,3366	3,366
50%	10,0000	0,8912	1,2409	0,3497	3,497

4. pH Test

Table 4. pH Test Results

Formula	pH
10%	5.00
15%	5.08
30%	5.12
50%	5.30

5. Bioinsecticide Effectiveness Test with the method Lethal Concentration 50

Table 5. Results of Bioinsecticide Effectiveness Test with the Method LC50

Tested

Concentration (%)	Log 10 Concentration	Number of Individuals	Number of Dead Individuals	% Mortality	% Corrected Mortality	Probit Value
0.00	0.00	10	0	0	0	0.00
10.00	1	10	4	40	40	4.76
15.00	1.18	10	6	60	60	5.25
30.00	1.48	10	9	90	90	6.28
50.00	1.70	10	10	100	100	7.73

Figure 1. Grasshopper Mortality in Each Treatment and Control

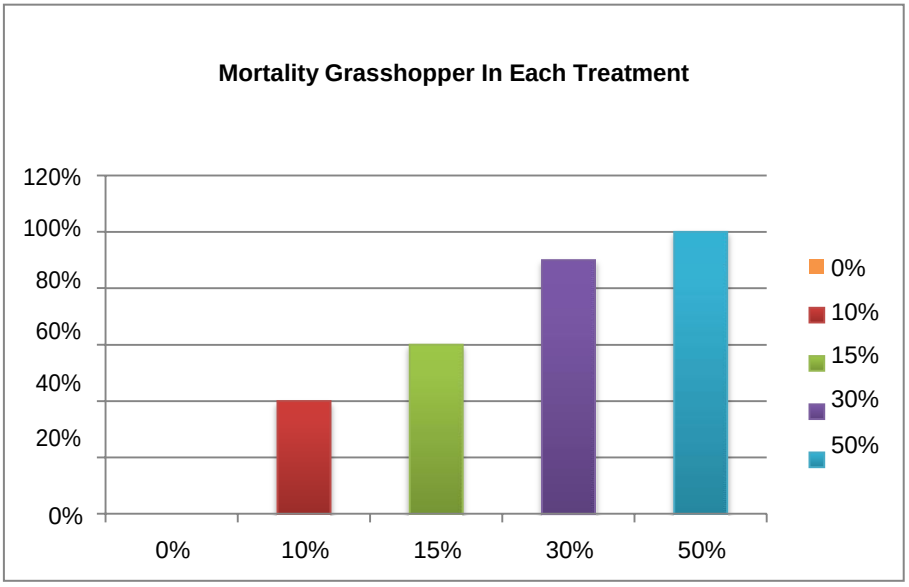


Figure 2. Log Linearity Curve 10 Concentration and Probit Value

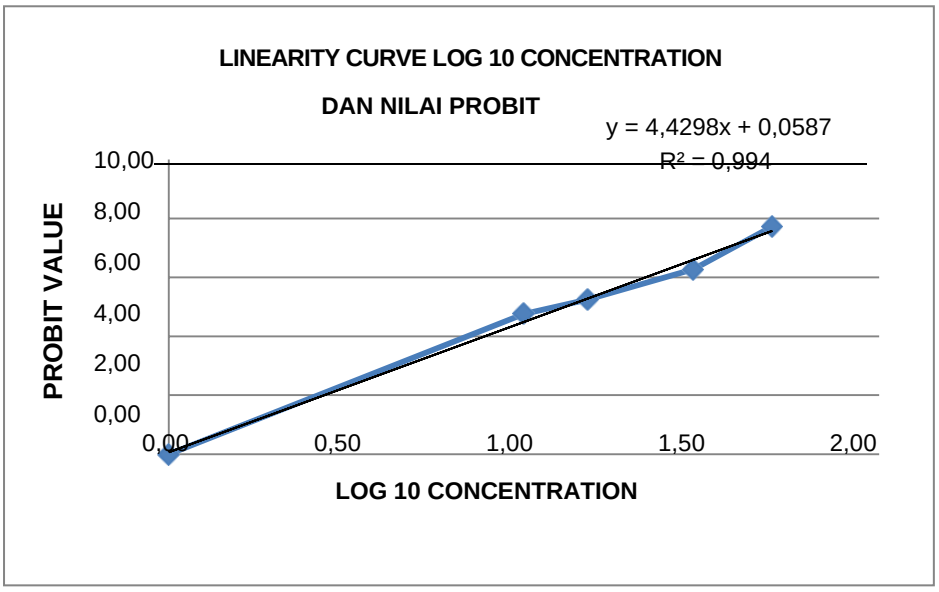


Table 6. Determination of LC50 Value

No	Time (hours)	Value a	Value b	y	X	LC50
1	6	0.0587	4,4298	5	1,115467967	13,04571741%

6. Test of the Effectiveness of Preservatives for Shelflife

Table 6 Results of the Preservative Effectiveness Test for Shelflife

Formulas	Dilution	Observation	Results (CFU/g)	Day 0 (CFU/g)	%Reduction
10%	10^{-1}	1	1.0×10^{1}	10	-
	10^{-2}	0			
15%	10^{-1}	1	1.0×10^{1}	10	-
	10^{-2}	0			
30%	10^{-1}	1	1.0×10^{1}	10	-
	10^{-2}	0			
50%	10^{-1}	1	1.0×10^{1}	10	-
	10^{-2}	0			
Formula	Dilution	Observation	Results (CFU/g)	Day 7 (CFU/g)	% Reduction
10%	10^{-1}	0	1.0×10^{1}	10	0
	10^{-2}	0			
15%	10^{-1}	0	0	0	100
	10^{-2}	0			
30%	10^{-1}	0	0	0	100
	10^{-2}	0			

50%	10^{-1}	0	0	0	100
	10^{-2}	0			

Formula	Dilution	Observation	Results (CFU/g)	Day 14 (CFU/g)	%Reduction
10%	10^{-1}	0	0	0	100
	10^{-2}	0			
15%	10^{-1}	0	0	0	100
	10^{-2}	0			
30%	10^{-1}	0	0	0	100
	10^{-2}	0			
50%	10^{-1}	0	0	0	100
	10^{-2}	0			

Formula	Dilution	Observation	Results (CFU / g)	Day 21 (CFU /	g)%Reduction
10%	10^{-1}	0	0	0	100
	10^{-2}	0			
15%	10^{-1}	0	0	0	100
	10^{-2}	0			
30%	10^{-1}	0	0	0	100
	10^{-2}	0			
50%	10^{-1}	0	0	0	100
	10^{-2}	0			

Formula	Dilution	Observation	Results (CFU/g)	Day 28 (CFU/g)	% Reduction
10%	10^{-1}	0	0	0	100
	10^{-2}	0			
15%	10^{-1}	0	0	0	100
	10^{-2}	0			
30%	10^{-1}	0	0	0	100
	10^{-2}	0			
50%	10^{-1}	0	0	0	100
	10^{-2}	0			

Discussion

The samples used in this study were ketapang leaves which were picked with the criteria that the leaves had a length of 17-20 cm and a width of 13-15 cm, while for the brotowali stems we used the young stems. The leaves of the ketapang and the brotowali stems that have been picked are then carried out in the preparation stages by washing, chopping, drying, and refining. The drying stage is

carried out using an oven to get maximum heat with a heating temperature of 70 ° C to avoid damaging the content of secondary metabolite compounds (Indah, 2018). Furthermore, a refining stage is carried out which aims to increase the surface area so that the contact between the solvent and the sample is maximized during the extraction process.

The sample extraction process in this study used the maceration method with 96% ethanol as a solvent which refers to the research of Agus et al. (2014). This method has the principle that the simplicia material (sample) is refined (generally cut into pieces or coarse powder) is combined with the extracting material. Then the bath is kept protected from direct light (preventing light catalyzed reactions or discoloration) and stirred again. (Romadhoni, 2017). The extracted compounds are secondary metabolites in ketapang leaves and brotowali stems, namely saponins, alkaloids, flavonoids, and tannins. (Dwiangga, 2015). The secondary metabolite compounds in the sample will dissolve in the solvent and then evaporate with rotary evaporator at a temperature of 50-60°C to prevent the secondary metabolite from being.

According to research conducted by Tutik, et al. (2012) to extract saponins, alkaloids, flavonoids, and tannins on the brotowali stem, 96% ethanol is used, as well as for ketapang leaf extraction, 96% ethanol is used, to extract saponins, alkaloids, flavonoids, and tannins. (Dwiangga, 2015). The yield obtained from the extraction of ketapang leaves and brotowali stems for the first extraction and used for testing was 24.70% and 31.91%, while for the second extraction and used for product manufacture were 24.77% and 32.30%. Based on the yield results from the first and second extraction, the% RSD for brotowali stem yields was 0.13% (% RSD requirements <0.60%) so that it can be concluded that the precision was accepted, while for the ketapang leaf yield was 0.20% (% RSD requirements <0.62%) so that it can be concluded that the precision is accepted.

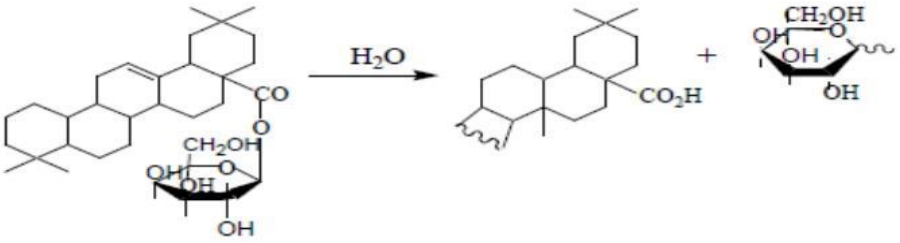
Testing on the process of making bioinsecticides from ketapang leaves and brotowali stems includes preliminary tests (tests *screening* phytochemical), product tests based on SNI 02-3182-1992, bioinsecticide effectiveness tests using themethod *lethal concentration* 50, and preservative effectiveness tests for determining storage time.

***The* Phytochemical Test**

a. Saponins

Saponin test showed positive results for the extract of ketapang leaves and brotowali stems because the foam formed after shaking lasted up to 15 minutes and did not disappear. According to Sangi, et al. (2008) saponins have glycosyls as polar groups and steroid/triterpenoid groups as non-polar groups so that they are surface-active and form micelles when shaken with water. In the micellar structure the polar groups are facing outwards while the non-polar groups are facing inwards and this is what looks like foam.

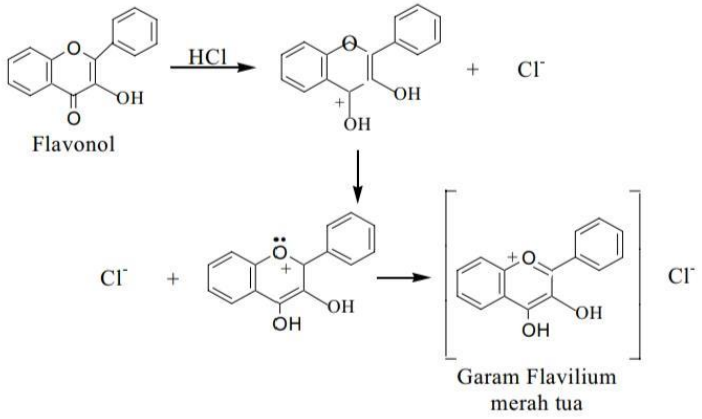
Testing saponin is based on the hydrolysis process saponins be aglycone and glycated (produce foam), the following is the mechanism reaction:



1-arabinospriosil-3th-acetyl olenolic aglycone glycated

b. Flavonoids

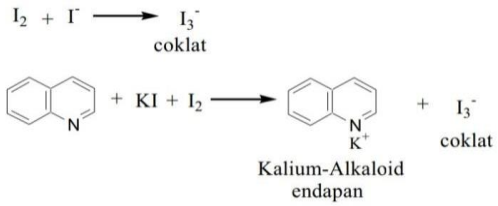
Rseult of flavonoid test from leaf extracts ketapang and brotowali shows positive results, this is because flavonoid compounds are polar so that they can dissolve in both polar and semipolar solvents. The polarity of the compound is because flavonoids are polyhydroxy compounds (having more than one hydroxyl group) (Harborne, 1987). The polyhydroxy from flavanones is reduced by magnetic metals and reacts with hydrochloric acid to form a benzopyrilium salt (flavilium salt) which is red or orange (Sastrohamidjojo, 1996). The following is the reaction mechanism according to Achmad (1996):



c. Alkaloids

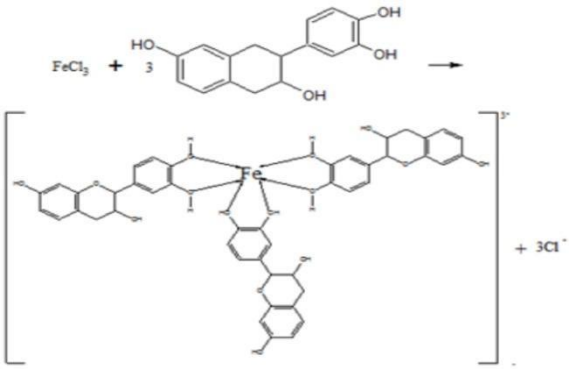
The alkaloid test was carried out using Wagner's reagent and showed positive results for the extract of ketapang leaves and brotowali stems due to the formation of brown deposits. In the alkaloid test, the addition of chloroform is used to dissolve the extract along with the alkaloids, then the addition of ammonia solution is carried out the alkaloid bound to the extract becomes free alkaloids. The addition of H_2SO_4 is used to form alkaloid salts into two layers, the chloroform layer and the H_2SO_4 layer (there is an alkaloid salt). The H_2SO_4 layer was separated and added Wagner's reagent.

Positive result for alkaloids in the Wagner test is indicated by the formation of a brown precipitate or a yellow solution. These deposits are potassium-alkaloid compounds. According to Soerya (2005) in the Wagner test, the metal ion K^+ will form a coordinate covalent bond with nitrogen in the alkaloid to form a precipitated potassium-alkaloid complex. The following is the reaction mechanism:



d. Tannins

In the identification of tannins using iron (III) chloride ($FeCl_3$) reagent is used to determine whether the sample contains phenol groups because tannins are polyphenolic compounds from plants and taste bitter. The presence of phenol groups is indicated by a blackish-green or dark blue color after adding $FeCl_3$. The formation of a blackish green or dark blue color according to Harborne (1987) is because Fe^{3+} ions will react with tannins to form complex compounds. The results obtained in the extracts of ketapang leaves and brotowali stems were positive for tannins by giving a dark blue color. The addition of the extract with iron (III) chloride in water pose a dark blue-black color due to the tannins will react with Fe^{3+} ions to form complex compounds, the following reaction mechanism:



Test Product Bioinsecticide Based on SNI 02-3182-1992

a. Insoluble Acetone Test

Insoluble acetone test results in the bioinsecticide product is much lower than that of the comparison product which is used as standard. The acetone insoluble test is needed to show the amount of suspension left by the insecticide. The high suspension affects the residue that will be left on the plant. The components that are insoluble in acetone show their level of solubility. The bioinsecticide product produced acetone insoluble material for the 10% formula of 3.145%, the 15% formula for 3.164%, the 30% formula for 3.366%, and the 50% formula for 3.497%. Meanwhile, the comparison product that is used as a standard according to research by Putri, et al. (2018) is 98.33%. This is because the comparison product is a synthetic insecticide that has systemic properties with physical or chemical properties that allow the residue to move to the applied plant and spread throughout the plant (Jamhour, 2014). The results showed that the bioinsecticide with active ingredients of ketapang leaves and brotowali stems for all formulas left a lower residue than the comparator insecticides.

b. pH Test

pH test on bioinsecticides was tested using a pH meter. Before testing, the pH meter needs to be calibrated. The importance of calibration is to prevent

irregularities or errors that can lead to decreased or incorrect accuracy. Calibration is done by immersing the electrode in a buffer of pH 4.00, 7.00, and 9.00. After the new calibration, the bioinsecticide pH test can be carried out by first rinsing the electrode with distilled water and desiring it with a tissue, then dipping it in the bioinsecticide solution to be tested, and recording the reading results.

The bioinsecticide product has a pH value for the 10% formula of 5.00, the 15% formula of 5.08, the 30% formula of 5.12, and the 50% formula of 5.30. Based on the test results, it was concluded that the four bioinsecticide formulas had met the requirements according to BALISTA (2014), with pH 4.50-6.00. The importance of this test is because in its use, bioinsecticides will have direct contact with plants and the environment, so it is necessary to pay attention to the level of acidity or alkalinity. If the pH of the bioinsecticide is too acidic or too alkaline, it will interfere with nutrient absorption, this is because in conditions that are too acidic, manganese and aluminum will be toxic while in too alkaline conditions some micronutrients such as copper, zinc, and iron will bond chemically and not absorbed by plants, so the plants will grow abnormally and with low productivity with poor quality.

c. Preservative Effectiveness Test for Determination of Shelflife

In the preservative effectiveness test, the preservative used was Na-benzoate. The choice of Na-benzoate as a preservative in bioinsecticide products is because Na-benzoate is included in the benzoate group organic preservative which effectively works as an antimicrobial preservative at low pH. After all, at low pH, the proportion of undissociated acids increases, and the acid that does not dissociate is the main determinant. the role of preservatives (Cahyadi, 2012). This is consistent with the characteristics of the bioinsecticide which has an acidic pH. Where the addition of Na-benzoate to each formula is 0.0500 grams, and the basis for adding Na-benzoate to an amount of 0.0500 grams is based on the experimental method (randomized design).

The mechanism of action of Na-benzoate as a preservative is by inhibiting the growth of microbes, namely mold and yeast, toxin-producing bacteria, spore bacteria, and non-decaying bacteria. According to Pujihastuti (2007), the mechanism of action of benzoates and their salts as antimicrobials is based on acid molecules that do not dissociate to interfere with the permeability of microbial cell membranes. The contents of microbial cells have a pH that is always neutral. If the microbial cells are acidic or alkaline, there will be disturbances in the cell organs so that metabolism is inhibited and eventually some cells die.

Testing the effectiveness of preservatives was carried out by a simple method, which was not using positive controls for *Escherichia coli*, *Staphylococcus Aureus*, *Pseudomonas aeruginosa*, and *Candida albicains* due to limited research funds. To overcome this, the researchers made modifications to the observation of tested bacteria, by observing the decrease or stability number of microbes in bioinsecticide that had not been added with Na-benzoate and after the addition of Na-benzoate for the day determined according to the fourth edition Pharmacopeia of Indonesia, on 0 day, 7th, 14th, 21st, and 28th. The microbial testing technique was carried out using the Total Plate Count (TPC) method which aims to grow all aerobic mesophyll microbes (microbes that can grow at an

optimum temperature of 25°C-40°C and require oxygen) using the general media Plate Count Agar and incubated at temperature 36 ± 1°C for 48 hours.

The results of testing the effectiveness of preservatives obtained data that the number of microbes in the formula that had not been added with Na-benzoate had a uniformity of $1x10^1$ CFU / mL for all formulas. This can be because the extract contains secondary metabolites which have antimicrobial properties so that the number of microbes in the sample is not too much, then on the 7th day, the 15%, 30%, and 50% formulas have decreased (reduction) by 100%, whereas the 10% formula has not decreased. For day 21 to day 28, all formulas were reduced with % reduction of 100%. From these data, it can be concluded that Na-benzoate with an amount of 0.0500 grams is effective for preserving bioinsecticides for all formulas within ± 1 month in storage at a temperature of 24.7°C-25.2°C, so it can also be concluded that The bioinsecticide product has a shelf life of ± 1 month in storage at a temperature of 24.7°C-25.2°C.

d. Bioinsecticide Effectiveness Test Using the Method *Lethal Concentration* 50

Testing the effectiveness of bioinsecticides was carried out using the method Lethal Concentration 50 (LC50). According to Rosma (2015), insect toxicity can be expressed by the LC50, which is a large insecticide that can kill 50% of the insect population tested. Judging from the graph of the effectiveness of biopesticides on grasshopper mortality, the concentration that can kill half the grasshopper population tested is the 15%, 30%, and 50% formula treatment with each percent mortality respectively 50%, 60%, and 90%. . Whereas for the control, there was no death so there was no change in the% mortality corrected value, this is because the wood grasshopper before the test was adapted first to the test environment for three days and was given food 2 times a day using reed leaves and guava leaves.

Furthermore, after it is known the% mortality of grasshoppers from each formula is followed by probit testing. Probit regression is a procedure used to estimate the effect of one or more independent variables on one binomial dependent variable. This probit test was carried out to see how the relationship between the amount of concentration and the mortality value in 4 different treatment segments, namely 10%, 15%, 30%, 50%, and control.

The results of the calculation of probit obtained linearity with the equation y = 4.4298x + 0.0587, which is obtained from the relationship between the log10 concentration and the probit value. Then the calculation is carried out by entering the value of 5 (the constant in the LC50 calculation) and the result is that the lowest concentration that has the potential to kill 50% of the tested grasshoppers is based on the LC50 value of 13.04571741% ~ 13.05%. Based on this, we chose the 15% formula as a concentration close to the concentration of LC50, this selection was also supported based on data from other tests where the 15% formula for the test parameters for insoluble acetone, pH, and preservative effectiveness had met the qualifications so that it could be It was concluded that the 15% formula was the best.

4. CONCLUSION

Based on the results of phytochemical analysis, it shows that ketapang and brotowali leaf extracts contain secondary metabolites in the form of saponins, flavonoids, alkaloids, and tannins. The bioinsecticide with the best formulation is the bioinsecticide with a formula of 15% extract because it has an LC50 value that is close to the LC50 value of the probit regression, which is 13.05%. Based on the results of the product test, it shows that the bioinsecticide of ketapang leaves and brotowali stems for all formulas has met the quality qualifications with the test method according to the requirements in SNI 06-0475-1996. So that it is effective for use in farming activities.

REFERENCES

[BPS] Badan Pusat Statistik. 2018. *Perkembangan Jumlah Produksi Padi Indonesia2013, 2015, dan 2018.* Jakarta: Badan Pusat Statistik

[BSN] Badan Standardisasi Nasional. 1992. *Cara Uji Fisika Kimia Insektisida Pekatan Yang Dapat Larut Dalam Air (Water soluble concentrate, WSC).* SNI 02-3128 1992. Jakarta: Badan Standardisasi Nasional.

Agung, Prastiyo (2016) Populasi Relatif Belalang Kembara (Locusta Migratoria Manilensis Meyen) Pada Beberapa Jenis Vegetasi Di Kawasan Perkebunan Tebu Di Lampung Tengah. Fakultas Pertanian, Universitas Lampung.

Agus, Nugroho,. dkk (2014) Uji Efektivitas Berbagai Konsentrasi Insektisida Nabati Bintaro (Cerbera manghas) Terhadap Hama Ulat Grayak

(Spodoptera litura) Pada Tanaman Kedelai. *Jurnal Pertanian, Universitas Muhammadiyah Yogyakarta*.

Arif, Ashari (2013) Uji Inhibisi Korosi Pada Baja Lunak Menggunakan Ekstrak Senyawa Tanin Dari Daun Ketapang (Terminalia Catappa L) Dalam Larutan Garam. Fakultas Mipa, Universitas Lampung.

Dwingga, Wisman (2015) Pemanfaatan Daun Ketapang (Terminalia Catappa) Menjadi Zat Warna Alami Tekstil Dengan Menggunakan Variasi Pelarut. *Other Thesis*, Politeknik Negeri Sriwijaya.

Latifah, Latifah (2015) Identifikasi Golongan Senyawa Flavonoid Dan Uji Aktivitas Antioksidan Pada Ekstrak Rimpang Kencur Kaempfira galangan L. Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil). *Skripsi*, Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Ma'rufah, Siti (2007) Efek Ekstrak Etanol Daun Salam (Eugenia Polyantha Wight) Terhadap Penurunan Kadar Asam Urat Pada Mencit Putih (Mus Muculus) Jantan. *Skripsi Thesis*, Universitas Muhammadiyah Surakarta.

Mubaroq, Irfan Abdurrachman (2013) Kajian Potensi Bionutrien Caf Dengan Penambahan Ion Logam Terhadap Pertumbuhan Dan Perkembangan Tanaman Padi (Oryza Satival.). *S1 Thesis*, Universitas Pendidikan Indonesia.

Nurinsani, Juniarti dkk (2015) Alkaloid. *Makalah*, Universitas Negeri Makassar.

Romadhoni, Febrina Putri (2017) Isolasi Pektin Dari Kulit Pisang Kepok (Musa Balbisiana Abb) Dengan Metode Refluks Menggunakan Pelarut Hcl Encer. *Other Thesis*, Politeknik Negeri Sriwijaya.

Subandono, Subandono (2006) Isolasi Dan Identifikasi Flavonoid Dari Daun

Ceremai (Phyllanthus Acidus [L.] Skeels.). *Skripsi Thesis*, Universitas Muhammadiyah Surakarta.

Sumartini, Sumartini (2016) Bioinsektisida untuk Pengendalian Hama dan Penyakit Tanaman Aneka Kacang dan Umbi. Jurnal Pangan. Pusat Penelitian dan Pengembangan Tanaman Aneka Kacang dan Umbi.Malang.

Tutik Novita , Syari (2012) Pengaruh Pemberian Ekstrak Brotowali (Tinospora Crispa, L.) Terhadap Perkembangan Folikel Ovarium Tikus Putih (Rattus Norvegicus, L.). *S1 Thesis*, Universitas Negeri Yogyakarta.

Warsani, Henki (2013) Kajian Pemanfaatan Lahan Sawah Di Kecamatan Kuantan Tengah Kabupaten Kuantan Singingi. *S1 Thesis*, Universitas Pendidikan Indonesia.

Yeni Hadiyanti, Yeni (2015) Efektivitas Daun Kacang Babi (Vicia faba L.) terhadap Mortalitas Larva Ulat Grayak (Spodoptera litura F.) pada Tanaman Kedelai (Glycine maxima L.) Varietas Grobogan. *Diploma thesis*, Perpustakaan UIN Sunan Gunung Djati Bandung.

Zainal, Ida Rizkayanti (2013) Evaluasi Kesesuaian Lahan Kualitatif Dan Kuantitatif Tanaman Padi Tadah Hujan (Oryza SATIVA L.) Pada Lahan Kelmpok Tani Karya Subur Di Desa Pesawaran Indah Kecamatan Padang Cermin Kabupaten Pesawaran. Fakultas Pertanian, Universitas Lampung.