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THE EFFECTS OF ETHANOL EXTRACT AND PEPAYA LEAF WATER EXTRACT (Carica papaya Linn.) AS BIOPESTICIDES AGAINTS SOIL TERMITES (Coptotermes curvinaghtus Holmgren.)

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#### **ABSTRACT**

This study aims to examine the effect of ethanol extract concentration and the effect of long storage of papaya leaf water extract (Carica papaya Linn.) as biopesticide against soil termite (Coptotermes curvinagthus Holmgren.). The study was conducted on February 8<sup>th</sup> – 23<sup>th</sup>, 2017. The treatment of each papaya leaf extract (*Carica papaya* Linn.) was sprayed into termites soil (Coptotermes curvinagthus Holmgren.) with the observation time of termite mortality was 10 minutes .This study was analyzed by using One Way Anava and continued with Bonferroni. The result showed that there was effect in each extract. In papaya leaf ethanol extract (Carica papaya Linn.) with concentrations of 20%, 40% and 70% had an effect on termite soil mortality (Coptotermes curvinagthus Holmgren.). The higher the concentration of ethanol extract, the higher the number of dead termites. In papaya leaf water extract (Carica papaya Linn.) with a storage duration of 1x24, 2x24, and 3x24 hours has an influence on the mortality of termites soil (Coptotermes curvinagthus Holmgren.). The lower of shelf life of the water extract, the higher number of dead termites.

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#### INTRODUCTION

Initially termites play an important role in the decomposition, nutrient turns and processes in the soil. Termites have a sensitivity to land use change and habitat destruction rates that can be used as bioindicators (Tarumingkeng in Ningsih et al., 2013). The decomposition process of various wood materials that fall on the forest floor is inseparable from the role of various types of termites as one of the invertebrate decomposers (Collins in Syaukani, 2013). Termites are able to decompose leaf litter more than 38 kg / ha / week. This amount is proportional to 32% of all fallen foliage on the forest floor (Thapa in Syaukani, 2013). But over time, termites in human life are often identified as destructive pests in buildings, housing, archives, books, plants, and so forth (Sabeth and Zulfahmi, 2010). In addition, termites also damage agricultural products, among which are recorded are coconut, rubber, cloves, cotton, sugarcane and various ornamental plants, among others, roses. Attacks are carried out in all growth rates from the nursery to adulthood (Hasan, 1986). In Indonesia, losses due to malignant termite attack can reach 224-238 billion rupiah per year (Tarumingkeng in Sabeth and Zulfahmi, 2010). Based on estimates, the economic losses caused by termite attack in Indonesia reached 1.67 trillion rupiah. Termites that have been studied by experts from 1758 to 1960 consist of 1959 species of 184 genera. Not all types of termites are human enemies because of its ferocity. From the data collected about the termites contained in buildings in various countries in the world can be known, that there are more than 100 species of termites that are destroyers (Hasan, 1986). The results so far show that some types of termites that are capable of causing significant damage to buildings are termites of the genus Coptotermes and Macrotermes (Nandika et al., 2003). One of the most harmful termites is termites of members of the Rhinotermitidae class of the Coptotermes genus Coptotermes sp. Much effort has been made for termite control. Most use highly toxic and environmentallyfriendly chemicals such as borax acid, CCB (Cooper-Chrome-Boron), CCA (Copper-Chrome-Arsen), CCF (Copper-Chrome-Flour), this will damage environment if not Anticipated because the material is difficult to overhauled by nature (Prasetyo in Desyanti et al., 2012). This is what drives to find ways to control the destruction of environmentally destructive termites. If thermisides are used continuously to control termites, their toxic active ingredients will accumulate in nature and greatly endanger human and ecosystem survival (Hasan in Sabeth and Zulfahmi, 2010).

The control of these Coptotermes termites has also been widely practiced using natural pesticides (biopesticides) that utilize the availability of flora present in nature. (Hadi in Desyanti et al., 2012) have

done research using kirinyuh leaf (*Eupathorium odoratum*) and got the best extract with concentration 2,5%. Control of termites with botanical pesticides has also been performed using bintaro extract (*Carbera oddolam* Gaertn) and amethyst (*Brugmansia candida* Pers) (Tarmadi in Desyanti *et al.*, 2012).

One of the plants known to be efficacious in many aspects is papaya. In addition to anti-tumor, papaya leaf is also potential to be used as a controlling termite. Compounds contained in papaya leaves that are expected to be used as termite controller are tannins, alkaloids, saponins and flavonoids (Tarmadi in Desyanti et al., 2012). In all parts of pepaya there is also a protease enzyme that serves to break the protein, the enzyme is the enzyme papain. It is expected that the technique of papaya leaf processing by extracting it can further strengthen the levels of natural substances that already exist in this leaf so it can be utilized properly.

#### **METHODS**

The type of this research is experimental research which is located in Basic Biology Laboratory Faculty of Teacher Training and Education and Basic Chemistry Laboratory Faculty of Mathematics and Natural Sciences Pattimura University, Ambon on 08-23 February 2017. The samples of this research were 360 tail termites. The variables of this research consist of two kinds: independent variable: papaya leaf ethanol extract (Carica papaya Linn.) With concentration 20%, 40% and 70%; Papaya leaf water extract (Carica papaya Linn.) By long storage of 1x24 hours, 2x24 hours and 3x24 hours. Dependent variable is Number of termite mortality (Coptotermes curvinagthus Holmgren.). The analytical technique used to test the effect of ethanol extract and papaya leaf water extract (Carica papaya Linn.) towards the termite mortality (Coptotermes curvinagthus Holmgren.) was single anova (one way anova).

#### 1. Tools and Materials

- Tools and Materials in Preparation of Papaya Leaf Ethanol Extract; Scissors, newspaper, analytic balance blender, aluminum foil, measuring cup, shaker, filter paper, vacuum rotary evaporator, papaya leaf and ethanol 96%
- Tools and Materials in Papaya Leaf Water Extract Making; Scissors, tablespoons, basin, papaya leaf, water, kerosene and detergent

# 2. Making Papaya Leaf Ethanol Extract

- Leaves that have been were obtained from Seed Holtikultura Bureau (BBIH) at Telaga Kodok village then they were washed and aerated for 1 hour.
- After that, the leaves were cut into small pieces and then dried at room temperature for 2 days using newspaper.
- Then in two days later, the small pieces of papaya leaves were blended until smooth to get dried powder as much as 300 grams, weighed using the analytical balance.
- 300 grams of dried powder is then divided into 2 erlenmeyer, with 150 grams of powder on each erlenmeyer
- Add 96% ethanol to both erlenmeyer, with a size of 600 ml per erlenmeyer, wiggled briefly by hand, then covered with aluminum foil on the whole of the erlenmeyer.
- The solution was ready to be macerated for 24 hours using a shaker.
- After one day, the solution was then filtered and was evaporated until we obtained papaya pure thick viscous extract
- The viscous extract is then diluted using aquades to obtain concentrations of 20%, 40% and 70%.

# 3. Making Papaya Leaf Water Extract

- Washing the papaya leaves as much as 250 grams
- The leaves of papaya were cut into small pieces
- Then the small pieces of papaya leaves were added 2.5 liters of water, 1/2 tablespoons of kerosene and 7.5 grams of detergent.
- The solution was then stirred until evenly distributed
- Separated into jars of jam based on variations of storage duration ie 1x24 hours, 2x24 hours and 3x24 hours

#### 4. Termite Selection

Preferably selected terminates were the active mobile ground termites taken from their acclimatized nests for 2 weeks using tree trunks.

The trunks were used as the path from the acclimation site to the test container.

# 5. Treatment Against Termites

The extracts of papaya leaf were sprayed on the animals. The distance of spraying used was 10 cm. There were 20 soil termites on each test container. Spraying time was conducted in the afternoon at 15.00 WIT for 3 days. Observation of soil termite mortality was 10 minutes using a loop. The termites were indicated dead if they did not show movements.

# **RESULTS**

**Table 1.** Raw Data Number of Dead Termites

Treatment	Variation	Deuteronomy		
	of	1	2	3
	treatment			
Papaya	$\mathbf{k}_{1}$	0	0	1
Leaf	k 2	2	0	1
Ethanol	k 3	4	4	5
Extract				
(A)				
Papaya	$1_{1}$	3	3	2
Leaf	1 2	4	3	3
Extract	1 3	1	1	2
Water (B)				

In each test container, 20 termite termites were used

# 1. Papaya Leaf Ethanol Extract (Carica papaya Linn.)

**Table 2.** Summary of One-Way Anova Result Concentration of Papaya Leaf Ethanol Extract (*Carica papaya* Linn.) against Deaths of Termites (*Coptotermes curvinagthus* Holmgren.)

ANOVA							
The death	of the						
soil termit	tes						
	Sum of	Df	Mean	F	Sig.		
	Squares		Square				
Between	27556	2	13778	24,800	.001		
Groups							
Within	3.333	6	.556				
Groups							
Total	30881	8					

Based on Table 2 data, it is seen that the value of F test is 24,800 with sig, 001 (P = 24,800>  $\alpha$  = 0,05). So it can be interpreted that there is significant effect of

papaya leaf ethanol extract (*Carica papaya* Linn.) towards the termite mortality (*Coptotermes curvinagthus* Holmgren). This is consistent with Setiawati's statement in Kubangun (2016) which revealed that the insecticidal power of *C. papaya* leaves is caused by repellent power and antifeedant power. This is caused by the compounds contained in papaya leaf extract *C. according* to Cahyadi in Kubangun (2016) compounds that can inhibit the

power of eating (antifeedant) are alkaloid compounds, triterpenoids, saponins and flavonoids.

The existence of significance on the results of anava analysis therefore infer that it is necessary to do further tests using Bonferroni, to determine the difference of each level of extract concentration of papaya leaves ethanol (*Carica papaya* Linn.) which has an effective effect on termite mortality (*Coptotermes curvinagthus* Holmgren).

**Table 3.** Bonferroni Test Result Concentration of Papaya Leaf Ethanol Extract (*Carica papaya* Linn.) Against Deaths of Termites (*Coptotermes curvinagthus* Holmgren.)

	M	Iultiple Compari	isons			
The number of de Bonferroni\	ead termites					
(I) extract	(J) extract	Mean	Std.	Sig.	95%	
ethanol papaya	ethanol papaya	Difference	Error		Confidence	
leaf	leaf	(IJ)			Interval	
					Lower	Upper
					Bound	Bound
Concentration	Concentration	66667	.60858	.946	-	1.3340
20%	of 40%				2.6673	
	Concentration	-4.00000 *	.60858	.002	-	-
	70%				6.0007	1.9993
Concentration	Concentration	.66667	.60858	.946	-	2.6673
of 40%	20%				1.3340	
	Concentration	-3.33333 *	.60858	.005	-	-
	70%				5.3340	1.3327
Concentration	Concentration	4.00000 *	.60858	.002	1.9993	6.0007
70%	20%	4				
	Concentration	3.33333 *	.60858	.005	1.3327	5.3340
	of 40%					
*. The mean diffe	erence is significant	at the 0.05 level.				

The result of the research as tested using one way anava and *post hoc* test using *bonferroni* showed that at each level of ethanol extract concentration papaya leaf has different insecticidal power (toxic effect) on termite mortality. The higher concentration ethanol extracts of papaya leaf the higher the toxic effect on termite mortality.

The death of the soil termite (*Coptotermes curvinagthus* Holmgren.) is characterized by the absence of signs of life that is not moving to the touch. The toxicity of papaya leaves (*Carica papaya* Linn.) is caused due to it contains several active compounds such as alkaloids, polyphenols, quinones, flavonoids, terpenoids and papain enzymes contained in papaya leaves can affect some physiological systems that regulate the development of pests (Sastrodihardjo in Siahaya and Rumthe 2014) . Saponin compounds enter the body of the larvae through the skin by adhesion

process and cause systemic effects. Penetration of the compound into the body of the insect is through the insecticide epikutikula, the compound get into the network under the integument to the target area. The entry of saponins results in the destruction of wax in the cuticle layer resulting in death due to the larvae experiencing a lot of water loss (Cottrell in Hidayati, et al, without years).

Flavonoid compounds enter through the cell membrane. Flavonoids are phenol compounds that are disinfectants that work by denaturing proteins. According to (Sastrodihardjo in Kubangun, 2016) in the hemolymph there are proteins, if the protein is denatured by flavonoids then the food can not be channeled from the digestive tract to the entire tissue of the larvae, resulting in the larvae shortage of ATP and die. According to Robinson in Paradise (2013) states that tannins can act as food repellent to termites because it

can inhibit the process of animal metabolism through the  $\alpha$ -amylase enzyme. In addition, tannin also enters the body of the larvae through the respiratory system which will then cause wasting on the nerves as well as damage to the respiratory system and cause the larvae can not breathe and eventually die (Purwani and Rogib, 2015).

### 2. Papaya Leaf Water Extract (Carica papaya Linn.)

**Table 4.** Summary of Anava Results One Direction Long Storage of Papaya Leaf Water Extract (*Carica papaya* Linn.) Against Deaths of Termites (*Coptotermes curvinagthus* Holmgren.)

ANOVA						
The death	of the					
soil termit	tes					
	Sum of	Df	Mean	F	Sig.	
	Squares		Square			
Between	6.222	2	3.111	9333	.014	
Groups						
Within	2,000	6	.333			

ANOVA
The death of the soil termites
Groups
Total 8.222 8

It is clearly shown in the data of Table 4 that the value of F test is 9.333 with sig 0.014 (P = 9.333>  $\alpha$  = 0.05). So it can be interpreted that there is significant effect of papaya leaf water extract to soil termite mortality.

This is in accordance to the study of Hasibuan et al (2011) that natural pesticides are short-term solutions to rapidly resolve pest problems. Natural pesticides should be part of an integrated pest control system, and only used when needed (not used if there are no pests that damage the plant). According to the significance of the anava analysis results, it is necessary to do further tests using *Bonferroni*, to determine the difference of each storage length of the extract Papaya leaf water that effectively affects the termite mortality.

**Table 5.** The Test Result of Bonferroni Long Storage of Papaya Leaf Water Extract (*Carica papaya* Linn.) Against Deaths of Termites (*Coptotermes curvinagthus* Holmgren.)

Multiple Comparisons							
The number of d Bonferroni	lead termites						
(I)	(J)	Mean	Std.	Sig.	95% Confidence		
extract water	extract water	Difference	Error		Interval		
papaya leaf	papaya leaf	(IJ)			Lower	Upper	
					Bound	Bound	
storage	storage 1x24	66667	.47140	.621	-	.883	
1x24 hours	hour				2.2164		
	storage 3x24	1.33333	.47140	.090	2164	2.883	
	hour						
storage	torage1x24 s	.66667	.47140	.621	8831	2.216	
2x24 hours	hours						
	storage 3x24	$2.00000$ $^{*}$	.47140	.016	.4503	3.549	
	hours						
storage	storage 1x24	-1.33333	.47140	.090	-	.216	
1x24 hours	hour				2.8831		
	storage 2x24	-2.00000 *	.47140	.016	-	-450	
	hour				3.5497		
*. The mean diff	ference is significant	at the 0.05 level.					

Results of the study as tested using one way anova and *post hoc* test using *bonferroni* showed that at each storage period papaya leaf water extract (*Carica papaya* Linn.) there was different insecticidal power (toxic effect) on termite mortality (*Coptotermes curvinagthus* Holmgren.). The faster the duration of storage of papaya leaf water extract (*Carica papaya* 

Linn.) The higher the toxic effect on termite mortality (*Coptotermes curvinagthus* Holmgren.) In the treatment of papaya leaf water extract (*Carica papaya* Linn.) Allegedly due to the influence of papain enzymes. With combination Proteases and peptidases can break up 90% peptide bonds. Papain enzyme is a contact poison that enters the body of the pest through the natural holes of

its body. Once inside, toxins will spread throughout the body and attack the nervous system so that it can disrupt the activity of pests. Papain enzymes can also work as protease enzymes that can attack and dissolve the components of the insect cuticle (Trizelia in Siahaya and Rumthe 2014).

Based on the classification of The International Union Of Biochemistry, papain includes hydrolase enzymes that catalyze the hydrolysis reaction of a substrate with the help of water molecules. The activity of papain catalysis is carried out through hydrolysis which takes place on the active sides of the papain. The separation of the amide groups present in the protein takes place through the breaking of the peptide bond.

This enzyme has catalytic activity as a proteinase and is able to hydrolyze peptides. Based on the chemical properties of the active site, papain includes the sulfhydryl protease, since the active part of the papain is the -SH group.

#### **CONCLUSION**

As one of the natural insecticidal ingredients, papaya leaf (Carica papaya Linn.) is very useful for killing. The higher concentration of papaya leaf ethanol extracts then the higher mortality rate of soil termites. Yet, the faster the storage duration of papaya leaf water extract the higher the termite mortality (Coptotermes curvinagthus Holmgren.).

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