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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 8th – 23th, 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 environmentally-friendly chemicals such as borax acid, CCB (Cooper-Chrome-Boron), CCA (Copper-Chrome-Arsen), CCF (Copper-Chrome-Flour), this will damage the 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 of treatment	Deuteronomy		
		1	2	3
Papaya Leaf Ethanol Extract (A)	k_1	0	0	1
	k_2	2	0	1
	k_3	4	4	5
Papaya Leaf Extract Water (B)	l_1	3	3	2
	l_2	4	3	3
	l_3	1	1	2

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 termites

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	27556	2	13778	24,800	.001
Within Groups	3.333	6	.556		
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.)

Multiple Comparisons

The number of dead termites
Bonferroni\

(I) extract ethanol papaya leaf	(J) extract ethanol papaya leaf	Mean Difference (IJ)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concentration 20%	Concentration of 40%	-.66667	.60858	.946	-2.6673	1.3340
	Concentration 70%	-4.00000 *	.60858	.002	-6.0007	-1.9993
Concentration of 40%	Concentration 20%	.66667	.60858	.946	-1.3340	2.6673
	Concentration 70%	-3.33333 *	.60858	.005	-5.3340	-1.3327
Concentration 70%	Concentration 20%	4.00000 *	.60858	.002	1.9993	6.0007
	Concentration of 40%	3.33333 *	.60858	.005	1.3327	5.3340

*. The mean difference 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 α-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 Roqib, 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 termites

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6.222	2	3.111	9333	.014
Within	2,000	6	.333		
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> α = 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 dead termites
Bonferroni

(I) extract water papaya leaf	(J) extract water papaya leaf	Mean Difference (IJ)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
storage 1x24 hours	storage 1x24 hour	-.66667	.47140	.621	-2.2164	.8831
	storage 3x24 hour	1.33333	.47140	.090	-.2164	2.8831
storage 2x24 hours	torage1x24 s hours	.66667	.47140	.621	-.8831	2.2164
	storage 3x24 hours	2.00000 *	.47140	.016	.4503	3.5497
storage 1x24 hours	storage 1x24 hour	-1.33333	.47140	.090	-2.8831	.2164
	storage 2x24 hour	-2.00000 *	.47140	.016	-3.5497	-4503

*. The mean difference 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 rate (*Coptotermes curvinagthus* Holmgren.).

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