Bio-Attractant of Termites Bait from Waste Paper and Extract *Ocimum basilicum* Linn against Subterranean Termites *Coptotermes curvignathus* Holmgren

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Abstract

Termites were the most destroying wood organism and one of the methods for controlling termites and suppressing their population is the baiting system. One of the conditions of the baiting system method is that the bait must be able to attract termites. Mostly the attractant is made from an extract of the plant. This research aimed to evaluate the attractants of termites bait from waste paper and extract of basil leaf (*Ocimum basilicum*) against subterranean termites *Coptotermes curvignathus* Holmgren. Leaves of *Ocimum basilicum* extracted with maceration process with ethanol. After extraction, the yield was made into five concentrations, consisting of 2%, 4%, 6%, 8%, and 10%. The waste paper was made into termite bait with a square bait with measure 2 cm long x 2 cm wide x 1 cm thick. After that, the extract of *Ocimum basilicum* was poured into the bait in each concentration. The termite's bait is then exposured to termites for 21 days. The number of termites was 50 workers and 5 soldiers. The variable of research was termite mortality and weight loss of termite bait. The result of the research showed that the average termite mortality value was 18.79% - 97.58%. The highest termite mortality was achieved at a 10% concentration of extract *Ocimum basilicum*. Its followed by a concentration of 8% with a mortality value of 91.52%; a concentration of 6% with a mortality value of 78.18%; a concentration of 4% with a mortality value of 61.82%, and a concentration of 2% with a mortality value of 51.52%. Meanwhile, in the control treatment, the mortality value was 18.79%. The average weight loss of termite bait was 3.41% - 8.36%. The highest weight loss was achieved on concentration 8% and this bait was more attractive to termites. The important results of the research was termite's bait from waste paper and extract of *Ocimum basilicum* was attractant to subteranean termites *Coptotermes curvignathus* and the optimum concentration was 8%.

Keywords: attractant, *Coptotermes curvignathus*, *Ocimum basilicum*, subterranean termites, termites bait.

Introduction

Termites are social insects that live in communities and are known as cellulose-eating insects that can damage wood and buildings. Every year the losses caused by termites in Indonesia are around Rp 224 billion – Rp 238 billion (Nandika *et al* 2015). An effort that can be made to reduce losses caused by termite attacks is to carry out controls aimed at suppressing termite populations and termites attacks (Kutana *et al* 2018). The termite baiting system technique is considered more effective than other termite control techniques because other control techniques such as spraying the chemical (termiticide) are considered to endanger human safety and health and the surrounding ecosystem can be contaminated with termiticide (Sucipto 2009). The baiting system technique is unique because this system should attract termites and makes termites eat the bait. Therefore the bait should have properties or aroma to attract termites to consume the bait (Permana dan Husni 2017).

Materials that have a scent that can attract termites to approach and consume the bait are called bio-attractants (Septiana dan Husni 2017). Currently, the attractant used on termites bait was chemicals thought to be not environmentally friendly. Natural attractants or bio-attractants can be obtained from various types of plants, especially plants with distinctive aromas (Permana dan Husni 2017). Basil leaf (*Ocimum basilicum*) hasthe potential a termite bait attractants because it contains aromatic compunds which attract termites to consume the bait (Indrayani *et al* 2017) and in the research of Simbolon *et al* (2015) resulted 41 termites from 55 termites moved to the samples that had been given basil leaf extract.

Basil leaf extract consists of aromatic compounds namely methyl eugenol (2.24%), methyl linoleate (1,49%), and toluene (4,46%), and this compound attract termites to consume the bait (Noviansari *et al* 2013). Basil leaf can be combined with materials that contain high cellulose to strengthen the bait because cellulose is the main food of termites. HVS paper waste is a material that contains high cellulose (Franceschin *et al* 2010). According to Muin *et al* (2015) termite bait formulations from degraded Pine wood, HVS paper waste, paperboard waste and newsprint waste with a mixture of boiled soybean water can be used as feed ingredients to support termites baiting system techniques.However, there have been no reports on the utilization of the HVS paper waste with basil leaf extract as a bio-attractant of termites bait to control termites with a baiting system. The study aimed to evaluate the extract of basil leaf (*Ocimum basilicum)* as a bio-attractant of termites bait, and analyze the optimum concentration of basil leaf as a bio-attractant against *Coptotermes curvignathus* Holmgren. The research outcome is to provide the information on effectiveness of basil leaf extract as bio-attractant of termites bait against subterranean termites and can be used as an environmentally friendly bio-attractant in wood preservation.

Materials and Methods

Sample Preparation

The research was carried out at the Laboratory of Wood Technology, Forestry Faculty Tanjungpura University. The young leaf of basil (*Ocimum basilicum*) around 10 kg was obtained from a plantation in Ahmad Yani Street Pontianak City. Subterranean termites *Coptotermes curvignathus* was from a secondary forest in Sungai Ambawang, Kubu Raya district. The termites were kept in laboratory for one month before being used as a sample for testing.

Basil Leaf Extraction

The process of extraction of basil leaf was referred to Kumalasari and Andiarna (2020) that air-dried basil leaf are crushed into powder and filtered using 40 mesh and 60 mesh screens. The sample used was the powder of basil leaf which passes 40 mesh filter and was retained by the 60-mesh filter. Furthermore, 200 grams of basil powder was dissolved in 800 ml of ethanol and macerated for 48 hours. The result of maceration is filtered and re-extracted until a clear solution is obtained. After that, the liquid extract was filtered on the rotary evaporator at a speed of 50 rpm and temperature 45°C for 1 – 2 hours to remove the ethanol solvent. The yield of the extract was counted as follows (Bihari *et al* 2011):

$$\text{Yield of extract (\%)} = \frac{a}{(1-x)b} \times 100\%$$

a = weight of basil extract (gram)
b = weight of initial basil powder (gram)
x = water content

Termite Bait Preparation

HVS paper waste which has something print in the paper was crushed using a hammer mill with a size of 20 mesh and made into termite bait refer to Severtson and Majer (2006). The density of the termite bait sample was made into 0.5 g/cm^3. The HVS paper waste was soaked in distilled water for 30 minutes and then air-dried using a filter cloth. After that, 2 grams of material was formed using a molded box with a size length of 2 cm, width of 2 cm, and of thickness 1 cm. The sample bait was then put in an oven with temperature of 40±2 °C for 48 hours. Then the termite bait was dripped with 1.2. ml of basil extract in each concentration (60% of the weight of the material) and drained for 5 minutes. The concentration of basil extract used were 2%, 4%, 6%, 8% and 10%. Five replication was made for termite bait on each concentration. Furthermore, the termite bait sample was put in an oven for 48 hours at a temperature 40±2 °C to protect it from fungi attack (Septiana and Husni 2017). Before testing termites all the termite bait was weight to determine the initial weight of the termite bait sample.

Laboratory Assay on Basil Termite Bait

Laboratory assay to evaluate the attractants of termites bait was conducted refers to Ohmura *et al* (2000). The test tools use a plastic cup with a diameter of 5 cm. Each test plate was filled with 10 grams of sterile sand and moistened with 2 ml of water to maintain moisture. Plastic gauze with a diameter of 3 cm is placed on the sand to prevent direct contact of the sample with the sand. Furthermore, the termites bait was put in front of plastic gauze and termites was put inside the cup (50 workers and 5 soldiers). Observations were made for 21 days, and all the plastic cups were stored in a dark room with a temperature of 26.9 °C – 28.3 °C and humidity of 70% - 82%. The research was conducted in five replications. The variable of research was termites mortality and weight loss of termites bait. Determination of the termites mortality and termite bait weight loss value was calculated by the following formula (Owoyemi *et al.* 2011):

$$\text{Mortality (\%)} = \frac{N_1 - N_2}{N_1} \times 100\%$$

N_1 is the number of initial termites
N_2 is the number of termites at the end of the test

$$\text{Termites bait weight loss (\%)} = \frac{B_1 - B_2}{B_1} \times 100\%$$

B_1 is the weight of the initial termite bait (gram)
B_2 is the weight of the termite bait at the end of the test (gram)

Data Analysis

Research on termite bait from basil leaf extract (*Ocimum basilicum*) and HVS waste paper against subterranean termite *Coptotermes curvignathus* using analysis of variance (ANOVA) with completely randomized design (CRD) according to Gaspersz (1994). The concentration of basil (*O. basilicum*) leaf extract were 5 concentration, consist of A_0= 0% (control), A_1= 2%, A_2= 4%, A_3= 6%, A_4= 8%, and A_5= 10%. Each concentration has 3 replications. The test results of the analysis of variance with the F test have a significant or very significant effect will continue to evaluation the difference between the treatment with honest significant difference (BNJ) test.

Result and Discussion

Yield of Extract *Ocimum basilicum*

The result of maceration process on basil leaf (*Ocimum basilicum*) was 30.93%. This result was good and higher compare to other research, such as Bilal *et al* (2012) reported the extractive values of basil were 4.0% in ethanol; 6.24% in water and 3.7% in ether. Meanwhile Diba et al (2022) reported the extractive values of basil was 29.38%. The form of extract was gel with a dark green color and smells like the original plant, this is thought to be due to the content of

eugenol or its methyl eugenol derivatives contained in the basil leaf extract. According to Islamy and Asngad (2018) the eugenol compound is a clear liquid to pale yellow, volatile and gives a certain aroma with the same distinctive aroma as the original plants. Extraction is a process of withdrawing secondary metabolites using solvents (Purushothaman *et al* 2018). The extraction used ethanol which is a polar solution. According to Ohmura *et al* (2000) ethanol can produce the optimal amount of extract.

Termites Mortality

The average value of termites mortality after feeding the termites bait from waste paper and extract of basil leaf (*Ocimum basilicum*) was 51.52% ~ 97.58%, meanwhile, on control treatment, the mortality value was only 18.79%. The concentration of extracted basil leaf of 6%, 8%, and 10% has a very strong activity to inhibit termites and resulted in mortality from 78.18% ~ 97.58%.

The analysis data of termites mortality with completely randomized design showed that the extract of basil leaf on termites bait has a significant effect on termites *Coptotermes curvignathus*. The mortality of termites happened on five days until the end of the evaluation test. Termite feed the bait then spread out the bait with trophallaxis system Ridhwan and Isharyanto (2016) stated the basil leaf has a potential as bio-insecticide. The highest concentration of extract basil in the bait resulted more termite mortality and the bait has a hig palatability to termites. The average termite mortality after feeding the bait from waste paper and extract of basil leaf value was shown in Figure 1.

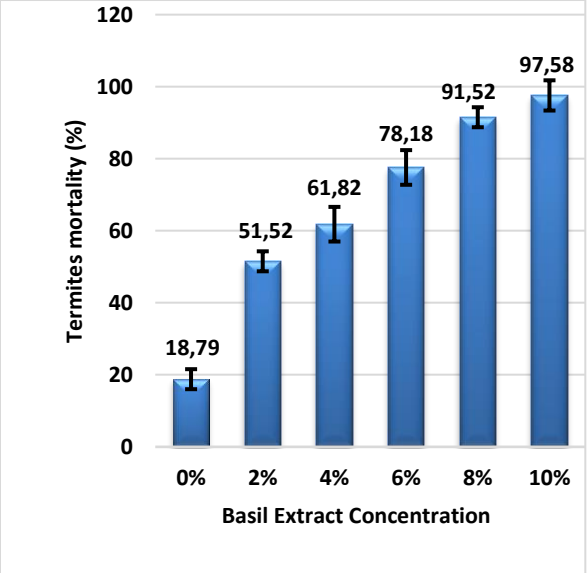

Figure 1. The average value of *Coptotermes curvignathus* termites mortality after feeding the bait from waste paper and extract of basil leaf (*Ocimum basilicum*) with different concentration.

Referring to the research of Suhara (2020), subterranean termite mortality in baits using low concentrations will react more slowly because the compounds contained in baits require time to be absorbed by the subterranean termite. Meanwhile, baits with higher concentrations reacted more quickly and increased subterranean termite mortality. From the experience, we found that the mortality of termites after feed the bait from waste paper and extract basil with concentration 2% was happened after 10 days, meanwhile on concentration 10% the mortality was happened after five days.

Umar and Majid (2020) stated that termites bait methods has the advantage to control termite. Elimination of termite colony population was successfull with the aid of termite behavior of trophallaxis which is the sharing of the bait toxicants among the termite nest mates.The bioatractan from basil leaf made the termites worker share the toxicants among the nest mates then eradicate the colony along the process. The mortality value of *Coptotermes curvignathus* termites after feeding the termite bait from waste paper and extract of basil leaf was from 78.18% ~ 97.58%, almost 100% mortality was achieved at basil concentration of 10%. This results showed that the basil extract has a potential used for bioatractant on bait formulations and to achieved the ellimination process, the bait require additional material, such as from monosccharide and sugar.

Bilal *et al*. (2012) stated the highest concentration of basil extract have the highest bioactive compound which made the highest mortality of termites. The bioactive compound from basil leaf was eugenol, methyl eugenol, and methyl clavical. Kardinan (2007) stated the eugenol and methyl clavical was inhibited and made mortality of flyhouse Musca domestica. Methyl clavical has the effect of anasteticum which works by interfering the nervous system. Meanwhile, eugenol works as a contact poison through the surface of the body. This is because eugenol is easily absorbed through the skin, causes burns and can cause death.

Eugenol can be toxic to termites and has the potential as a termite control bioactive, besides that the eugenol content in plant extracts can also be a bio-attractant for termites, but depending on the type of plant and the concentration used (Indrayani *et al*. 2016). Furthermore, the lowest mortality was at a concentration of 0% with a mortality value of 18.79%. The low mortality at 0% concentration was due to the absence of bioactive components from basil leaf extract, then termite mortality tends to be low. Mortality at a concentration of 0% occurred even though basil leaf extract was not given, presumably due to the inability of termites to adapt to their new environment, and cannibalism between termites was occur (Nandika *et al*. 2015).

Based on the research of Indrayani *et al*. (2017), ethanol extract of basil leaf contains methyl eugenol (2.24%) which has the potential as a bio-attractant and control subterranean termites Coptotermes curvignathus Holmgren. According to Kardinan (2007) methyl eugenol ($C_{12}H_{24}O_8$) is a

semi-chemical material that is an attractant that can stimulate the olfactory (sensory device) of insects. Methyl eugenol can affect the behavior of insect animals, such as the behavior of looking for food, laying eggs, sexual relations and others. This statement is supported by Lewis and Forschler (2016) who stated that eugenol was significantly more toxic to subterranean termites *Coptotermes* sp than other chemical compounds such as citral, citronellal, geraniol, and pyrrolidine. The content of eugenol in basil plants is higher than other compounds. Pandey *et al* (2014) stated the main components of essensial oil from basil plant is eugenol with a percentage of 6.6%.

Termite mortality is also influenced by several other factors such as termite conditions, temperature, humidity, and light intensity. In laboratory bioassay, the termites used must be in good condition. Termites are sensitive insects and their activities are easily disturbed, so it is necessary to condition the termite colony before testing. In addition, Oramahi *et al*. (2021) stated subterranean termites Coptotermes curvignathus are termites that require a moist place to live, the ideal temperature and humidity conditions such as their natural habitat. Therefore during the test the temperature should be measured, if it is too high and the humidity is too low, it can trigger an increase in the mortality of subterranean termites. In this study, the average temperature is in the range of 28.5 °C - 30.8 °C, such a temperature is considered to be optimum and ideal for termite life because it is under the living conditions of subterranean termites in nature. This is supported by the statement of Cao and Su (2015) which states that the optimum temperature for the activity of wood-destroying termites is between 24 °C – 32 °C. Meanwhile, the humidity ranges from 70% - 82% and is in accordance with the termites nest. Nandika *et al* (2015) said that the optimum humidity of subterranean termites in living habitat was range between 75% - 90%. In addition to termite conditions, temperature and humidity, during termite laboratory bioassay, it is necessary to store the termites in a dark room, because termites has a cryptobiotic character. Exposure of too much light can interfere termites activity.

Bait Weight Loss

The average value of bait from waste paper and extract of basil leaf (*Ocimum basilicum*) weight loss after feeding by termites was 6.51% ~ 8.36%, meanwhile on control treatment the bait weight loss value was only 3.41%. The concentration of extract basil leaf of 8% has a highest bait weight loss. The average bait weight loss value was shown in Figure 2.

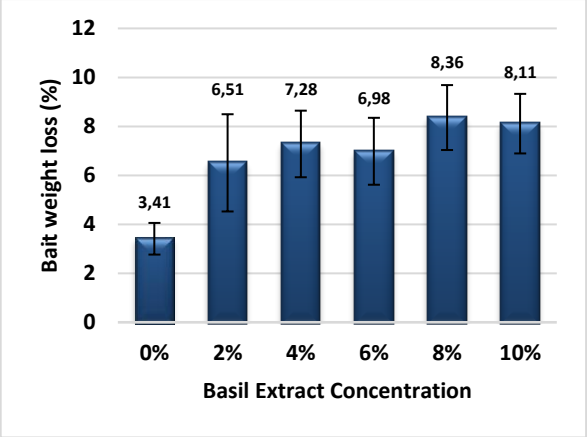

Figure 2. The average value of bait from waste paper and extract of basil leaf (*Ocimum basilicum*) weight loss after feeding by *Coptotermes curvignathus* termites.

Basil leaf extract has a significant effect on termites feeding to the bait. The weight loss of bait without basil leaf extract was 3.41%, meanwhile, the weight loss of bait with basil extract was higher, 6.51% ~ 8.36%. This condition reflect the effect of bio-attractant from basil leaf extract. The rate of termite consumption tends to increase with the higher concentration of basil leaf extract. In line with this result, Carnohan *et al*. (2021) stated the concentration of extract was influence the feeding activity of termites. Some extract with higher concentration tend to increase the preference of termite to feed the bait. According to Diba *et al*. (2017) termite bait active ingredients should be non-repellent and slow acting to ensure the bait will be transport to the nest by worker foraging termites and distribution the active ingredients throughout the colony by trofalaksis system. Bio attractants from basil leaf have a potential in controlling termites. Diba *et al*. (2022) stated the active ingredients in basil leaf extract consist of linalool, methyl chavicol, eugenol, methytl eugenol, flavonoid and saponin. Research of Hikmawanti *et al*. (2019) analysis extract of Ocimum basilicum with GCMS and it had 13 components with the major compound were methyl eugenol (52.60%), caryophyllene (18.75%) and germacrene-D (9.19%). This bio-active compound as an attractant for termites to feed the bait. Nandika *et al*. (2015) states that worker termites use their sense of smell to find food sources because this caste does not have eyes.

The value of bait weight loss is influenced by several other factors, one of which is the material of bait. In this study, the material used was HVS paper waste. HVS waste paper was different from natural food sources for termites. According to Pivnenko *et al* (2015) waste paper contain many chemicals from printing ink, especially polychlorinated biphenyls. The ink remaining on the bait is thought to be toxic to subterranean termites. However, according to Santos *et al*. (2007) subterranean termites genus *Coptotermes* sp and *Macrotermes* sp was able to metabolize and remove lipophilic

xenobiotics in the waste paper, and thus the two genera of termites has a potential candidates for the biogenic conversion of waste paper. This research also prove that HVS waste paper was suitable as material for termite bait and not repellent to subterranean termites. The bait after feeding by termite was shown in Figure 3.

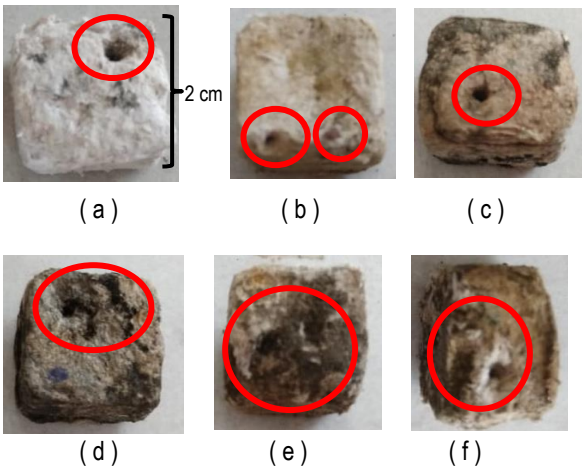

Figure 3. Condition of termite bait from waste paper and extract of basil leaf (*Ocimum basilicum*) after feeding by Coptotermes curvignathus termites
(a) : control; (b) : concentration 2%;
(c) : concentration 4%; (d) : concentration 6%;
(e) : concentration 8%;(f) : concentration 10%;

Figure 3 shows that termites feed on the bait on the sides or bottom of the sample by making holes (red circle in the image). From the results of the study, it can be seen that the feeding activities of termites tend to be in the same hole, so that the change in the shape of the bait is not too significant on the outside, but is dominant on the inside. This is presumably because the termites tend to make hole as part of termite burrow which can be in direct contact between the bait and the sand. In this study there were several components that caused the weight loss of the bait, namely basil leaf extract which have a distinctive aroma that can attract termites and HVS waste paper as a source of cellulose in bait which is very popular as termite food. The mixture of these components can be the main trigger for termites to eat the bait.

According to Kadian and Parle (2012) basil leaf extract contain of methyl eugenol, methyl clavical and linalool. Methyl eugenol has potential as bio-attractant, methyl clavical has an anesthetic effect, which is works by interfering with the work of nervous system. Silalahi (2018) stated linalool is the main constituent of basil leaf extract (52.42%) and has a fragrant aroma and smells good and suspected to be a natural attractant and can attract termites to approach the bait. In addition, according to Sarma *et al* (2011) basil leaf extract has a compound og 1.8 cineol (5.61%) and this compound has a distinctive aroma in plants and can stimulate sensory or olfactory devices, which termites can be attracted to approach the bait and consume the bait.

Basil leaf extract and HVS waste paper are materials favored by termites, and termites are attracted to eat the bait. Simbolon *et al*. (2015) stated that basil leaf extract with a concentration of 10% was the optimum concentration as a bio-attractant against subterranean termites Coptotermes curvignathus. Utilization of HVS waste paper as a basic materials for bait become and additional attractant so that termites are increasingly attracted to consume the bait. This is in line with the research of Muin *et al*. (2015) which stated that HVS waste paper, cardboard waste and news paper waste can be formulated into bait and attractant to termites. In sample testing, the use of HVS waste paper as bait was thought to be good enough to meet the termite feeding needs because all treatments showed a loss of bait weight.

Termite baiting system use small amounts of active ingredients and the main goals was colony elimination. Evans and Iqbal (2015) studied and evaluated about termites baiting used in commercial treatment. They study 15 active ingredients, 23 termite species and 16 countries with mostly the active ingredients was chitin synthesis inhibitor. The result of research showed that baiting system was success to eliminate the colony of termites from genus Coptotermes and Reticulitermes with bait material from paper and 0.5 gram active ingredients. They stated the need of future research to use other active ingredients for termite baiting system. Basil leaf extract could be one promising as active ingredients on termite baiting system.

The baiting system is considered environmentally friendly on termite control method compare to other methods such as spraying with chemical solution or fumigation system. The main goal of termite control is to prevent or remove termites infestations in the area such as plantation or structures. In line with the goal of a termites baiting system, the baiting treatment is to eliminate termites colony and to suppress the populations of termites.

Bait matrix is an important factor of successful on termite baiting system because the bait will be in competition with alternative feeding sites (Iqbal *et al*. 2021). Therefore the bio-attractant compound in termites bait will increase the preference of termites in consume the bait. Result of research showed that the active ingredients of basil leaf extract has a function of bio-attractant compound and acceptance by subterranean termite Coptotermes curvignathus.

Some factor on successful of the termite baiting system is dependent on the knowledge of termite characteristic and behavior. Yii *et al*. (2016) researched the feeding preference test and found that bait size and nutrients supplemented in bait play a role in the food selection of termites. Iqbal *et al*. (2017) stated the size of the bait and the bait station was an important component on termites baiting system. They studied found that termites Macrotermes gilvus and Macrotermes carbonarius was prefer the large bait station. Evans and Iqbal (2015) stated the commercial termite bait was consist of two categories of active ingredients, namely insect growth regulator, and metabolic inhibitor. The

metabolic inhibitors such as hydramethalnon, sulfluramid, borates and mirex. The insect growth regulators with chitin synthesis inhibitors such as noviflumuron, hexaflumuron and triflumuron. Extract of plant was a promising active ingredients due to environment consider of the termite control method. The results showed that the treatment with bait from HVS waste paper and concentration of basil leaf extract 8% showed a mortality of termites of 91.52% and the weight loss was 8.36%. Based on these results, it can be stated that termite bait from HVS waste paper and basil leaf extract with concentration 8% was optimal as bio-attractant against subterranean termites. Therefore it can be stated that the mixture of basil leaf extract with HVS waste paper has potential as an attractant to termies to feeding the baiting.

Conclusions

Basil (*Ocimum basilicum*) leaf extract has a function as a bio-attractant to subterranean termites Coptotermes curvignathus. Termite bait using HVS waste paper and basil leaf extract was non-repellent to termites and a promising used for termites bait in control the termites. The optimum concentration of basil leaf extract was 8% with a classification a very strong termite's activity, the average termites mortality value was 91.52% and the weight loss of bait was 8.36%.

Acknowledgements

This research was funded by Forestry Faculty, Tanjungpura University. The author are appreciation to Laboratory of Wood Technology, Forestry Faculty, Tanjungpura University for providing the facilities used during the research.

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