The Efficacy of Pine Wood Pellets reinforced with Active Hexaflumuron Against the Subterranean Termite Coptotermes curvignathus Holmgren

Arinana¹, A R E Silalahi¹, D Hendra² and D Nandika¹
¹Departement of Forest Products, Faculty of Forestry and Environment, IPB University, Bogor, 16680, West Java, Indonesia
²Forest Products Research and Development Center, Jl. Gunung Batu No. 5 Bogor, West Java, Indonesia
E-mail: arinana@apps.ipb.ac.id

Abstract. Bait formulation using hexaflumuron is the most used baiting techniques because it is relatively friendly to the environment. One of the potential wood species for bait formulation for termites is pine wood. The resin in pine wood can reduce termites preference. Therefore, the resin contained in pine wood needs to be reduced so that the level of termites preference for pine wood can be increased. This study aims to determine the efficacy of the bait formulation in the form of pellets from pine wood (Pinus merkusii Jungh de Vries) which was pre-treated with presto (0.4 bar, 100 °C) for one, two, three, four, and five hours with active hexaflumuron (0.25%) against subterranean termites Coptotermes curvignathus. Pine wood chips were treated with presto for one hour, two hours, three hours, four hours, and five hours. The treated pine wood chips then made into sawdust by 40-60 mesh. Pine sawdust then added with hexaflumuron (0.25%) and formed into pellets without adding any adhesive material and then fed to the subterranean termites C. curvignathus (JIS K 1571: 2010). Solid pine wood used as a control sample. The result showed that palatability of formulation pellet bait with active hexaflumuron (14.33%) was higher than solid pine wood (6.58%). The formulation of pellet bait with 0.25% active hexaflumuron with presto pre-treatment was able to eliminate C. curvignathus termites.

1. Introduction
Subterranean termites Coptotermes curvignathus Holmgren (Isoptera: Rhinotermitidae) is the most important termite that causes a lot of damage to wood building structures [1]. Termite C. curvignathus has wide distribution area and the cases of attacks almost occur in all parts of Indonesia [2]. The losses are very large. Nandika [3] stated that the economic loss due to termite attacks on residential buildings in Indonesia in 2015 was 8.86 trillion rupiah. The value of the loss is expected to increase if no control measures are taken

Baiting method is one of the most recently used methods to control termites. This is because baiting method has advantage that it does not pollute the soil, right on target, specific and easier to take samples [4]. Baiting method use the trophalaxis behavior of termites where worker termites will feed other members of the colony and also the nature of termites preferences for active ingredients so that termites will consume the bait and spread it to all the colony members [2]. One of the active ingredients used as termite bait formulation is Hexaflumuron (benzoylphenyl urea). Hexaflumuron is known to be a substance that has low toxicity to mammals, non-repellent, and slow action. Hexaflumuron stops the
growth of termites by interfering the synthesis of chitin which is needed by termites to form a new exoskeleton [5]. As a result, the termite cuticle does not form properly and causes termite integument cannot protect its body and the termite become dehydrated and then die. But the production of termites bait requires expensive cost so that the alternative materials is needed for more efficient feed formulations and can reduce the cost.

Arinana et al. [6] stated that pine wood is preferred by subterranean termites C. formosanus compared to acacia (Acacia mangium), rubber wood (Hevea brasiliensis), and sengon (Falcataria moluccana). However, pine wood contains resin which can reduce the level of preference for subterranean termites Coptotermes spp. against pine wood so its need to be reduced [7]. The research also stated that heat treatment up to 100 °C applied for pine wood could increase termites palatability and the best treatment was presto (0.4 bar, 100 °C). Meanwhile, termites bait with active ingredients hexaflumuron in the form of pellets have the highest weight loss percentage than block form [8]. But in that research, the termite bait formulation did not use pine wood as an additive and the dose of hexaflumuron used was 0.5%.

Therefore, its necessary to perform research to evaluate the efficacy of pellet bait originated from pine wood (Pinus merkusii Jungh er de Vries) which was pre-treated with presto and mixed with hexaflumuron against the subterranean termites Coptotermes curvignathus.

2. Methods
Wood samples for this study were collected from Ciherang, Darmaga, Bogor. Hexaflumuron came from SIGMA ALDRICH. Healthy and active subterranean termites worker and soldier C. curvignathus, alcohol 70%, dental cement, and distilled water were used in this research. The tools used in this study includes wood chopper, wiley mill, semi-automatic hydraulic pellet mill, presto pot, oven, scale, desiccator, caliper, acrylic tube, plastic net, tissue, microtome slides, microscope, axiovision software, water bath and camera.

2.1. Microscopic observation of pine wood
Observations of the anatomy of pine wood were carried out microscopically by observing the cross section, radial, and tangential sections of the wood using a light microscope. Preparation of microtome preparations refers to the Gasson et al. [9]. Microscopic images of pine wood at cross-sections, radial, and tangential were obtained through a microscope camera and axiovision software.

2.2. Sample preparation
Pine wood chips were treated with presto (0.4 bar, 100 °C) for one, two, three, four, and five hours and then air-dried (8-12%). The treated pine wood chips then made into sawdust by 40-60 mesh. Pine sawdust then added with 0.25% hexaflumuron and formed into pellets without using any adhesive material. Pellet were pressed by semi-automatic hydraulic pellet mill with pressure 170-175 kg/cm² at 125 °C for 10 minutes. This study used solid pine wood (2 cm x 2 cm x 1 cm) as a control sample. Each treatment was replicated three times.

2.3. Pellet characteristics
Pellet characteristics measured were moisture content and density. The measurement refers to SNI 8021:2014 [10] about Wood Pellets. The moisture content (MC) measurement was carried out by weighing 2 gram of pine sawdust (W.). The sawdust placed in a porcelain cubicle, then placed in an oven (103±2 °C) for 24 hours until constant. The sawdust then placed in desiccator and then weighed (W.). Meanwhile, pellet density (D) is the ratio between pellet weight (W) and pellet volume (V) measured under the same conditions. The moisture content and pellet density is calculated by the following equation:

\[ MC(\%) = \frac{W_1 - W_2}{W_2} \times 100 \]

\[ D \left( \frac{g}{cm^3} \right) = \frac{W}{V} \]
2.4. Termites bait testing
The test refers to the JIS K 1571: 2010 [11] standard, which uses a test container made of an acrylic tube with a diameter of 8 cm and a height of 6 cm, the bottom of which is closed with a dental cement 1 cm thick. The center of the acrylic tube is placed with a plastic net (3x3 cm). Then the test container is placed on a tissue sheet that has been given distilled water. Each termite bait material consisting of solid pine wood control, pellets with pressure treatment for one hour, two hours, three hours, four hours, and five hours was pre-heated for 48 hours at a temperature of 60±2 °C and then weighed (W₁). The termite bait was placed on a plastic net in an acrylic tube. Furthermore, into each test container subterranean termites C. curvignathus are inserted with total 165 termites consisting of 150 termites’ workers and 15 soldier termites. The test container was kept for 21 days in a dark room. During the test, if any termites die, they are taken immediately, and distilled water added if the tissue is not as wet as at the beginning of the test. The subterranean termite palatability test of C. curvignathus against termite pellet bait from pine wood is presented in figure 1.

![Figure 1. Testing of the termite palatability of C. curvignathus based in JIS K 1571: 2010.](image)

After 21 days, the test sample was dismantled and counted for termites who are still alive. Each termite bait was cleaned and put into the oven (60±2 °C) for 48 hours, and then weighed (W₂). Furthermore, the measurement of the weight loss (WL) of the termite bait and termite mortality (M) of C. curvignathus is calculated by the following equation:

\[
WL (\%) = \frac{W_1 - W_2}{W_2} \times 100
\]

\[
M (\%) = \frac{N}{150} \times 100
\]

Where:
- \(W_1\) = Dry weight of bait before baited (g)
- \(W_2\) = Dry weight of bait after baited (g)
- \(N\) = Number of termites that died at the end of testing

2.5. Measurement of the chemical components of pine wood
The control pine wood chips and pre-treatment presto (one, two, three, four, and five hours) pine wood chips were ground using wiley mill and sieved through 40-60 mesh sieve. Each sawdust was then measured for its chemical components (holocellulose, cellulose, clason lignin, and solubility in ethanol-benzene). The determination of holocellulose and cellulose content refers to method in Browning [12]. Meanwhile, the standard used for clason lignin refers to TAPPI T-222 om-88 standard [13], and solubility in ethanol-benzene refers to TAPPI T-204 om-88 [14].
2.6. Statistical analysis
Qualitative data was presented in histogram and described qualitatively, meanwhile the quantitative data were calculated using Microsoft Excel 2016 and analyzed using IBM SPSS Statistics 26. If the result of the variance analysis at the 95% confidence interval were significant, the analyzing process will be continued using Duncan test.

3. Result and Discussion
3.1. Pine wood anatomy (Pinus merkusii Jungh)
Pine wood used has yellowish white color with smooth texture and surface and straight grain direction. The anatomical structure of pine wood which was microscopically observed was made from slide microtome. Microscopic picture of cross section, radial, and tangential of pine wood can be seen in figure 2. Small intercellular canals (resin canals) are seen in the cross section and are diffusely scattered. In the tangential cross section uniseriate ray is seen which there are parenchyma ray and tracheid cells. The main characteristics of pine wood (P. merkusii) is that it does not have pores but has axial canals of small size that are diffuse and infrequent [15]. Karlinasari et al. [16] stated that P. merkusii has fiber length of 5155 µm, fiber diameter 48.74 µm, lumen diameter 32.80 µm and wall cell thickness 7.97 µm. Based on the macroscopic and microscopic characteristics, the wood used as the test sample is classified as P. merkusii [15]. In addition, based on Jensen [17] identification key, P. merkusii has leaves in needle-shaped and there are two needle-shaped leaves in one fesicle.

3.2. Pellet characteristics
Pellet density shows the ratio between the weight and volume of wood pellets. Result shows that pine wood sawdust measuring 40-60 mesh which was compressed at temperature 125 °C and pressure 170-175 kg/cm² for 10 minutes produces pellet with range density from 1.02-1.07 g/cm³. The density value of pine wood is 0.62 g/cm³. These result show that there is an increase in the value of density after pine wood forming into pellets by hot pressing. According to Utami [18], the density value of pulai wood pellets with sawdust size 40-60 mesh and compressed at temperature 180 °C is 1.55-1.67 g/cm³. The difference in density value caused by difference in wood species and the temperature used during compression. The hotter temperature used during compression, the higher density of wood pellets formed. This is due to the presence of high compression pressure and temperature, the stronger the bond between the particles, thus increasing the density value [19].

Determination of moisture content (MC) was to determine the hygroscopic of resulting pellets. Based on the results of the study, the highest value of MC was in one hour presto pellet (12.36%), followed by five hours presto pellet (11.11%), two hours and four hours presto pellet has the same value (10.50%) and the lowest MC is the three hours presto pellet (7.53%). The value of the MC of pellets with particles
size 40-60 mesh ranged between 7.65-10.48% [20]. The difference result is caused by differences in the ability to absorb water from surrounding environment and it is also affected by the pressure applied during the pellet compression [21].

3.3. Termites bait weight loss
The result shows that highest weight loss was termite bait in the form of pellets with pre-treatment of presto for four hours (15.15%), followed by pellets with pre-treatment presto for three hours (14.665%), pellets with pre-treatment presto for five hours (14.60%), pellets with pre-treatment presto for two hours (13.65%), pellets with pre-treatment presto for one hour (13.60%), and the lowest weight loss was on control solid pine wood (6.59%). The details are presented in figure 3.

![Figure 3](image)

**Figure 3.** Percentage of weight loss of the termite bait formulation with 0.25% active hexaflumuron with various treatments.

The result of the analysis of variance at 95% confidence intervals showed that the bait formulation had a significant effect on weight loss. Furthermore, based on Duncan’s test result showed that the formulation of bait with active ingredient hexaflumuron and pre-treatment presto was not significantly different. Termites C. cuvignathus palatability was higher for the treatment bait than the control. This shows that applying pre-treatment presto and added active ingredient hexaflumuron for formulation of bait influences weight loss of the test sample. Based on the test result of the chemical properties, the solubility value of the extractive substance become smaller as the time used for presto is increased. This is because when the pressure treatment is given, hot water and steam have an effect so that the resin content in pine wood chips comes out. The longer time used for presto, the more resin is released. This is supported by Widyorini et al. [22] and Arinana et al. [7] that the application of heat treatment up to 100 °C for pine wood causes the resin content to decrease.

In addition, termite behaviors that prefer the most suitable type of food which contains a lot of cellulose, easy to bite and chew so that the weight loss value of pellet bait is higher than the control solid pine bait. Other factors that affect termite consumption are the environmental conditions, body size, and termite colony size [23]. The presto treatment with the difference in the length of time used for presto was not significantly different from each other, which means that the length of time used for presto did not affect the level of termite preference on bait.

Based on the result of testing for 21 days, in the test sample there were holes due to termite’s attack (figure 4). According to Arinana et al. [7] the higher level of termite’s attack on bait indicates termites most preferred bait wood. Visually, the control test sample has a mild level of damage compared to the
The pellet test sample, it can see from the termite’s attack marks which only ate the edge of the sample. The pellet test samples that were given pre-treatment presto with 0.25% active hexaflumuron had a higher level of damage than the control test sample, it can see the holes from the termites bite in the pellet bait that were clearly visible. This shows that given pre-treatment presto on bait that’s contains active hexaflumuron can increase termites attacks on the bait.

![Figure 4](image-url)

**Figure 4.** Condition of (a) control test sample, (b) pellets with pre-treatment presto for one hour, (c) pellets with pre-treatment presto for two hours, (d) pellets with pre-treatment presto for three hours, (e) pellets with pre-treatment presto for four hours, (f) pellets with pre-treatment presto for five hours after 21 days of feeding against subterranean termites *C. curvignathus*.

3.4. Termites mortality

The result showed that mortality in termites *C. curvignathus* consuming pellet bait with active ingredient hexaflumuron caused 100% mortality, while for the control mortality of termites was 71.56%. The details are presented in figure 5. After termites are given pellet bait with active ingredient hexaflumuron, the termites that consuming the bait experienced symptoms of death. Hexaflumuron is non-repellant so termites would continue to consume the bait and will affect the workings of enzyme on termites, especially on the work of the chitinase enzyme [24]. Hexaflumuron will inhibit the formation of termite chitin when termites molting so that the termite cuticle cannot form perfectly. As the result, the termites integument cannot perform its function perfectly as body protection. Then the termites will become dehydrated which causes death of the termites. Diba et al. [24] stated that no matter how much hexaflumuron was consumed by the termites, the toxic effect would not appear until the termites had molted and depended more on the period of exposure time. Su [25] also stated that hexaflumuron is a chitin synthesis inhibitors (CSI) that satisfy the three attributes required to eliminate termite colonies such as be slow acting, be non-repellent, and have dose-independent lethal time.

The result of the analysis of variance showed that pre-treatment factor for the pellet bait with active ingredients hexaflumuron had a significant effect on termite mortality *C. curvignathus*. Duncan test result showed that mortality of termites *C. curvignathus* in pellet bait with active ingredients
hexaflumuron and pre-treatment presto was not significantly different. The formulation of pellet bait with 0.25% active ingredients hexaflumuron and pre-treatment presto had a higher mortality than control bait and was able to eliminate termites *C. curvignathus*. This means that the bait formulation is able to function as the termite bait and has high efficacy against the mortality of subterranean termite *C. curvignathus*.

![Figure 5](image)

**Figure 5.** Mortality of subterranean termites *C. curvignathus* after 21 days of feeding.

3.5. **Chemical properties analysis**

Chemical components of pine wood with various treatment presented at Table 1. The result showed that the given pre-treatment presto for pine wood chips with different lengths of time (one, two, three, four, and five hours) did not have a significant effect on the chemical components of pine wood. The result of the measurement of the chemical component are presented in table 1. The degradation of wood cellulose components occurs at a temperature of 275-350 °C, hemicellulose in the temperature range of 180-350 °C, while lignin is degraded at a temperature of 250-500 °C [26]. According to Hadiyane [27] that temperature is very influential on changes in the chemical components of wood. The use of temperature in this study is estimated to be less than 200 °C so that the degradation of wood components has not occurred completely.

| Chemical components | Chemical component content (%) in treatment |
|---------------------|--------------------------------------------|
|                     | Control | Presto 1 hour | Presto 2 hours | Presto 3 hours | Presto 4 hours | Presto 5 hours |
| Holocellulose       | 72.82   | 75.84         | 76.72          | 78.27          | 76.25          | 75.65          |
| α-cellulose         | 54.21   | 55.20         | 54.88          | 59.05          | 55.41          | 61.21          |
| Lignin              | 19.24   | 22.23         | 21.62          | 21.61          | 22.42          | 21.47          |
| Ethanol-benzene solubility | 8.04   | 7.95          | 6.95           | 6.38           | 4.91           | 4.40           |

In testing the solubility of wood in ethanol-benzene, the highest solubility value was obtained in the control and the lowest in the tusam powder under pressure treatment for 5 hours. Extractive substances dissolved in organic solvents are resins, fats, fatty acids, oils, and tannins [28]. Giving pressure treatment will reduce the resin content in the wood, this is because the longer the heat treatment is given to the wood, the resin content in the wood will significantly reduce [7].
4. Conclusion
The pellet bait formulation with 0.25% active ingredient hexaflumuron with presto pre-treatment was able to eliminate termites $C.\ curvignatus$. Presto pre-treatment for pine wood chips can increase the palatability of termite bait. The weight loss of pellet bait with 0.25% active ingredients hexaflumuron (14.33%) was higher than solid pine wood (6.58%). Pre-treatment presto for one hour is the optimal treatment.

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