TERMITICIDAL ACTIVITY OF CHITOSAN ON PAPER

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Abstract

Termites are insects that can damage buildings, paper and plants. Termites are controlled by using termiticides (chemicals). Besides polluting the environment, termiticides may have harmful effects to organisms, including humans, and destroy metal. The use of chemicals will be environmentally and economically profitless. A joint effort to utilize biodegradable material will help to reduce the negative impacts of termiticides. One of the materials that can use to control termites is chitosan. The purpose of this study was to determine the termiticidal activity of chitosan on paper against termites. The termiticidal activity test followed method JIS K 1571 2004. The resistance of paper to termite damage was determined by calculating the percentage of weight loss and termite mortality rate. The following concentrations of chitosan in acetic acid were used: 0.5%, 1%, 1.5% and 2%. The test result showed that weight loss percentages were 20.49%, 16.37%, 15.77% and 10.80%, respectively. On the other hand, the weight loss percentage of paper without chitosan was 32.69%, which shows that termites do not favour chitosan used in the paper. The activity of termites was successfully inhibited when the concentration of chitosan was increased, and the percentage of paper weight loss decreased from 10.80% to 20.49%. Paper without chitosan had a termite mortality rate of 18.3%; The addition of chitosan increased termite mortality to 28.2%–30.4%. The percentage of weight loss decreased with increasing termite mortality. The termite mortality rate show that chitosan is nontoxic and potential as biotermiticide for paper.

Keywords: *chitosan, termite, percentage weight loss, mortality, paper.*

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1. Introduction

Termites are insects that can cause severe damage to buildings, houses, books and plants. Paper is a printed and packaging material of tangled cellulose fibres. Termites can damage the material containing cellulose, such as paper. Various termite control methods have been developed, ranging from control, feeding, preservation and others. Termite control employed to deal with their attacks generally uses chemicals (conventional termiticides). Some types of termiticides are toxic to termites, unstable in the open air and relatively unfriendly to the environment. Besides polluting the environment, it may have harmful to organisms, including humans, and destroy metal. The toxicity of termiticides is of particular concern. It is essential to develop environmentally friendly technologies to avoid the use of chemicals and to provide a safer alternative for humans and the environment. The use of chemicals will be environmentally and economically profitless, mainly because chemicals are nonbiodegradable material. A joint effort to utilize biodegradable material will help to reduce the negative impacts of synthetic termiticides. The topic of the development of new biodegradable materials has gained growing interest from researchers in recent years.

Chitosan is derivative that is commonly obtained by chemical deacetylation of chitin using highly concentrated sodium hydroxide (NaOH). Chitosan is increasingly used in industries due to its biodegradability, biocompatibility, nontoxicity and antimicrobial activity [1]. Chitosan can protect plants by killing pest insect pathogens and soil infectious diseases that attack plants [2, 3]. Chitosan reportedly possesses antifungal, antibacterial and antifeedant activities and is effective against insects and fungi [4, 5]. Chitosan has been found to be useful as insecticide within a concentration range of 5–11 mg/g, resulting in insect mortality range of 15%–100% [6, 7]. Chitosan is widely used to control termites and it has been used as a wood preservative. Treatment of chitosan applied at sufficient concentration effectively protected wood from termites [8, 9–11]. The materials used for paper must be nontoxic to humans and other organisms. Chitosan has been widely applied as a biomaterial in industries including food, textile, pesticide and medicine [12].

Chitosan can be produced from chitin through a process of deacetylation, by using a strongly alkaline solution at high temperature. The difference between chitosan and chitin is present in the acetyl group (-CH_3-CO) in the unit of the polymer, whereas chitosan contains a free amine group (-NH_2). Chitosan is nontoxic, biodegradable and cationic polyelectrolyte because it contains amino groups and hydroxyl groups, both primary and secondary, each of which is bound to primary and secondary carbon atoms. The presence of these functional groups means that chitosan has high chemical reactivity, with the ability to adsorb heavy metals, macromolecules, lipids and other compounds [13]. Chitosan has the capacity to adsorb dyes such as mono azo dye, methyl orange [14, 15] and carbon black pigment of printing ink in the deinking process [16]. Chitosan, which is used as a filler and sizing material, can increase the surface and mechanical strength of paper [17–19]. Chitosan can be used as a crosslinking agent in paper and thus increase the strength. Chitosan can increase mechanical (physical) properties of paper such as tensile strength, bursting index, modulus of elasticity, folding endurance and more resistant to moisture [20, 21]. Fibres in the paper interact via hydrogen bonds, and chitosan affects the distance between fibre crosslinking [22]. Chitosan used as an adhesive (surface sizing agent) in papermaking to improve paper surface characteristics [23, 24]. Chitosan has high activity in controlling termite attack on wood or plants. According to this feature, chitosan has termiticidal activity and it is potential to be used as a biotermiticide on paper. This research aimed to study the termiticidal activty of chitosan on paper.

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2. Materials and Methods

2.1. Material

The materials used in this experiment were shrimp shells, acetic acid (Merck), NaOH (Merck), HCl (Merck), Aqua Dest, waste paper (photocopier paper), filter paper and *Coptotermes curvignathus* Holmgren. That organism is the main termite species that has caused economic losses; it has very high population densities in Indonesia and other tropical countries. *C. curvignathus* is the most important pest species of wooden buildings and wooden products. Termites used in this research were obtained from termite colonies at the Bogor Agricultural University, Forestry Department, Indonesia. The instruments required and used for this research were a glass apparatus, analytical scale, hotplate stirrer, a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu), pH meter, oven, screen and jar.

2.2. Preparation of Chitosan

The chitosan was prepared by deacetylation of chitin from shrimp shells obtained from the fish market in Jakarta, Indonesia. There are three sequential steps to obtain chitosan: deproteinization, demineralization and deacetylation of chitin. Shrimp shells were washed with distilled water and dried at 35°C in an oven. For deproteinization, shrimp shells were treated with 4% NaOH solution (w/v) and boiled for 8 h. The shells were cooled and then washed with distilled water until it was a neutral pH. For demineralization, deproteinized shell were treated with 1 M hydrochloric acid (HCl) for 1 h to remove calcium carbonate ($CaCO_3$). The chitin was deacetylated with a 40% NaOH solution at 110°C for 10 h to yield chitosan. After deacetylation, the chitosan was drained and washed with distilled water until it was a neutral pH. Chitosan was dried at 70^0C for 4 h in the oven and was prepared for the characterization. The ash content was analysed and determined according to ASTM method D2866-94. The chemical properties of chitosan are given in Table 1.

Table 1. Chemical properties of chitosan.

Parameter	Value
Moisture content (%)	2.06
Ash content (%)	28.45
Degree of deacetylation (%)	72.58

2.3. Characterization of Chitosan

The isolated chitosan was characterized to determine its functional groups using FTIR in the range of 4000–400 cm^{-1}. The chitosan spectrum is presented in Fig. 1.

The band at 3476 cm^{-1} is due to stretching vibration of -OH groups, which overlaps with a stretching vibration of -NH groups. The band at 3387 cm^{-1} is a stretching vibration of -NH groups. The stretching vibration of CH of the polymer backbone occurs at 2952 cm^{-1}. The band at 1622 cm^{-1}–1673 cm^{-1} is due to the stretching vibrations of C-O (acetyl groups). The band at 1373 cm^{-1} is assigned to the stretching vibration of -NH groups. The band at 1154 cm^{-1} is due to stretching vibration of C=O groups. The other band at 1055 cm^{-1} is assigned to stretching vibration of C=O groups.

2.4. Preparation of Handsheet Paper

Fifty grams of photocopier waste paper was ripped into small pieces and immersed in 10 L deionized water for 2 h. Then, the paper was blended until it turned into pulp.

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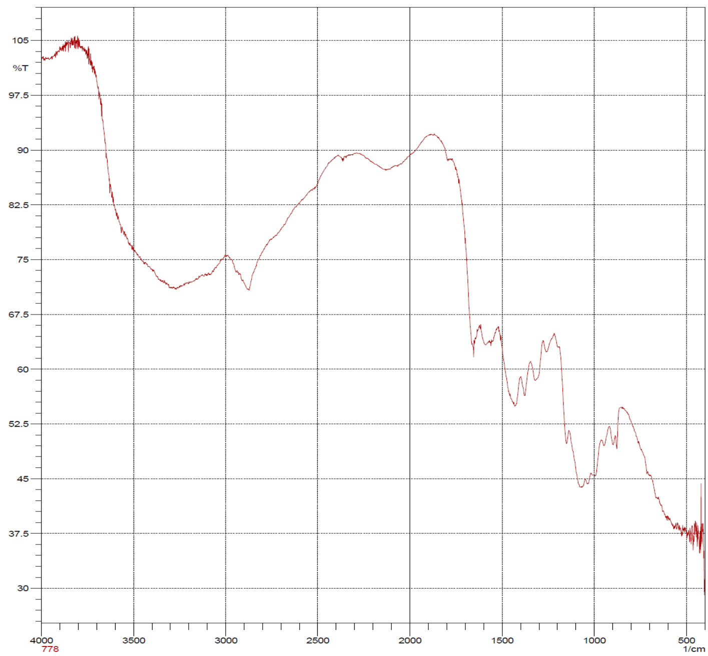

Figure 1. Fourier transform infrared spectrum of the isolated chitosan.

Chitosan solutions (0.5%, 1%, 1.5% or 2%) in 1% acetic acid were added to the pulp suspension. Handsheet paper was obtained by screening the pulp slurry with chitosan. The resulting handsheet paper was then dried in the air.

2.5. Termiticidal Activity Test

Termiticidal activity was evaluated to determine the effectiveness of chitosan against termites, following method JIS K 1571 2004. This method is suitable to determine the acceptance of treated paper as a food source to termites [25]. This research was conducted under two tests, each with three replications and ten 2 × 2 cm paper samples. The 10 samples were tested in a random order. The paper samples with chitosan were placed on a jar. One hundred and fifty *C. curvignathus* workers were added to each test jars. Test jars were maintained at 28 ± 2°C and 80% relative humidity for 4 weeks [26].

2.6. Calculations

A measure of termiticidal activity (the paper's resistance to termite damage) was conducted in a laboratory with controls by calculating percentages of weight loss and termite mortality rate after 4 weeks. The percentage of weight loss of the paper sample was calculated as the difference between the initial and final weight with respect to initial weight:

$$\text{Percentage weightloss (\%)} = \frac{(w1-w2)}{w1}\ x\ 100, \quad (1)$$

where w1 is the weight of the paper sample before the test (initial weight) and w2 is the weight of the paper sample after the test (final weight). The resistance class of the paper was determined according to the Indonesian Standard [27], as shown in Table 2.

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Table 2. Resistance classes against the subterranean termite *Coptotermes curvignathus* Holmgren (Indonesian Standard SNI 01.7207-2006).

Sample condition	Weight loss (%)	Resistance class
Very resistant	< 3.52	I
Resistant	3.52–7.50	II
Moderate	7.5–10.96	III
Poor	10.96–18.94	IV
Very poor	> 18.94	V

The termite mortality rate was determined using the following equation:

$$\text{Termite mortality rate (\%)} = \frac{(number\ \ of\ dead\ \ termite\ )}{150}\ x\ 100. \qquad (2)$$

3. Results and Discussion

3.1. Effect of Chitosan on Weight Loss Percentage of Paper

The result of the percentage of weight loss of paper are shown in Fig. 2. The weight loss percentage of untreated samples (control) was highest at 32.69%. The addition of chitosan decreased the weight loss percentage. The paper with 0.5% chitosan had a percentage weight loss of 20.49%, which according to Table 2, is categorized as class V (very poor). Paper with 1% or 1.5% chitosan was classified as class IV (poor). By contrast, paper with 2% chitosan showed greater resistance to termite attack and was classified as class III (moderate). The results demonstrated that paper sample treated with chitosan generally exhibited a lower percentage of weight loss compared to untreated sample. The percentage of weight loss significantly decreased with an increasing chitosan concentration ($p < 0.05$). Hence, termites do not favour the addition of chitosan in paper.

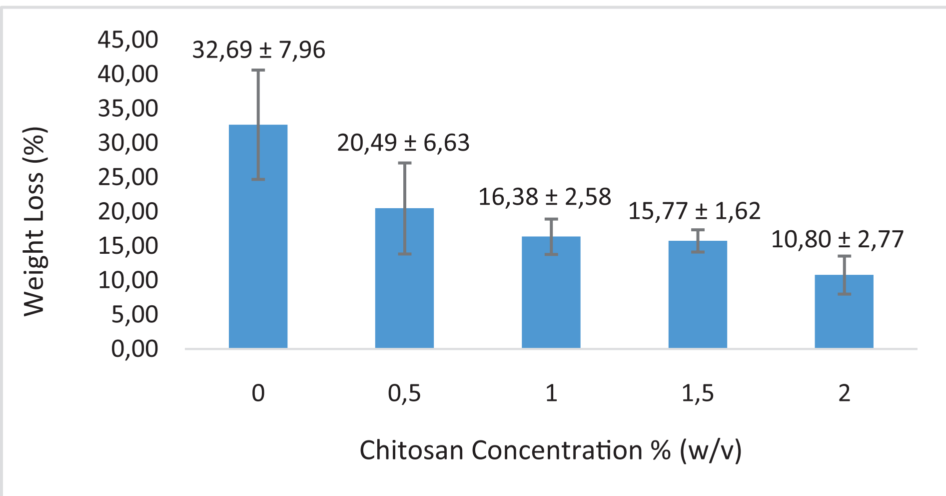

Figure 2. The effect of chitosan concentration of on the paper weight loss percentage. The values indicate the mean ± standard deviation.

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3.2. Effect of Chitosan on Termite Mortality

The termite mortality rate data are shown in Fig. 3. Dead termites had an abdomen that changed from brown to black and their bodies dried out. Chitosan increased the termite mortality compared with the control sample. There was no statistical difference in termite mortality rates among the chitosan concentrations ($p > 0.05$). Low termite mortality rate (< 80%) on paper with chitosan demonstrated that chitosan is nontoxic and suitable as a biotermicide on paper.

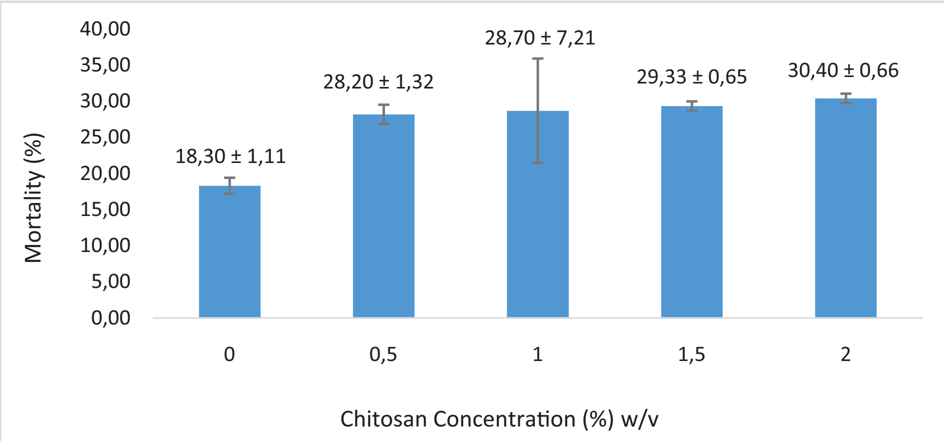

Figure 3. The effect of chitosan concentration on termite mortality rate. The values indicate the mean ± standard deviation.

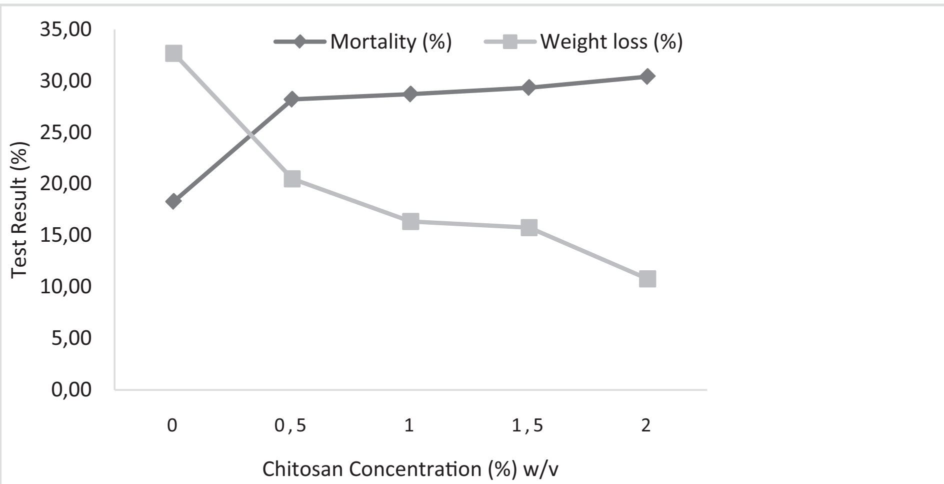

Figure 4. The relationship between weight lost percentage and termite mortality rate.

The relationship between percentage weight lost and termite mortality rate is shown in Fig. 4. The percentage of weight loss significantly decreased with an increasing termite mortality rate ($p < 0.05$). While the addition of chitosan in paper will not directly kill termites, it seems to act as a slow-acting termiticide. Chitosan can control termites by

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interfering with feeding, namely disturbing the ability of protozoa within the termite's digestive tract to digest food. Consequently, termites starve and slowly die. Termites that have been exposed to chitosan show early symptoms of behavioural changes, such as being less active in terms of movement, decreased eating activity and morphological changes occurs in terms of colour. Trophallaxis distributes the toxicant in baits throughout the colony [28]. This behaviour is how termites transfer and distribute food to others. Healthy termites can contract an infection with the toxin from chitosan in their shared food. Termite mortality, in paper samples without chitosan, probably occurred due to the inability of termites to adapt to new environmental conditions. In this case, the environment greatly influences the feeding activity of *C. curvignathus,* and because this species consumes a single food, there is no other food choice [29]. *C. curvignathus* that can survive will adjust their food orientation, while those that are unable to adapt will die. The mortality rate was not high (< 20% most likely due to lack of the concentration of chitosan in paper and low degree of chitosan deacetylation). In this study, the chitosan degree of deacetylation was 72%. Physical and biological properties of chitosan such as activity and reactivity are dependent on its molecular weight and degree of deacetylation [30, 31]. Hence, a higher degree of deacetylation may also affect the activity against termites on paper.

In a previous study, high termite mortality (> 94%) was caused by the exposure to wood treated with ≥ 2% chitosan solution; concentrations < 2% caused < 50% mortality [32]. For this study, a chitosan concentration that is too high will affect the mechanical properties of the paper. The increase of chitosan affects physical and mechanical properties, such as stiffness and bonding strength. This could be attributed to the increase in the stiffness of the film that formed over the paper fibres, and the decrease in mechanical properties [22]. A previous study showed that the addition of up to 1% chitosan improved the mechanical properties of paper. However, the rate of increase in strength properties was higher from 0% to 0.5% compared with 1.5% to 2% [33]. Those data indicate that an increase in chitosan concentration (> 1%) leads to a decrease in physical and mechanical properties such as stiffness and bonding strength. The use of low chitosan concentrations in papermaking is expected not to reduce the mechanical properties of paper.

The low termite mortality rate showed that chitosan is nontoxic, suitable for use in papermaking and harmless to humans. The fact that the percentage weight loss decreased with an increasing termite mortality rate indicates that chitosan has termiticidal activity against termites on paper. Chitosan has a potential as a biotermiticide and is an appropriate option to control and prevent termites replacing chemical termiticide. The study of the feeding rate and its mechanism as termite antifeedant require further research.

4. Conclusion

The conducted research demonstrated that the addition of chitosan to paper affects weight loss percentage and termite mortality rate. The percentage weight loss significantly decreased with the increasing chitosan concentration. Chitosan can act as an antifeedant in the paper, which termites dislike. The fact that the percentage weight loss decreased as termite mortality increased indicates that chitosan has termiticidal activity against termites on paper. Application of chitosan in paper is appropriate to be used as biotermiticides to control and prevent termites. This application will reduce or eliminate the use of chemical termiticides.

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