Retention and concentration of *Reticulitermes speratus* feeding-deterrent from fungus-decayed Japanese red pine extract

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Abstract. Wood-feeding termites and wood-decaying fungi share a niche and likely interact, impacting wood degradation processes. Prior research has primarily examined termite the preferences for decayed wood species, revealing instances of termite feeding deterrence in response to fungal decay products. The compound that caused the deterrence effect was extracted and studies regarding the suspected compound and its toxicity has been previously conducted. In this study, further observation of the extract retention and concentrations were asessed against *Reticulitermes speratus.* Result indicated that the decayed wood extract retains its deterrent activity for up to four days post-application, with a peak effect observed within the initial hours. Furthermore, the concentration of the extract correlates with both feeding deterrence and termite mortality, suggesting a concentration-dependent relationship. Results show a significant decrease in feeding and increase in mortality with longer exposure durations, indicating sustained deterrent activity for up to four days. Higher extract volumes correlate with reduced feeding and increased mortality, supporting concentration-dependent deterrence. Highest mortality was observed after 24 hours of exposure, suggesting continued toxicity despite diminished deterrence.

1 Introduction

Reticulitermes speratus is one of the subterranean termite species that are economically important [1]. During the forganing process, termite forms tunnels that can lead to structural damage or collapse in wooden structure [2]. Methods to minimize the possible damage caused by Reticulitermes activities has been developed, one of it is through the study of negative interaction of termite and wood-decay fungus [3]. Both wood-feeding termites and wood-decaying fungi degrade and digest wood for energy source therefore naturallym crates an interaction between the two organisms. Studies on the interactions between termites and

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wood-decaying fungi have mainly focused on investigating the preference of termites to different species of decayed wood. Several reports and studies have shown the termite feeding deterrence phenomenon in relation to wood decayed by fungi. Amburgey and Beal [3] showed that white rot-decayed southern pine stakes were not a preferred food substance for Reticulitermes flavipes (Kolar) and stakes of three pine sapwoods infected with a white rot fungus were not damaged by termites. Grace et al. [4] reported that secondary metabolites released by the brown-rot fungus *Gloeophyllum trabeum* deterred *Coptotermes formosanus* feeding on filter paper.

Fungi and insects use chemosensory systems to communicate and interact with the environment. Sharing of living space by these two organisms has led to the evolution of a wide range of beneficial as well as adverse interactions [5]. In termite-infested areas, termites tend to avoid feeding on the decayed parts of wood, suggesting that they are prevented from approaching or feeding on wood by decay-related chemical substances secreted by the fungi. In higher concentrations, these substances might act as chemosensory signals for excessive degradation of wood, hence leads to the absence of nutritional content. The secretion of feeding deterrents is also beneficial to fungi because they eliminate other competitors for the same cellulosic resource [4].

Based on the previous studies, it is suggested that chemical cues related with the signalling process were concentration-dependent and can be retained in the wood in certain time range. In this study, the feeding deterrence phenomenon of decayed Japanese Red Pine (Akamatsu) stakes from the field in previous studies [6, 7] were tested and evaluated again under laboratory conditions. The extracts of decayed field stakes and laboratory-decayed Akamatsu were applied to paper disks to investigate the effect of extract concentration and retention towards termite mortality and feeding.

2 Materials and Methods

2.2 Feeding test of Decayed Akamatsu Extract

Paper disks (8 mm, ADVANTEC TOYO, Tokyo, Japan) were treated with the extract obtained from the laboratory-decayed wood. Untreated paper disks serve as control. Decayed wood extract was prepared in accordance with previous studies in [6] and [7]. The volume of decayed-wood extract given were: 50, 100, 200, and 300µL. The no-choice feeding tests were conducted with the paper disks consisted of 50 Reticulitermes speratus (Kolble) workers that were introduced in each. A single colony from Oarai City, Ibaraki Prefecture, was used in this test. The no-choice feeding chamber were replicated three times and and kept inside a 27 °C incubator for 10 days. After the test, the paper disks were removed, cleared of any attached debris, oven-dried at 60 °C for 48 h, and weighed. The mass losses of the disks were calculated from the difference in dry weight before and after exposure.

2.3 Retention of feeding deterrent in paper disk

Paper disks were oven dried at 60℃ for 48 hours and weighed. The paper disks were treated 100µl of decayed wood extract, placed in a clean glass petri dish, and kept in an ambient temperature for 0, 24, 36, 48, 60, and 72 hours. Paper disks treated with 100µL of n-hexane prepared with the same manner as decayed wood extract treatment untreated paper disks serves as control. Three replications were conducted for each treatment. Test chamber were kept inside a 27°C incubator for 4 days with 60-70% relative humidity. Test chambers lid were opened slightly to expose test containers with fresh air. After the test period ended, paper disks were removed from test containers, dried at 60 °C for 48 hours, cleaned from

debris or termite excrements, and weighed to determine mass loss from termite feeding. Survived termites are counted to calculate the mortality caused by feeding. Flow chart of the experimental procedure can be seen in Fig 1.

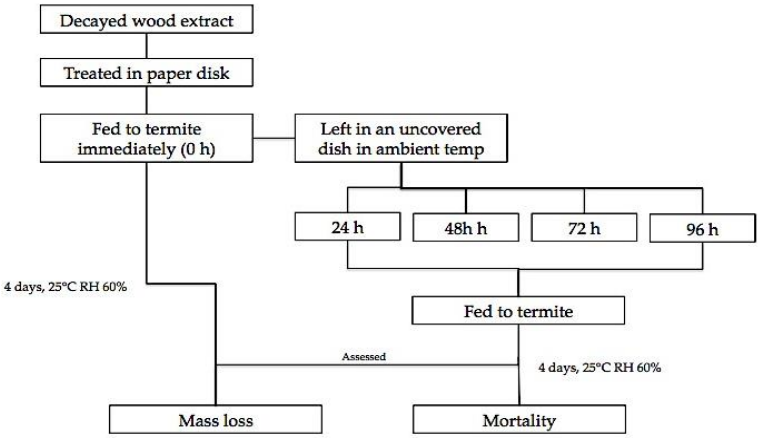

Fig. 1. Flow chart of retention of feeding deterrent in paper disk test.

Termite mortality percentage is counted by:

$$\text{Mortality (\%)} = (\text{Number of dead termite})/(\text{Number of termite on the beginning of the test}) \times 100$$

Termite feeding (Mass loss) percentage is counted by:

$$\text{Mass loss (\%)} = (\text{Initial mass of paper disk - mass after termite consumption})/(\text{Initial mass of paper disk}) \times 100$$

3 Results

3.1 Mass loss of disk treated decayed wood extract kept in various duration

Kruskal-Wallis H test shows that both of the termite feeding as represented by the mass loss of treated paper disks was significantly influenced by extracts' exposure duration to open air. Termite feeding of paper disks treated with decayed wood extract that have been left in open air for 96, 72, 48, 24, and 0 hours were lower than that of control ($p = 0.03$) this result suggest that even after 96 hours post application, decayed wood extract retained its bioactivity, in this case as feeding deterrent. Differences were also detected between control and paper disks left for 0 hours after extract application ($p = 0.036$). This may indicate that the deterrent effect was more potent in fresh samples. Ties can be seen between paper disks that have been left for 96, 72, and 48 hours post extract application, which may suggest that feeding deterrent compound has partly removed but still enough to inhibit termites' feeding (Fig. 2).

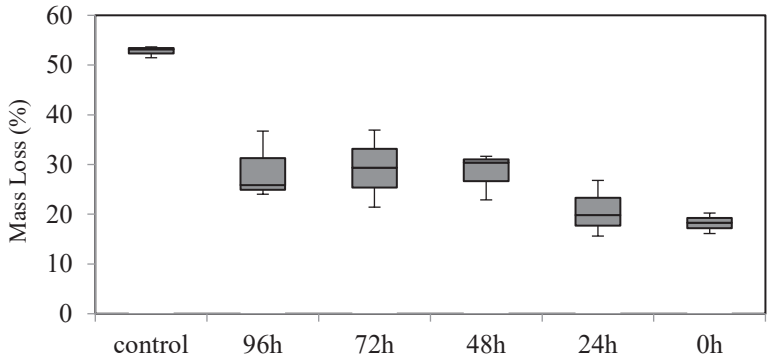

Fig. 2. Mass loss of paper disks kept for 96, 72, 48, 24, and 0 hours under ambient temperature prior to no-choice feeding test.

3.2 Mortality of termite fed with disks treated with decayed wood extract

Similar result is also obtained in the case of termite mortality (Fig. 3). There are significant differences between treated paper disks than that of control/untreated paper disk ($p = 0.01$). The highest mortality was caused by the feeding of treated paper disks that have been left in room temperature for 24 hours instead of 0 hour. This may suggest that compounds that related to deterrence and toxicity were not the same. The compound related to feeding deterrent activity may have been reduced after 24 hours but the compound related to toxicity is still apparent after the same period of time. In pairwise comparison, there are no strong correlation in the term of mortality between treated paper disks that has been exposed to open air for 72 and 96 hours than that of untreated.

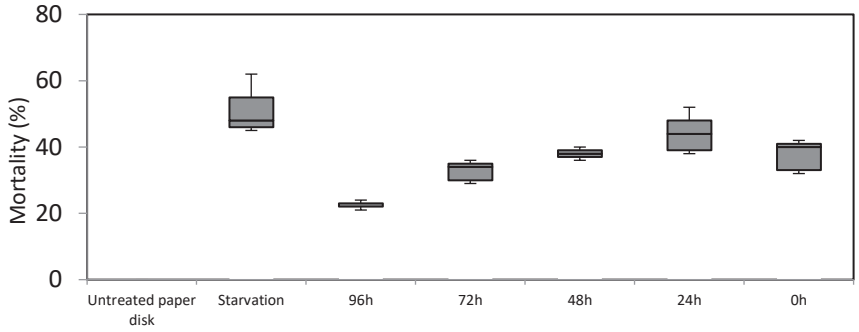

Fig. 3. Termite mortality due to the consumption of paper disks kept for 96, 72, 48, 24, and 0 hours under ambient temperature prior to no-choice feeding test.

3.3 Mass loss of disk treated decayed wood extract in various concentration

Differences were detected in feeding of paper disk various extract volume and control by Kruskal-Wallis H test ($p = 0.031$). Mean rank of the feeding test shows termite consumption of decayed-wood extract treated disks decreased as the volume treatment increases. Pairwise comparison displayed a significant difference between paper disks treated with 300µl of extract and control ($p = 0.043$) as shown in Fig. 4.

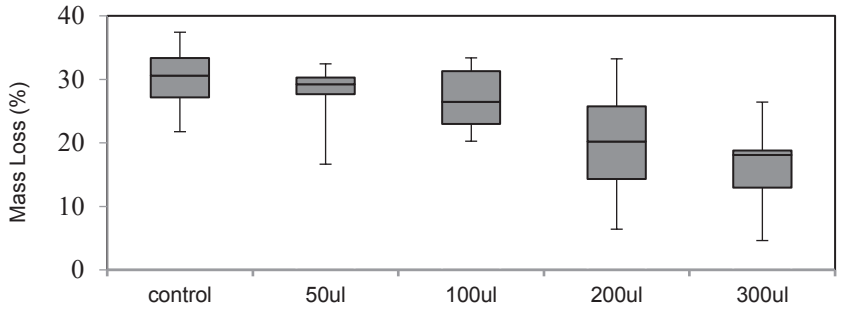

Fig. 4. Mass loss from feeding after various volume treatment in no-choice feeding test.

3.4 Mortality of termite fed with disks treated with decayed wood extract in various concentration

In the case of mortality, differences between treatments were also detected ($p = 0.008$) although in pairwise comparison, there were no notable differences between paper disks treated with lower volumes of extract (50 and 100µl). The concentration of toxic compound also increases with the treatment, shown by highest mortality was caused by 300µl treatment followed by 200µl (Fig. 5).

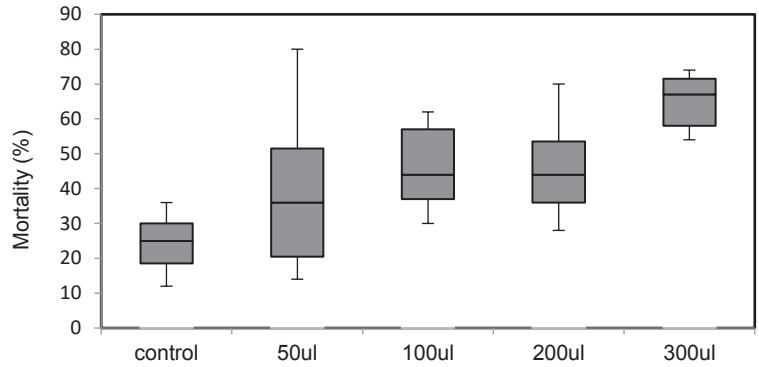

Fig. 5. Termite mortality percentage after feeding of paper disk treated with various volume in no-choice feeding test.

4 Discussion

Termites rely heavily on its chemosensory for feeding. Extract in high volume will evaporate and saturate termites surrounding and antenna longer, this may interfere the food recognition process and may result deterrency in termite feeding. Furthermore, high concentration of extract may turn compounds that acts as feeding stimulants into deterrent or even repellant. Raina [4] reported paper disks treated with 20ng/cm2 of hydroquinone is a termite repellant, but on the concentration of 2ng/cm2, hydroquinone acts as feeding stimulant.

Based on the result shown in Fig 4 and Fig 5, there was a linear relationship between concentration is mass loss and mortality. Interestingly, although the feeding was relatively low, termite mortality was not induced as well. This suggests that the extract from decayed wood served as feeding deterrent but not toxic towards termites. This clarifies the production

of the compounds that mediates *F. radiculosa* and termite was due to antagonistic relationship competition and possibly only occurred in a restricted time range. Relationship between fungi and termite is often ephemeral [9]. The deterrent compound released by *F. radiculosa* in the decayed wood may signal that the wood is unpalatable (e.g., heavily degraded) or nutritionally inadequate for termite consumption.

Low termite feeding as indicated in Fig. 2 and Fig. 4 may indicate the different amount of feeding deterrent present in the paper disks. Insects' first initiation of feeding is the detection of phagostimulant and deterrent. If the deterrent content is higher than the stimulant, insect will not feed on the food any further [10]. which indicates that the feeding deterrent is concentration dependent

5 Conclusions

Reticulitermes speratus feeding were deterred in paper disks treated with decayed-wood extract. Various retention and concentration were examined and showed that the extract can retain its feeding deterrence up until 96 hours, but the activity is higher in the early hour of application. After 72 hours, the lethal effect of decayed wood decreases but the effect is still apparent up until 96 hours. The feeding deterrent and toxicity ascends with the increased volume application which suggest that the compound is concentration dependent.

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