# Characterization of Termite Feeding Deterrents from *Fibroporia radiculosa* (Peck) Parmasto

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## Abstract

This study was conducted to clarify characteristics of the termite feeding deterrent produced by brown-rot fungi *Fibroporia radiculosa* (Peck) Parmasto. From previous research it is known that wood blocks decayed by *F. radiculosa* and its *n*-hexane extract are not preferred by *Reticulitermes speratus* (Kolbe). It is suggested that there are compounds that can inhibit termite feeding in extracts of decayed wood blocks. Compounds were extracted from growth media using *n*-hexane and subjected to a series of no-choice feeding tests. Various no-choice feeding test results showed that paper disks immersed with the *n*-hexane extract of *Fibroporia radiculosa* were less preferred than not-treated which indicates that the deterrent compound exists in the purified extract. The deterrence of the extract was concentration dependent. In order to get more information about the compound(s), analysis of crude and purified extract were done using Gas Chromatography – Mass Spectometry. It is assumed that this compound is produced by the fungus itself and displays between 30 to 45 minutes retention time.

**Keywords:** Feeding deterrent, *Fibroporia radiculosa*, subterranean termite.

## Introduction

Nishizawa's [i] research displayed *F. radiculosa* decayed wood blocks are avoided by *Reticulitermes speratus* (Kolbe) and the *n*-hexane extract treated filter paper was less preferred than the controls in a no-choice feeding test. It is suggested that this fungus also produces termite a feeding deterrent compound that is prospectus for further investigation. Beside its crucial function during wood degradation, there is also evidence that fungal extracellular emissions' are potential termite feeding deterrents. Jones *et al*.[ii] showed that siderophore treated paper was less consumed compared to not-treated paper in a two-choice feeding assay. Grace *et al*.[iii] also demonstrated the same result, where not-treated paper was completely consumed after 3 days, while the siderophore-treated paper had only slightly roughened edges, indicating very minor feeding. Conversely, the presence of a high concentration of fungal siderophore could act as a chemosensory cue to termites that the substrate is excessively degraded and therefore nutritionally inadequate. The feeding deterrent compound could also be produced as a response to termite and fungus competition. Because both the termite and decay fungi compete for the same cellulosic source, the compound is

released to benefit the fungus (Grace et al., [10,iv]). This suggests a fascinating prospect for wood protection against termite attack by stimulation of this behavioral response. In this research, we try to clarify the production, structure, and *Reticulitermes speratus* (Kolbe) response to fungal microbial volatile organic compounds (MVOC) from brown rot fungi *Fibroporia radiculosa* (Peck) Parmasto.

## Materials and Methods

### Media Preparation of *F. radiculosa* and decayed wood under the laboratory condition

Four types of medium were prepared in order to study the characteristics and optimum conditions for feeding deterrent production. The media were PDA (Potato Dextrose Agar), 1/3-diluted PDA, PDA for decayed wood, and wood and agar media. Each bottle was inoculated with the fungus *F.radiculosa* and incubated at 27°C for 10 days.

### Extraction from Growth Medium and Decayed Wood Blocks

*Fibroporia radiculosa* growth medium was ground using a glass stirrer. About 100 gram of medium was extracted with 100 ml of *n*-hexane at room temperature for 24 hours. 30 grams of decayed wood blocks were soaked with 300ml *n*-hexane. Extracts were concentrated with a rotary evaporator into 1 ml.

### Thin Layer Chromatography (TLC)

Extracts were analyzed by TLC plate (Merck Silica 60 RP-18F 254s) and developed with an Ethyl Acetate and Ethanol (1:1) mixture. Materials were detected by Ultra Violet light. Crude samples were purified using glass TLC plates (Merck PLC Silica Gel 60 $F_{254}$, 2 mm) developed with the Ethyl Acetate and Ethanol (1:1) mixture. The separated materials were collected using a micro spoon spatula into a 10 ml screw capped vials and soaked with 1 ml *n-hexane*. After 24 hours the extract was separated using a vacuum filter to obtain purified extract. This extract was stored in a screwed top vial in a refrigerated box until needed for further analysis.

### Gas Chromatography Mass Spectrometry (GC/MS)

A JEOL MS-600 gas chromatograph/mass spectrometer with Agilent Technology's DB-5MS Phenyl Arylene polymer capillary column (24m x 0.25 mm) coupled to the instrument, and helium as carrier gas were employed. Electron Ionization (EI) spectra was 70eV and the ion source

temperature was 200ºC, samples were injected using the split less mode. Oven temperature was kept at 50°C for 5 minutes and increased to 200°C, kept for 10 minutes then increased to 300°C for 2 minutes.

**Test Termite**

Colonies of Yamato termites (*Reticulitermes speratus* (Kolbe)) were collected from Oarai, Ibaraki Prefecture in September 2012. Termites are maintained in plastic container boxes containing wood stakes in the laboratory. The container was opened twice a week to ensure good aeration and watered regularly to maintain a humid condition.

**Extract's Bioassay (No-choice Feeding Test)**

Paper disks (8 mm diameter, thick, ADVANTEC, Tokyo) were oven dried at 60ºC and weighed. The paper disks were immersed with the concentrated extract and air dried at room temperature to remove solvent. Extract and solvent treatments were based on a volume basis; 10µl of solvent, 5µl of all crude extracts (except for decayed wood block extract which is 1µl). Meanwhile, after GC-MS analysis, treatments were based on the intensity of crude decayed wood extract as the base value, which was: 1µl of crude decayed wood block extract, 10µl of purified decayed wood block extract, 5µl of crude PDA extract and 10µl of purified PDA extract.

Fifty workers of *Reticulitermes speratus* were introduced into each cup. The chamber was maintained in an incubator at 25ºC for 10 consecutive days. After exposure, the paper was taken out and cleaned of any debris, oven dried at 60ºC for 48 hours and weighed. Percent weight loss of the paper disks were calculated from the difference of dry weight before and after exposure. Three replications were conducted per group.

## Results and Discussion

Extracts from several media were tested to determine which media can produce the highest amount of the feeding deterrent compound(s). Figure 3 shows high inhibition of paper disks immersed with an extract from fungi grown in Potato Dextrose Agar (PDA) medium. The second highest compound is Diluted PDA, but due to small nutrition content, this medium cannot effectively support fungal growth.

GC-MS analysis was done to compare 2 samples, extract from decayed wood blocks and PDA medium. This sample selection was to assure that the feeding deterrent compounds were secreted by *Fibroporia radiculosa* regardless of the growth media; wood blocks as natural nutrition, PDA as an artificial or the same with a small-ranged nutrition source. From the chromatogram results it is

obvious that the crude extract displayed a higher intensity than the purified one even though the amount of crude sample injected into the instrument (0.5 µl) was smaller than the purified extract (2µl). This indicates that the purified samples are diluted. The decayed wood block showed 11 times higher intensity than purified extract. Furthermore, chromatogram results show that there are some removed and accumulated compounds between 0 to 25 minute retention time and a simpler peak at 30 to 50 minute retention time (Figure 1 and 2) in both decayed wood and PDA extracts.

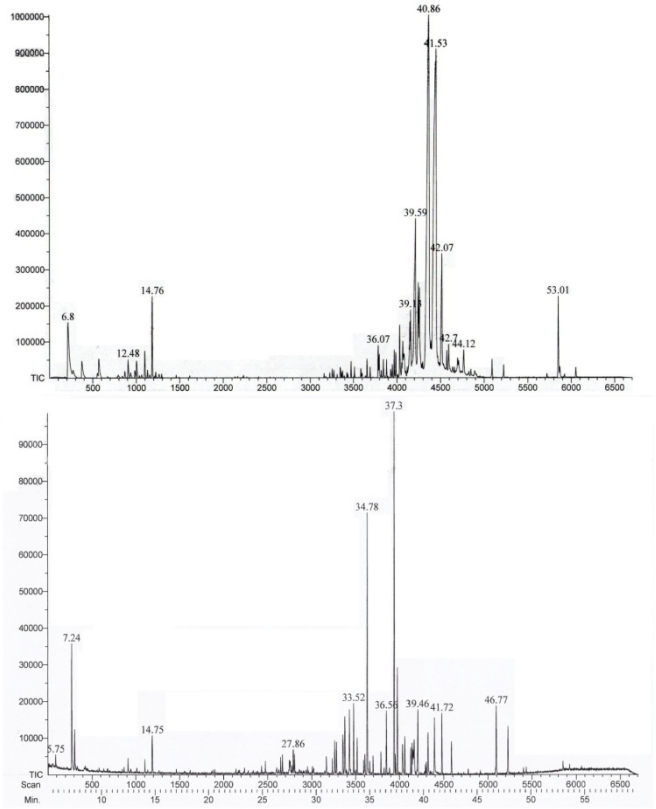


Figure 1. GC-MS Chromatogram of Crude (top) and Pure Extracts from Decayed Wood Blocks

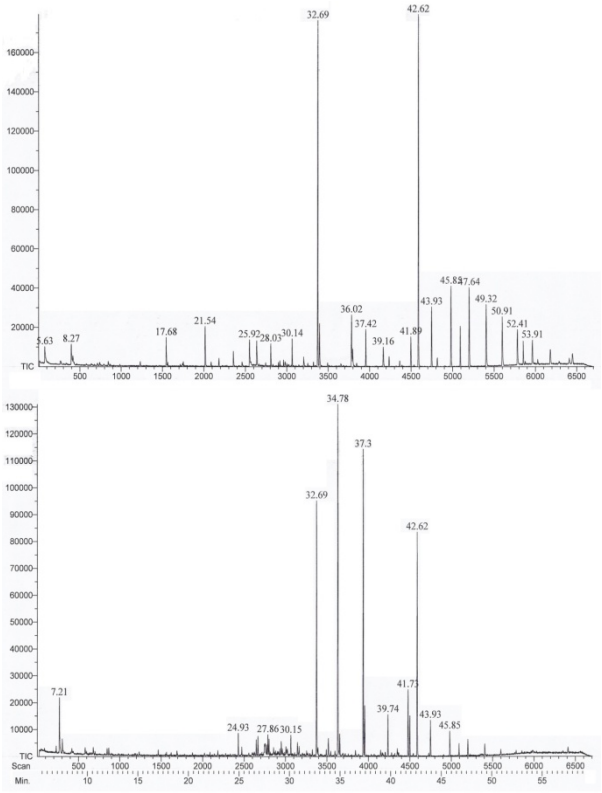


Figure 2. GC-MS Chromatogram of Crude (top) (bottom) and Pure (bottom) Extract from PDA Extract

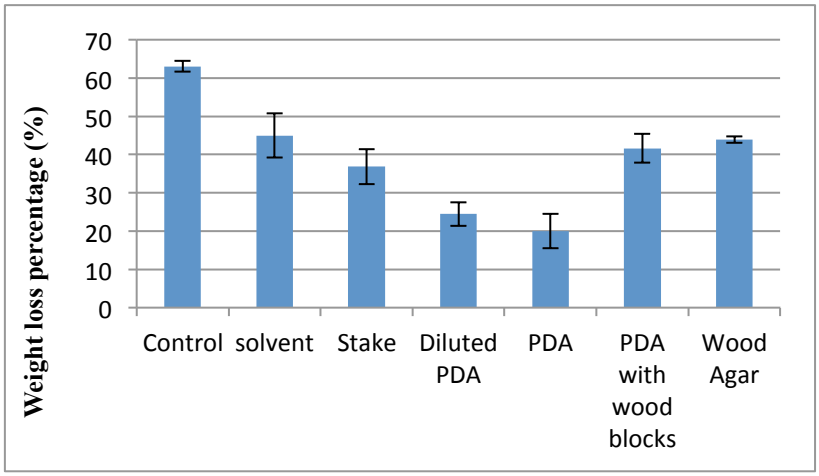


Figure 3. No-choice Feeding Test Result of Extract from Various Media

To confirm that the feeding deterrent was not removed during purification all of the extracts used for GC-MS analysis were subjected to bioassay. Bioassay using a no-choice feeding test showed that the purified extract still includes the termite feeding deterrent. Purified extract showed a smaller percent weight loss on filter paper, 52.91% for wood block extract and 47.64 % for PDA extract, which indicates that the compound still exists in the purified extract. Although the result of the no-choice feeding test showed lower percent weight loss in all extracts, the variation exhibited in this feeding test is quite high possibly due to uneven termite condition and unknown extract concentration. Grace[16] suggests that this compound is concentration dependent; therefore it is crucial to know the compound's concentration in order to get uniform results in a feeding test.

Beside the unknown concentration, the bioassay test could be less efficient because MVOCs can easily transform into gaseous phase at temperatures exceeding 20°C (Morath[10]). The feeding tests were done at 27°C according to the Nishizawa[13] procedure. The high temperature is likely to cause evaporation of any volatile compounds, resulting in a deviating bioassay result. It is important to keep the bioassay test optimum temperature in order to preserve the compound's efficiency.

Some of the compounds obtained from GC-MS analysis are known to have certain effects on insect behavior. 1-*octoen*-3-*ol* released by wood-rot fungi *Cariolus versicolor* fruiting bodies can influence Ciid bettles *Octotemus glabricus* and *Cis boleti* ovulating behavior (Guevara[v]). Steiner[vi] also showed that the same compound from *Aspergillus sydowii* and *Aspergillus versicolor* effects *Lariophagus distinguendus*, (parasitoid beetle larvae developing in stored grain) females host habitat assessment, in order to avoid negative fitness consequences due to secondary mold infestation of host patches. Napthalene, according to Daisy[vii] can be exclusively produced by *Muscodor vitigenus* grown on a liana in the understory of rainforests in the Peruvian Amazon, which can effectively repel the adult stage of the stem sawfly *Cephus cinctus*.

The presences of these volatiles in Fibroporia radiculosa are important to understanding the biology of the fungus and its relationship or response on competitor feeding behavior. Chemical

ecologists have elucidated the role of many fungal volatiles as attractant or deterrent to insect and other invertebrates (Morath[10]). Therefore the prospects are good that such an extract can be applied in the wood or paper preservation sector as a wood-feeding insect repellent. Further investigation is required in order to develop an applicable nature-based wood preservative.

## Conclusion

Based upon all of the observations in this study, the following conclusions may be made:

1. An extract was produced by *Fibroporia radiculosa* without the presence of wood blocks as demonstrated by the low feeding on paper disks immersed in extracted growth medium.
2. Based on GC-MS analysis, it is known that the extract consists of carboxylic acid, alkanes, terpenoid, and double bond hydrocarbons.
3. Based on percent weight loss in a feeding test, it is assumed that the extract can be produced effectively in Potato Dextrose Agar (PDA) medium.

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