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A study of a termite chemical defense fluid compound of *Macrotermes carbonarius*

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Abstract: Termite caste consists of reproductive individuals, worker, and soldier. Soldiers defend their colony by physical and chemical means. For *Macrotermes carbonarius*, they use mandibles as their physical defense and defense fluid for chemical defense. Their defense fluid plays a vital role to defend their caste from any harm from another insect such as ants. This can be related to the fact that *M. carbonarius* is an urban pest that has many threats to their colony. However, less study had been focused on their defense fluid composition and how the compounds function. This study was carried out to determine the compound present in the defense fluid of *M. carbonarius*. The methods used in this study were thin layer chromatography (TLC) and gas chromatography mass spectrometry (GCMS). TLC detection indicated presence of three unknown compounds and one important compound. GCMS analysis had confirmed the compound as lauric acid methyl ester that was obtained from the TLC. This information would contribute to fill up the research gap and assist in better understanding the compound composition found in *M. carbonarius* defense fluid to protect their colony, an update on the defense fluid research since 1979 by Prestwich.

1. Introduction

Termites are commonly known as an economic pest. However, fewer people have knowledge about termite ecology. It lives in a caste system that consists of reproductive individuals, worker, and soldier [1]. Termite workers help in maintaining nests while soldiers help to defend the colony [2]. Soldiers defend their colony by using mechanical and chemical defense mechanism. Mechanical means employing mandibular snapping and chemical means using secretion from the salivary gland, frontal, and cibarial gland [3]. The interesting fact about chemical secretion for the defense of termite is that consists of various types of fluid such as greases, irritants (majority in biting termites) and glue [3]. The chemical fluids are known as defense fluid, secreted by *Macrotermes carbonarius* to defend their colony from threats such as ants attack. In addition, *M. carbonarius* defense is resilient with dimorphism soldier, major and minor soldier that commonly exist in *Macrotermes* genus. Major and minor soldiers both have the ability to secrete the defense fluid. Furthermore, since 1977, it had been found that the volume of the secretion by the major soldier is higher than minor soldiering about five hundred times in *Macrotermes subhayalinus* [4].

The defense fluid content is yet to be fully known and discovered. Termite defense fluid composition study started early in 1986 by Prestwich and lately by Chuah in 2010. From the 2010 study, it had been



found that *M. carbonarius* defense fluid contains quinones [5]. Naphthoquinones and other quinonoid compounds characterize as a major natural product class with various biological activities including insecticidal, antifeedant and antifungal properties [6-11]. With this property, it helps *M. carbonarius* to defend themselves from the enemy. However, other compounds responsible for chemical defense mechanism had not been discovered. Thus, this study had been carried out to investigate the compound in the defense fluid of *M. carbonarius*. The information could reveal more understanding of the mechanism of termite defense fluid that functions to protect their colony.

2. Methodology

2.1. Collection and Identification

The soldier termites were collected in Agropark of Universiti Malaysia Kelantan Jeli Campus (N 05°44'45.7", E 101°52'02.5" at 70 meter elevation) and stored in a plastic aquarium together with its origin soil and litters. The species were brought back to the laboratory and maintained at room temperature with relative humidity >50% in dark condition. The morphology identification of the termites was done according to Tho [12].

2.2. Extraction of Defense Fluid

This method was adapted according to Green [13] with modifications. The termites were knocked down by wetting the cotton with 80% ethanol. Termites were rinsed using distilled water to remove dirt and air dried. Then, the termites were weight, homogenized and placed into 80% methanol in a falcon tube. The tube was shaken for 10 minutes to make sure all defense fluid was extracted. After 24 hours, the termites were removed from the extract by filtering firstly using muslin cloth, then filter paper. Solvent removal was done by using rotary evaporator. The crude extract was weighed and kept at -20°C until further use.

2.3. Thin Layer Chromatography (TLC)

The extract was fractioned using thin layer chromatography as the preliminary steps for GC/MS. It was done in duplicates. Silica gel F254 Merck was used as a stationary phase. Meanwhile, the plate was developed in chloroform/methanol/water (65:30:5, v/v/v) until 4 cm. Then, the plate was run again in hexane/diethyl-ether/acetic acid (80:20:1.5). The fractioned component was visualized by spraying sulphuric acid in ethanol and charred.

2.4. GCMS

The extract was analyzed by GC on a capillary column (HP-5; 0.25 mm 3 30 m; J&W Scientific). The temperature program was 70°C for 2 min, 20°C/min to 260°C, and hold for 20 min. The solvent delay was 2 min with Helium as the carrier gas.

3. Result and Discussion

The thin layer chromatography shows four compounds separations as in table 1. Three compounds are still unknown (band 1, 2, and 3) and one compound has been identified as lauric acid methyl ester as can be seen in Figure 1 when compared to standard purchased by Merck at $R_f = 0.7$ on band 4.



Figure 1. Four compounds had been found. Three compounds are unknown and one compound had been identified as lauric acid methyl ester.

Table 1. The retention factor of *M. carbonarius* defense fluid for two replicates. The TLC result show four compounds with different retention time.

Band	Rf	Rf	Mean Rf
1	0.12	0.10	0.11
2	0.26	0.28	0.27
3	0.40	0.44	0.42
4	0.70	0.70	0.70

Further confirmation had been done by using GCMS at 7.99 retention time in Figure 2. The content of lauric acid methyl ester can be understood when compared to the previous study of lauric acid effect on another insect. This is supported by Mohamad [14] who made a pesticide formulation against aphid, *Aphis gossypii* that consist 50% of lauric acid. The previous study shows lauric acid-based pesticide formulation has important effect as pest control. This could be related to the presence of lauric acid in *M. carbonarius* defense fluid. The presence of the compound may help the toxicity against another insect which aid in the chemical defense mechanism. In contrast, the study by Mohamad was confined to show the effect of lauric acid only, compared to the compound found in this study which is lauric acid methyl ester. Nevertheless, few literature reviews had been published on lauric acid methyl ester effect and mechanism. However, the compound with methyl ester has toxicity at level III which has effect of acute dermal on rabbit and level IV that has an acute oral effect on rat.

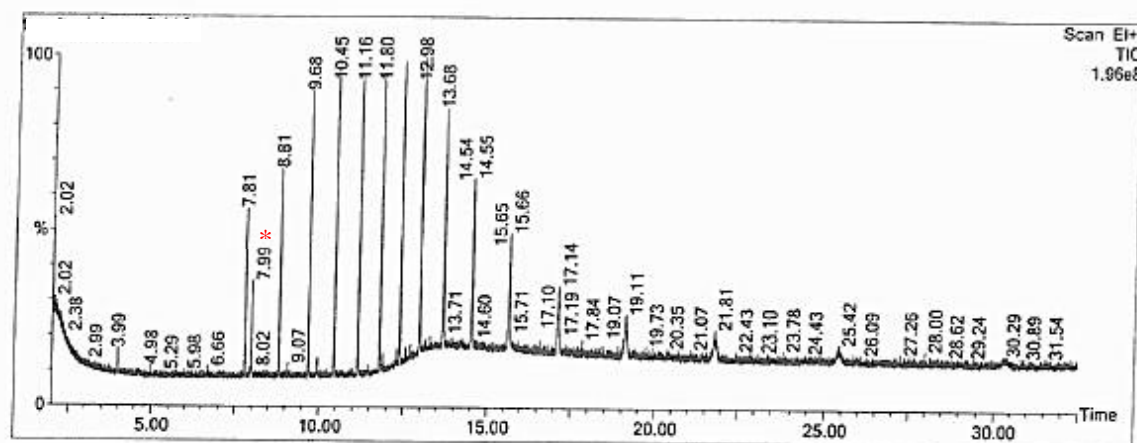


Figure 2. The gas chromatogram of *M. carbonarius* defense fluid with lauric acid methyl ester marked

(*) on the chromatogram.

The presence of lauric acid methyl ester had been discovered as one of the important compound other than quinones. More study is needed for complete understanding of the compound as this is an initial study for defense fluid composition of *Macrotermes carbonarius*. The information could help to fill the gap on defense fluid produced by this species and contribute in the understanding of defense fluid mechanism and how they function.

4. References

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