Dimethyldecanal Analysis on Body Extract and Head Space Sampling Method

N Subekti¹*, Saputri R¹, Partaya¹, T Kartika²

¹ Department of Biology, Faculty of Mathematic and Natural Science, Semarang State University.
² Research Center for Biomaterial, Indonesian Institute of Science.

*Corresponding author: nikensubekti@gmail.com

Abstract. Tribolium castaneum is warehouse pest for flour commodity. The usage of chemical pesticide to control Tribolium castaneum is harmful to humans and environment. One of effective and eco-friendly method is utilization of dimethyldecanal specific pheromone which act as attractant that affect both sex. The purpose of this research is to analyze the influence of dimethyldecanal towards flour beetle behaviour respons. This research conducted by sample preparation, dimethyldecanal extraction, like dislike assay, and GC-MS analysis. Dimethyldecanal extract was obtained using body extract and head space sampling methods was then tested for its effectiveness in influencing the behavior of Tribolium castaneum using like dislike test and analyzed using GC-MS. Like dislike test based on One Way Anova analysis showed that there was significant difference between dimethyldecanal extract from body extract and head space sampling in influencing the behavior of Tribolium castaneum (P = 0.001; P <0.05). Based on GC-MS analysis, dimethyldecanal compounds can only be detected in head space sampling extract. Like dislikes test result and GC-MS analysis showed that dimethyldecanal can be extracted using head space sampling method but cannot be extracted using body extract method.

1. Introduction

Many pests of stored products belong to the order Coleoptera and one of the most destructive secondary insect pests of durable stored products is the red flour beetle, Tribolium castaneum (Herbst) [1]. This species can be found in the storage warehouse of various food commodities such as wheat, spices, beans, chocolate, dried fruit, flour and some agricultural produce [2]. Tribolium castaneum can breed throughout the year in warm areas and live for two years or more, during which time females produce nearly 1,000 eggs [3]. The presence of Tribolium castaneum in storage warehouses caused the degradation in quality and quantity of food commodities and the disadvantage can reach 15% [4]. The heavy attack of Tribolium castaneum makes the commodity has been contaminated by benzoquinon poison (the result of the excretion of flour flea) so that the commodity is not suitable for consumption and causes brown flour [5]. Many studies indicated that benzoquinon produced or secreted by flour beetles may have toxic and carcinogenic effects on human [6]. Therefore, Tribolium castaneum control technology must be developed to produce effective, efficient, and environmentally friendly Triboliumm castaneum compound.

Nowadays, control of Tribolium castaneum was done by chemical pesticide, methyl bromide. Tribolium castaneum infestation control is done by chemical fumigation, however, acquisition of
resistance to the substance has been described for T. castaneum populations [7]. The use of chemical pesticides is very dangerous to human health, primarily in food products, not environmentally friendly and harmful to non-targeted animals and plants [8]. Based on this fact, environmentally friendly Tribolium castaneum control system is required to be developed. One of the solutions is using dimethyldecanal compounds derived from the Tribolium castaneum body itself.

Dimethyldecanal is an aggregation pheromone produced by the male Tribolium castaneum. The sequence that produces dimethyldecanal is the exocrine gland under sex spots located on the ventral side of the first femur [9]. As aggregated pheromones, dimethyldecanal can attract both male and female Tribolium castaneum [10]. The purpose of this research is to analyze the influence of dimethyldecanal towards flour beetle behavior respons. The extraction methods are using head space sampling and body extract. There has been no study comparing dimethyldanal aggregation pheromone extract with head space sampling and body extract.

2. Methods

2.1. Sample Preparation

Tribolium castaneum was obtained from the Insect Pest Control Laboratory, Biomaterial Research Center, Indonesian Institute of Science. The medium that used consists of wheat flour and yeast with a ratio of 20: 1. Tribolium castaneum maintained at 28°C and 70% moisture. Separation of males and females was performed at the pupa stage based on standard methods performed by Beeman et al. [11] (ARS USDA) using a stereo microscope (Leica Sapo).

![Female pupa](image1.png) ![Male pupa](image2.png)

**Figure 1.** (a) Female pupa (b) Male pupa

The difference between male and female pupa is seen in the genital papilla located below the urogomphi. Female genital papilla is larger than male genital papilla and has a fingerlike shape, whereas male genital papilla is shaped like small fingertips [11]

2.2. Dimethyldecanal Extraction

2.2.1. Head Space Sampling Method

Adult male Tribolium astaneum (7-14 days) is used for dimethyldecanal extraction. The dimethyldecanal compound was extracted by modification method of Lu et al [2]. The extraction process was done by Head Space Sampling method using Charcoal tubes. Fifty individuals Tribolium castaneum male were fed into 50 mL aeration tube with 2.5 grams medium. Aeration was carried out at 28°C with 24 hours of exposure to maximize pheromone production. The flowing air was regulated using a flow meter at 200 mL / min speed and moistened with aquades on a 200 mL glass. The dimethyldecanal compound was collected using Charcoal tubes which were changed daily for 4 days and each sample was eluted using 500 mL of hexane. The extract result was stored in a vial glass at -30 °C.
2.2.2. Body Extract Method
The extract of dimethyldecanal compound from the entire body of the *Tribolium castaneum* was carried out by extracting 100 individual male *Tribolium castaneum* added with 2 ml n-hexane for 24 hours. Dimethyldecanal extract is stored in a glass vial at -30 °C.

2.3. Like Dislike Assay
Like-Dislike test was used to compare the interest of *Tribolium castaneum* flour beetle to dimethyldecanal compound extract which was carried out using aeration method of Head Space Sapling and Body Extract. Each extract was tested as Like Dislike based on the modification of the method carried out by Kartika et al [12] used a 15 cm diameter petri dish and paper disc. A total of 20 µl of dimethyldecanal compound extract was dropped on the paper disc and dried air for 1 minute with n-hexane solvent used as a control. Each paper disc is placed 2.5 cm from each side and placed opposite. A total of 10 female flour mites were placed in the release area. Behavior of flour infestation *Tribolium castaneum* was observed for 10 minutes for 10 repetitions.

The response of flour infestation *Tribolium castaneum* to each dimethyldecanal extract was calculated using the Aggregation Index (AI) formula as follows:

\[ AI = \frac{T - C}{N} \]

with T= *Tribolium castaneum* that attracted to the treatment
C= *Tribolium castaneum* that attracted to the control
N= total of *Tribolium castaneum* that used (10)

2.4. Gas Chromatography Mass Spectometry (GC-MS) Analysis
The extracted solution was analyzed by Gas Chromatography Mass Spectrometry (GC-MS) at the Center for Forensic Laboratory of Polri Headquarters based on modification of the method undertaken by Lu et al. (2011). This solution was analyzed using GC / MS Aglient 5977B in spitless mode with helium as carrier gas and electron impact (EI) of 70 eV. The oven injector was set at 250°C and the transfer point toward MS was set at 250°C. The oven temperature was adjusted at 40°C for 0.5 min, then increased at a rate of 10°C / min to 200°C and held for 1 min, then increased again at 20°C / min to a temperature of 240°C and held for 1 minute. MS was set in the SIM (Selected Ion Monitoring) mode on 41, 83, 97, 111, 125 and 141 ions. The GC-MS results then analyzed using the W10N14.L database to determine the presence of dimethyldecanal compounds produced by *Tribolium castaneum* starch fleas.

2.5. Data Analysis
Data obtained from the test calculated the standard deviation value then tested its homogeneity. Data that has a homogeneous variant are then analyzed using One Way Anova to determine the difference in response of *Tribolium castaneum* to dimethyldecanal extracts with different extraction methods. The difference between groups of extraction methods was determined by P <0.05.

3. Results and Discussion
In this study, dimethyldecanal compounds were extracted from male Tribolium castaneum using two different methods, head space sampling and body extract method then tested with Like-Dislike assay to determine the response of *Tribolium castaneum* in both extracts. Based on two different extraction methods, dimethyldecanal compounds were only obtained by the head space sampling aeration method. This is evidenced by the response of the *Tribolium casatneum* to the dimethyldecanal extract obtained using a higher head space sampling aeration method than the response to the body extract calculated using One Way Anova analysis of aggregation index (AI) values. Based on One Way Anova analysis, it can be seen that there is a significant difference between the response of Tribolium castaneum flour infestation to dimethyldecanal compounds with head space sampling and body extract.
aeration methods. It is known based on the significance value of less than 0.05, which is equal to 0.001. The average AI in the head space sampling method is 0.30 with a standard deviation of 0.20 while the average AI in the body extract method is -0.27 with a standard deviation of 0.266.

The presence of dimethyldecanal compounds in extracts was also confirmed by analysis of Gas Chromatography Mass Spectrometry (GC-MS). Based on GC-MS analysis dimethyldecanal compounds only appear on aerated head space sampling extracts and are not present in body extract (Figure 2).

![Figure 2](image.png)

**Figure 2.** GC-MS result of dimethyldecanal. Head Space Sampling Method (a), Body Extract (B).

The results of GC-MS analysis of dimethyldecanal extract obtained through the Head Space Sampling method proved that the peak of dimethyldecanal appeared 14 times, one of which was at the 14.33 and 14.84 minutes. This is supported by research conducted by Lewis [13] which showed that dimethyldecanal compounds appeared at the 14th minute retention time. Dimethyldecanal compounds are not obtained through the body extract method because these compounds are only produced under certain conditions, for example due to the presence of food sources and related to reproductive behavior. In the extraction method of Head Space Sampling, wheat flour was used as a food stimulus so that male Tribolium castaneum produced dimethyldecanal compounds in the form of gas and then absorbed by charcoal tubes, whereas in the body extract method no stimulus was used so that dimethyldecanal compounds were not obtained. Dimethyldecanal is a volatile gas-shaped compound so that for the identification and extraction a gas extraction method is needed which is required by the Head Space Sampling aeration method [14]
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