Comparison of pheromone extraction methods for *Callosobruchus maculatus* (F.) warehouse pest control

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**Abstract.** *Callosobruchus maculatus* is a warehouse pest that attack many various of nuts. One of the food preservation techniques from the threat of pests in Indonesia is the use of chemical pesticides and fumigants. However, it can interfere the human health, contaminate the environment and non-target organisms. The aims of this study is to compare the extraction method of dimethyloctane dioic acid and analyze the effectiveness of dimethyloctane dioic acid nanoparticles in controlling *C. maculatus*. The research method consisted of sample preparation, dimethyloctane dioic acid extract, like-dislike test and GC-MS analysis. The results showed that compounds extracted using the sampling headspace aeration method were more effective and right on target than body extract method. The compound of dimethyloctane dioic acid from body is very high evaporation rate than headspace aeration. The Independent Sample T Test gave significant results between *C. maculatus* response to headspace aeration and body extract method.

1. Introduction

Indonesian Ministry of Agriculture stated that peanut production in Indonesia in 2016 reached 613,365 tons. The average of peanut requirement increased 2.8% a year, while the average increase production was still 1.0% a year. This facts show that the production of domestic peanuts cannot satisfy the needs and demands. It makes the dependence of peanuts import in Indonesia increases.

Post-harvest storage is the most important and influential stage [1]. At this stage there is an interaction between environmental and organisms, including warehouse pests, that can decrease the quality and quantity of peanuts. According to Ummah [2] that insect pest can cause the greatest damage and have an important role to human health. One of the nut warehouse pests is *Callosobruchus maculatus*. These pests attack many various of beans such as cowpea, green beans, red beans, gude beans, soybeans, peas and bogor nuts.

Food preservation from insect threat is usually done by using chemical pesticides made from organochlorine, organophosphate and carbamate [3]. However, continuous use of chemical pesticides can disturb human health and contaminate the environment. Fumigants are generally easily absorbed by planted plants that are harmful when consumed by humans [4]. This fumigant is also easily absorbed by human skin which will cause edema in the lungs. Therefore, a more environmentally friendly of pest control is needed. One of the methods is by using pheromones. Pheromones are sex pullers that produced by insects that usually attract both male and female insects. In contrast to chemical pesticides, pest control with pheromones is non-toxic and effective at very low
concentrations. These pheromones will be used as a bait because this technique is more effective, targeted and high contact with pest mortality [5].

The pheromone in *Callosobruchus maculatus* pest is dimethyloctane dioic acid. This sex pheromone *Callosobruchus maculatus* (F.) compound evaporates easily because it is released by females to attract the opposite sex from a distance. Finding the right extraction method is important to reduce compound evaporation. Research by comparing extraction methods and GC-MS testing is very important to know about this. This research is expected to provide a solution for the application of pest control using targeted bait techniques that only target the pest.

2. Methods

2.1. Sample Preparation

The *Callosobruchus maculatus* colonies were obtained from rearing on *Vigna angularis* bean medium in a 90 x 60 mm plastic jar covered with a tile cloth. Female colonies that used are virgin female and male colonies are 1 month old. The bean flea is reared at 27 °C, 70% relative humidity and separated by sex for the next stage.

2.2. Extraction of Dimethyloctane dioic acid compounds

The extraction of dimethyloctane dioic acid compound from the 400 virgin female *Callosobuchus maculatus* (F.) was done using charcoal tube aeration and body extract method. The extraction stage begins by inserting 200 insects into a glass container with a diameter of 3.5 cm and a height of 10 cm which is connected to the inlet outlet pipe toward the charcoal tube. The wind source for aeration is obtained from the air pump connected with the teflon tube to the flowmeter at a rate of 1.5 ml / min. Aeration charcoal tube is changed every 12 hours and repeated 3 times. The results of the charcoal tube extract were eluted using 600 μL hexane on each charcoal tube. The result was stored in a vial bottle with a temperature of -20°C. Body extracts method were produced by extracting 200 female pests from *Callosobruchus maculatus* (F.) in the virgin female phase with 4 ml of hexane for 24 hours in a vial bottle. After 24 hours, the liquid on the vial bottle and the insect's body are separated using micropipet

2.3. Bioassay like-dislike test

The results of extracting dimethyloctane dioic acid using body extract and headspace aeration sampling were tested like dislike. Like-dislike tests are carried out using a 9-cm diameter petri dish. The bottom outside the petri dish is given paper that has been lined with three sides. Each side has a 3 cm line from the edge. On the right and left side is a part of treatment and control. Papper disks with a diameter of 0.6 cm were placed in the treatment section which was dripped with 20μl dimethyloctane dioic acid and dried for 30 seconds. In the control section a papper disk was placed dripping 20μl hexane and air dried for one minute. 10 test insects were put into a petri dish, then observed for 5 minutes. Then do the same thing 10 times by replacing the new papper disk. Each group of 10 replications was calculated using the Aggregation Index with the formula (T-C) / N, T is the number of insects that chose the treatment paper disc, C is the number of insects that chose the paper disc control, and N is the number of insects used in the test 1 repetition [6]. Here is a like-dislike test scheme using a petri dish.

2.4. Gas Chromatography Mass Spectometry (GC-MS) Analysis

The The extract from the aeration result was analyzed using Agilent Gas Chromatography Mass Spectrometry (GC-MS). The oven injector was set at 250°C and the transfer point to MS was set at 250°C. The oven temperature was adjusted at 40°C for 2.5 minutes, then increased at a rate of 10°C/min to 200 °C, increased again at 20°C/min to 240 °C and held for 1 minute . MS was set in the SCAN mode. The GC-MS results were analyzed using the W10N14.L database to determine the presence of dimethyloctane dioic acid compounds.
3. Results and Discussion

3.1. Like-dislike test
Making samples of dimethyloctane dioic acid extract was carried out with two methods, namely from the overall body or body extract of the warehouse pest Callosobuchus maculatus (F.) and headspace aeration sampling with charcoal tube. Each sample was tested using a like-dislike test for 10 replications, once repeated 10 insects. The amount of insect attraction towards the treatment of each method is headspace sampling of 82% and body extract is 32% of the total test insect for each method.

The value of aggregation index (AI) was tested for homogeneity which showed the results that the two variants of the groups were the same and tested for normality which showed the results that the data were normally distributed. After testing homogeneity and normality, the AI value was analyzed using the Independent Sample T Test which showed a significant difference between the response of *Callosobruchus maculatus* (F.) warehouse pests to dimethyloctane dioic acid from body extract and headspace sampling aeration with values in the Sig column amounting to 0.38. The average AI obtained by the body extract method is -0.36 while the AI average with the sampling headspace method is 0.64.

This shows that the headspace sampling method is more effective to be used to extract dimethyloctane dioic compounds from warehouse pests *Callosobruchus maculatus* (F.) which can be seen from the amount of insect attraction and average AI.

3.2. Gas Chromatography Mass Spectometry (GC-MS) Analysis
The dimethyloctane dioic acid compound is produced by virgin female *Callosobruchus maculatus* (F.) which is extracted using the body extract and aeration headspace sampling method. The presence of dimethyloctane dioic acid can be known through analysis of chemical components using Gas Chromatography Mass Spectometry (GC-MS).

GC-MS results from body extract showed that the presence of dimethyloctane dioic acid appeared 8 times at 9 to 14 retention time while extracts from aeration headspace sampling showed that the presence of dimethyloctane dioic acid appeared 32 times at 7 to 7 minutes retention time. From the results of GC-MS and previous tests, namely like-dislike, it can be seen that dimethyloctane dioic acid compounds from the headspace sampling aeration method more effectively affect the response of *Callosobruchus maculatus* (F.) and produce more pheromone compounds than the body extract method.

![Figure 1](image-url)  
*Figure 1*. Results of GC-MS (a) Chromatogram of dimethyloctane dioic acid compound from aeration headspace sampling. (b) Chromatogram of dimethyloctane dioic acid compound from body extract.
Insects have a way of communicating for sexual activity between members of their species using a molecule called sex pheromone. Sex pheromones are released by one sex and attract the other sex as is done by the insect pest warehouse Callosobruchus maculatus (F.). Pheromone compounds in Callosobruchus maculatus (F.) are a type of sex pheromone produced by females used as the strength of sexual selection among species as a signal for mating [7]. The structure of the Callosobruchus maculatus (F.) pheromone is identified as a mixture of C16 aldehyde which can be called dimethyloctane dioic acid [8,9]. These pheromones are used as bait because this technique is more effective, on target, and has high contact with pest mortality [4]. Extraction of dimethyloctane dioic acid pheromone compound in this study was carried out using two methods, namely body extract and headspace sampling aeration. Both of these methods are compared using the like-dislike test using petri dish. The results of this test indicate that there is a significant difference between the response of the warehouse pest Callosobruchus maculatus (F.) to the dimethyloctane dioic acid compound from body extract and headspace sampling aeration. The dimethyloctane dioic acid compound from aeration headspace sampling was more effective in influencing the response of Callosobruchus maculatus (F.) warehouse pests which can be proven from the number of insect attractions towards dimethyloctane dioic acid rather than hexane as control of each method namely 82% headspace and 32 body extract % of the total test insects for each method. The use of hexane as a solvent and control tends to be safer to use for food applications because hexane has a low boiling point. Hexane solvents have a boiling point of 68 °C so that during the process of cooking food, hexane evaporates into the air. The value of aggregation index (AI) from the like-dislike test is tested for homogeneity which shows the results that the variants of the two groups are the same and tested for normality which shows the results that the data are normally distributed. After testing homogeneity and normality, the AI value was analyzed using the Independent Sample T Test which showed a significant difference between the response of Callosobruchus maculatus (F.) warehouse pests to dimethyloctane dioic acid from body extract and headspace sampling aeration with values in the Sig column. equal to 0.38. The average AI obtained by the body extract method is -0.36 while the AI average with the headspace sampling method is 0.64. In addition, dimethyloctane dioic acid is a volatile gas-shaped compound so that for identification and extraction a method using gas extraction such as headspace aeration sampling is needed. Dimethyloctane dioic acid compounds in pheromone extract can be found in the presence of gas chromatography followed by mass spectrophotometry through GC-MS analysis. Chromatography functions to separate the components of compounds in the extract, while spectrophotometry functions to calculate the abundance of each compound that has been separated [10,11]. GC-MS results from body extract showed that the presence of dimethyloctane dioic acid appeared 8 times at 9.6, 9.8, 9.9, 10.1, 11, 11.2, 12.2, and 14.8 while extraction from aeration headspace sampling showed that the presence of dimethyloctane compounds dioic acid appeared 32 times at minute retention times to 7.3, 7.4, 7.7, 8.5, 9.1, 18.3, 19.2, 19.3, 19.6, 20, 20.1, 20.8, 20.92, 20.98, 21, 21.1, 21.2, 21.4, and 22.1. This is supported by research conducted by Nojima [6] which shows that dimethyloctane dioic acid is a derivative of methyl ester with a retention time of ± 20.00 minutes. From the results of GC-MS and previous tests, namely like-dislike, it can be seen that dimethyloctane dioic acid compounds from the sampling headspace aeration method more effectively affect the response of Callosobruchus maculatus (F.) and produce more pheromone compounds than the body extract method.

4. Conclusion

Based on this research can be concluded that the dimethyloctane dioic acid compound with headspace sampling method affects the number of insect attraction towards treatment by 82% with an average AI of 0.64 and body extract of 32% with an average AI of -0.36 from the total test insects for each method. So that the compounds obtained through the headspace sampling aeration method are more effective in influencing the behavior of the warehouse pest Callosobruchus maculatus (F.) compared to the body extract method.
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References
[1] Sari D K and Cahyono E 2016 *Indones. J. Chem. Sci.* 5
[2] Ummah A K 2012 *Kajian Kondisi Komoditas, Serangga Hama Gudang dan Upaya Pengendaliannya (Studi tentang Penyimpanan Komoditas di Gudang Bulog 105 Bawen Sub Dolog Wilayah I Semarang)* (Semarang: Universitas Diponegoro)
[3] Rahman K 2007 *Clin. interv. aging* 2 219.
[4] Yadav I C and Devi N L 2017 *Environ. Sci. Eng.* 6 140
[5] Hutabarat N K, Oemry S and Pinem M I 2015 *J. Agrotek.* 1 103
[6] Kartika T, Shimizu N, T Yoshimura 2015 *PLoS ONE* 10 0141799.
[7] Nojima S, Shimomura K, Honda H 2007 *J. Chem. Ecol.* 33 923
[8] Shimomura K, Shimpe M, Kanju O, Shunsuke Y dan Shimo 2017 *Chemoecology* 27 65
[9] Oni M O., Ogungbite O C, Oguntuase S O, Bamidele O S and Ofuya T I 2019 *J. Basic Appl. Zool.* 80.47
[10] Sahil K, Prashant B, Akanksha M, Premjeet S and Devashish R 2011 *Int. J. Pharm* 2 1544
[11] Qiao X, Song W, Ji S, Wang Q, Guo D A and Ye M 2015 *J. Chromatogr. A.* 1402 36