SEMIOCHEMICAL INTERACTION BETWEEN *Myopopone castanea* SMITH WITH ITS PREY *Oryctes rhinoceros* LINN. LARVAE

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ABSTRACT

Ant species, *Myopopone castanea* is a predator for immature of *Oryctes rhinoceros*. The ants used semiochemical compounds in communications between individuals both in the same species or different species. This study aims to determine the semiochemical compounds that play a role in the relationship between the interaction of *M. castanea* as predators and their prey larvae *O. rhinoceros*. The results showed that the volatile compounds from GC-MS analysis of *M. castanea* ants wood nest were hydrocarbons compounds. In contrast, volatile compounds from GC-MS study of *O. rhinoceros* larvae were 5H-1 pyrindine compounds, carboxyclic acid an hydrocarbon compounds. The results of the odour detection test on *M. castanea* ants in the Y tube showed that the choice of *M. castanea* in the combined aroma of palm oil sawdust and *O. rhinoceros* larvae was higher (78%) than the choice only for palm oil stem sawdust (54%) and only *O. rhinoceros* larvae (48%). The combination of palm oil sawdust and *O. rhinoceros* larvae makes the volatile compounds stronger so that the ants prefer this scent. The semiochemical compounds in rotting oil palm stems can be a medium of interaction between *M. castanea* as and *O. rhinoceros* larvae.

Keywords: *Myopopone castanea*, *Oryctes rhinoceros*, semiochemical compounds, volatile compounds

ABSTRAK

Spesies semut, *Myopopone castanea* merupakan pemangsa bagi pramatang *Oryctes rhinoceros*. Serangga ini menggunakan sebatian semiokimia dalam berkomunikasi diantara spesies yang sama atau berlainan spesies. Tujuan kajian ini adalah untuk menentukan sebatian semiokimia yang memainkan peranan dalam interaksi hubungan pemangsa *M. castanea* dan mangsanya larva *O. rhinoceros*. Keputusan analisa GC-MS mendapati bahawa sebatian meruap daripada sarang semut *M. castanea* adalah sebatian hidrokarbon. Sebaliknya hasil analisis GC-MS sebatian meruap larva *O. rhinoceros* adalah sebatian pyrindine 5H-1, asam karboksilat dan senyawa hidrokarbon. Keputusan ujian pengesanan bau pada semut *M. castanea* yang dilakukan pada tabung Y menunjukkan bahawa pilihan semut *M. castanea* gabungan aroma habuk kayu kelapa sawit dan bau larva *O. rhinoceros* lebih tinggi (78%) daripada pilihan hanya habuk kayu kelapa sawit saja (54%) dan hanya larva *O. rhinoceros* sahaja (48%). Kombinasi habuk kayu kelapa sawit dan larva *O. rhinoceros* menjadikan sebatian lebih kuat meruap dan menyebabkan semut lebih menyukai aroma yang dihasilkan. Sebatian semiokimia pada kelapa sawit yang reput boleh dijadikan sebagai medium interaksi antara semut pemangsa *M. castanea* dan mangsanya larva *O. rhinoceros*.

Kata kunci: *Myopopone castanea*, *Oryctes rhinoceros*, sebatian semiokimia, sebatian meruap

INTRODUCTION

Semiochemical compounds in insect communication consist of two groups namely pheromones (alarms, traces, sexual, aggregation) which are used for communication between individuals in one species and allelochemicals (allomon, kairomon, synomon and apneumon) which are used for communication between individuals of different species (Norin 2007). These semiochemical compounds play an important role in the process of implementing biological control and Integrated Pest Management (IPM) programs (Khan et al. 2008; Witzgall et al. 2010). In controlling *Oryctes rhinoceros* pests, ethyl 4-methyloctanoate pheromone compounds have been used as traps to reduce *O. rhinoceros* beetle populations (Bedford 2014; Kamaruddin & Wahid 2004). This compound is a sex pheromone compound released by female imago to invite or call male imago to mate.

Ants as social insects communicate using trace pheromones among individuals in their colonies. Ants that find food sources leave pheromone traces on the ground through the sting at the tip of their abdomen. The trail made helps other ants find food sources or enlist the help of other ants in the colony to transport the food (Blum & Brand 1972; Morgan 2009; Vander Meer et al. 1998). Besides using chemical signals, ants also use abiotic signals of temperature and humidity in finding and foraging for food. *Tetraponera rufonigra* ants will be more active foraging for food when the temperature is around 27-30ºC (Norasmah et al. 2012). *Tetraponera rufonigra* could become pests in settlements and can interfere human health because of their highly toxic stinger (Sabtu & Ab Majid 2020; Somala et al. 2020).

As predators, ants also utilize semiochemical compounds in the interaction between predators and prey. Predatory ants such as *Pachycondyla analis* use chemical compounds such as kairomone released by termite prey from the *Macrotermes* group (Yusuf et al. 2014). Schatz et al. (2003) also found that *Crematogaster scutellaris* ants use chemical compounds to detect its prey, the fig wasps *Blastophaga psenes*.

Myopopone castanea is a predator for *O. rhinoceros* larvae. It lives on weathered logs. On oil palm plantations, *M. castanea* ants live and build nests in old and weathered oil palm trunks, or in fallen palm oil stems which are weathered and weathered due to stem rot disease. *Oryctes rhinoceros* larvae also live in weathered stems or stumps of oil palm or other organic material. The similarity in the recesses between predators and prey offers an excellent opportunity to utilize *M. castanea* ants as biological agents for *O. rhinoceros* larvae. To be able to optimize the role of *M. castanea* ants as predators for larvae of *O. rhinoceros* it is necessary to identify specific chemical compounds that guide the ants of *M. castanea* in find their prey *O. rhinoceros*. This research was carried out to ascertain the volatile compounds contained in these weathered stems of palm oil which are the nests of *M. castanea* ants. In addition, this research also revealed the volatile compounds found in the larvae of *O. rhinoceros*, and this information may later enrich the activity for integrated pest control for *O. rhinoceros* in Palm oil plantations.

MATERIALS AND METHODS

Sampling Site

The study was conducted at the Plant Pest Laboratory of the Faculty of Agriculture University of Muhammadiyah Sumatera Utara (UMSU) Medan and in the *Pusat Penelitian Kelapa Sawit* (*PPKS*) Medan chemical laboratory.

Test Insect Collection

The research was carried out by collecting ant predators *M. castanea* from oil palm trunk rotting in the community's oil palm cultivations in Tanah Merah village, Binjai Selatan sub-district, Binjai. The *M. castanea* ants obtained from the field were kept in the Plant Pest Laboratory of the Faculty of Agriculture, UMSU. The ant colony was maintained in a glass box with the size of 70 x 30 x 30 cm. Inside the glass box are two pieces of weathered palm stems with a capacity of 20 x 20 x 3 cm used as a medium where the ants build their nests. In the middle of the palm trunk, a small hole measuring 5 x 5 cm was made as a place to lay the prey larvae of *O. rhinoceros*. The provision of *O. rhinoceros* larvae was given according to ant predation needs. When seen prey is dead and shriveled, it will immediately be given the live and healthy *O. rhinoceros* larvae as a prey.

Extraction of Compounds and Chemical Analysis

Approximately five grams of oil palm trunk of ant nest *M. castanea* was inserted into a test tube and then added 2.5 ml of n-pentane solution was added to it. This solution was rotated for about 10 minutes so that it has mixed homogeneously. Next, the solution was extracted at room temperature for 2 hours and cleaned as well as filtered using a charcoal-purified nitrogen (charcoal purified with nitrogen) solvent to around 100 µL. When the sample was not analyzed immediately, the sample was stored in the freezer at -20 ^{0}C until was used for further analysis.

Two individual second instars of *O. rhinoceros* larvae were killed by ice and then extracted in 2 ml of n-pentane solution. Then, this solution has frozen for 2 hours. After extracting, the extraction solution is filtered and concentrated under nitrogen to 100 µL. This extraction was immediately analyzed or stored at -20 ^{0}C until it was used.

Analysis of GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out in the Medan PPKS laboratory using the 7890A Agilent Technologies gas chromatography equipped with a HP-5 MS capillary column (30 m x 0.25 mm ID × 0.25 µm film layer) and combined to 5795C mass spectrometer. One µL of each sample has injected in splitless mode, and helium used as a gas carrier at 1.0 mL/min. The temperature was programmed at 35°C for 5 minutes, and increased 10°C / minute to 250°C, and was maintained at this temperature for 15 minutes. The analysis was carried out at 70 eV in ionization mode affecting electrons. The resulting identity was produced by comparing it with the mass spectra and the resulting retention index.

Semiochemical Testing

Ant semiochemical testing was carried out using a Y tube in which each branch was connected to a glass tube. Ant semiochemical experiments were carried out by inserting ten individuals *M. castanea* worker ants at the bottom of the Y tube and this process was repeated five times for each unit of the test. The analysis carried out by comparing: (1) One Y tube sleeve contains the chip of oil palm trunk, and the other arm contains clean air (control), (2) One Y tube sleeve contains two individuals of the second instar of *O. rhinoceros* larvae, and the other arm contains clean air (control), (3) One Y tube sleeve contains two individuals of second instar larvae of *O. rhinoceros* + the chip of oil palm trunk, and the other arm contain clean air (control).

At each end of the Y tube arm is given a small fan that serves to blow air so that it flows in each Y tube arm. If the ant goes to one arm of the Y tube that is given the odours and stay on the odour for 5 minutes, then this indicates that the ant like the odour, but if the ant does not move and does not choose one of the odours on the arm of the Y tube and stay is under the Y tube, then this indicates the ant does not choose the given smell.

RESULTS AND DISCUSSION

GC-MS Analysis

The results of the GC-MS analysis of weathered oil palm trunks showed that the volatile compounds obtained were all hydrocarbon compounds except for tetracosanoic acid which is a compound from the carboxylic acid group (Table 1).

Table 1. GC-MS analysis of volatile compounds from GC-MS analysis from oil palm trunks as the nest of *M. castanea*

No	RT (Minutes)	Molecular Formulas	Compound Name	% Area
1	12.985	$C_{11}H_{10}$	Naphthalene, 2-Methyl- (CAS)2-Methylnaphthalene	17.20
2	13.257	$C_{10}H_8$	1-4Methanonapthalene, 1,4-Dihydro-(CAS) 5,6-BENZOBICYCLO(2,2,1)HE	9.09
3	14.879	$C_{12}H_{12}$	Napthalene, 1,3-Dimethyl-(CAS) 1,3-DIMETHYLNAPTHALENE	6.99
4	15.164	$C_{15}H_{22}$	(-)-Dehydroaromadendrene	6.64
5	15.213	$C_{18}H_{36}$	Dodecane, 2-Cyclohexyl-	6.40
6	15.393	$C_{11}H_{24}$	Decane, 2 Methyl-(CAS) 2-Methyldecane	14.37
7	15.497	$C_{13}H_{28}$	Undecane, 2,3 Dimethyl-(CAS) 2,3-Dimethylundecane	10.40
8	15.887	$C_{14}H_{30}$	Decane, 2,3,5,8-Tetramethyl-	15.36
9	16.598	$C_{12}H_{24}$	Cyclohexane, (1,3-Dimethylbutyl)- (CAS)	4.85
10	20.887	$C_{25}H_{50}O_2$	Tetracosanoic Acid, Methyl Ester (CAS) Methyl Lignocerate	8.70

The results of the GC-MS analysis of the larvae of *O. rhinoceros* obtained various volatile compounds. The compounds obtained were pyrindine (5H-1 Pyrindine). Also, compounds from the carboxylic acid group (Eicosanoicacid, methyl ester (CAS) Arachidic acid methyl ester, 9-Octadecenoic acid (Z)-(CAS) Oleic acid, Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate, 9-Octadecenoic acid (Z)-(CAS) Oleic acid, Ethyl Octadec-9-enoate). Other compounds are the hydrocarbon group (Cyclooctane, Cyclooctylidene, Naphthalene, 2-methyl-(CAS)) 2-Methylnaphthalene) and sulfur (Sulfur, mol. (S8) (CAS)) Octa-sulfur) (Table 2). The most dominant volatile compound is pyrindine compound.

Table 2. Volatile compounds resulting from GC-MS analysis from *O. rhinoceros* extraction larvae

No	RT (Minutes)	Molecular Formulas	Compound Name	% Area
1	12.955	$C_8H_{10}N$	5H-1-Pyrindine	44.89
2	13.249	$C_{11}H_{10}$	Naphthalene, 2-methyl-(CAS) 2-Methylnaphthalene	1.98
3	20.887	$C_{21}H_{42}O_2$	Eicosanoicacid, methyl ester (CAS) Arachidic acid methyl ester	3.48
4	21.209	$C_{36}H_{68}O_4$	9-Octadecenoic acid (Z)-(CAS) Oleic acid	7.13
5	21.576	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate	3.54
6	22.303	S_8	Sulfur, mol. (S8) (CAS)) Octa-sulfur	12.16
7	22.655	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)- methyl ester (CAS) Methyl Oleate	2.80
8	22.984	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)-(CAS) Oleic acid	2.83
9	23.241	C_8H_{16}	Cyclooctane, Cyclooctylidene	3.95
10	23.291	$C_{20}H_{38}O_2$	Ethyl Octadec-9-enoate	17.23

Semiochemical testing

Results of semiochemical tests conducted on the Y tube found that *M. castanea* choose the ants nest oil palm trunk and clean air (control). Notably, an average of 5.4 individuals of *M. castanea* ants (54%) would prefer to go to the ant nest of oil palm trunk rather than clean air ($\overline{X}$ = 3.2 individuals; 32%). In contrast, 1.42 ants (14%) did not choose either oil palm trunk or clean air (Figure 1).

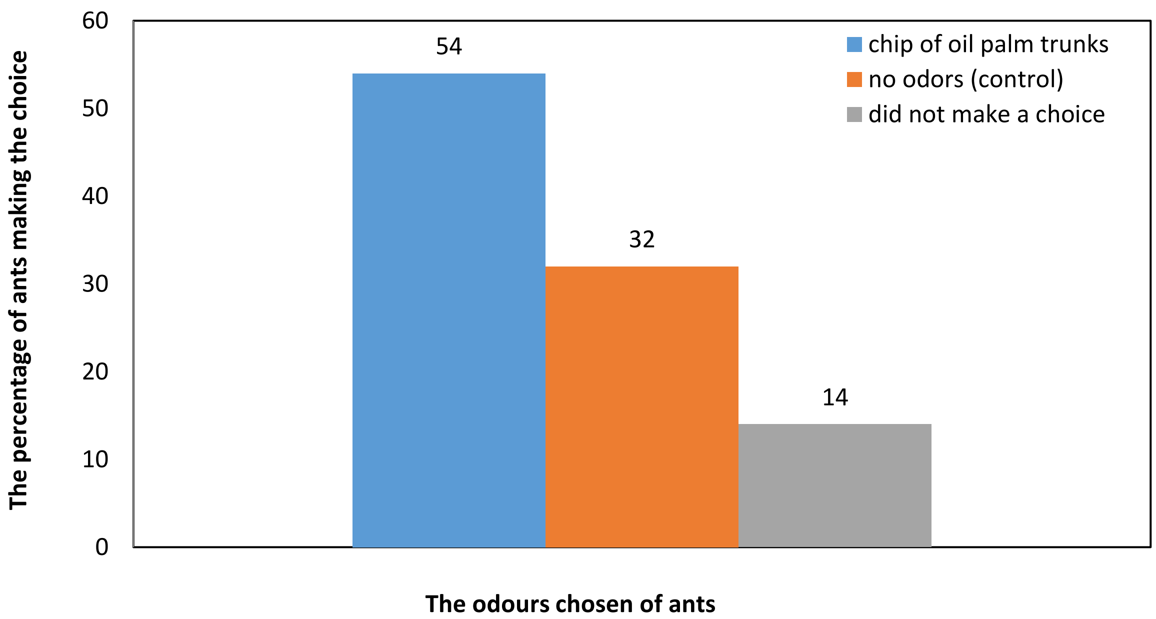

Figure 1. The odours of a semiochemical compound chosen by *M. castanea*, which was between the odour of chip of oil palm trunk and the clean air (control).

When the ants were given a choice between *O. rhinoceros* larvae and no odours (control), then 48% of the ants choose the odours of the larva *O. rhinoceros*, 16% choose the control, and 36% did not make a choice (Figure 2).

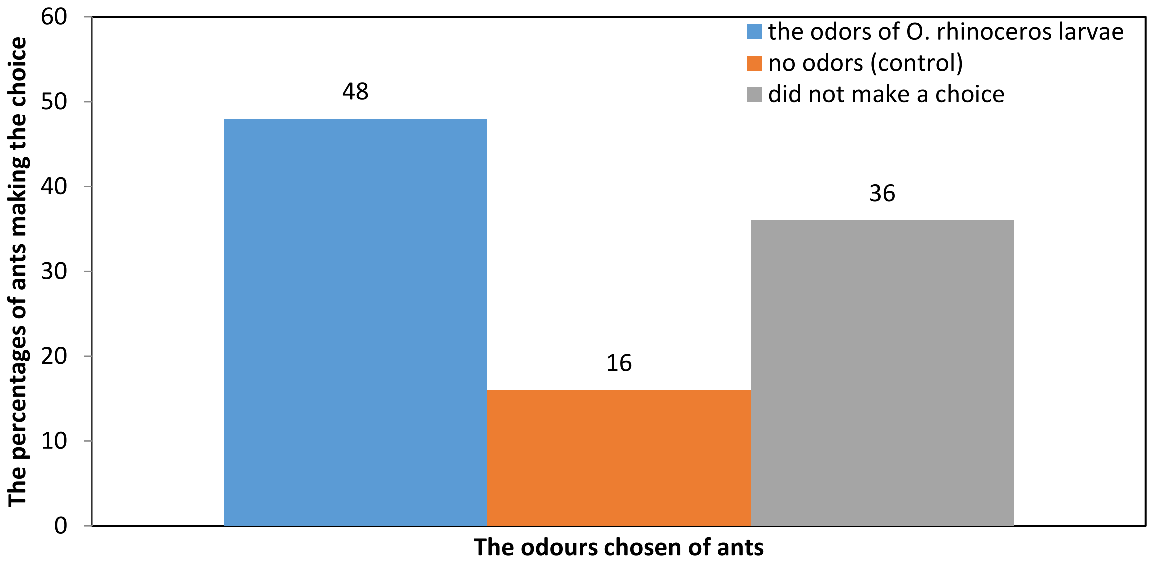

Figure 2. The odours of a semiochemical compound chosen by *M. castanea*, which between the odour of *O. rhinoceros* larvae and the clean air (control).

The results of the combined odours testing of oil palm trunk chips and *O. rhinoceros* larvae showed an increase in the number of ants that led to the Y tube arms that were scented (Figure 3).

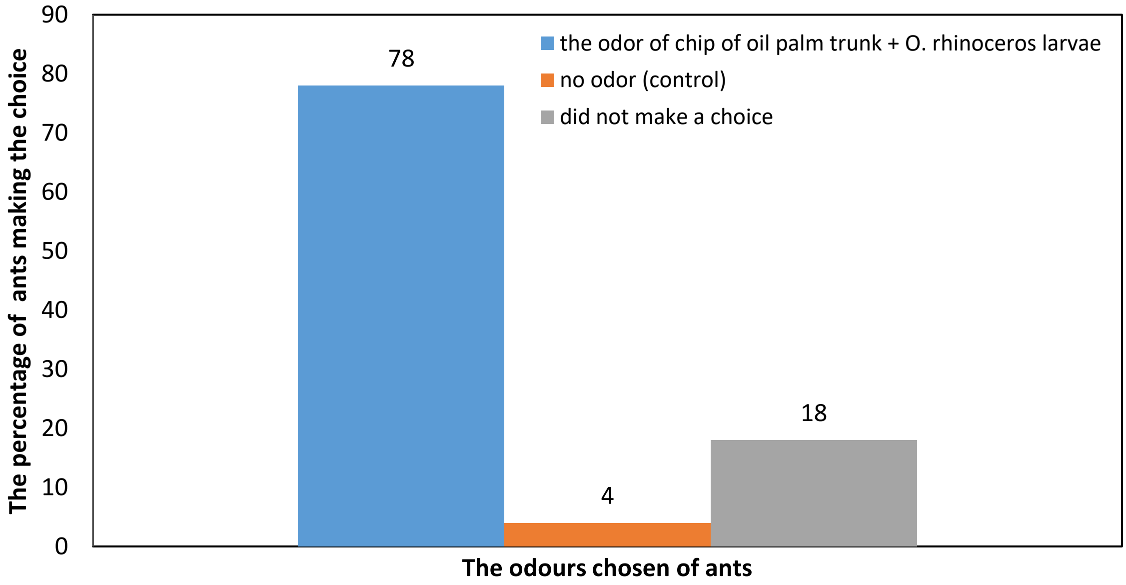

Figure 3. The odours of a semiochemical compound chosen by *M. castanea*, which between the odour of chip of oil palm trunk + *O. rhinoceros* larvae and the clean air (control)

Chemical compounds obtained from the results of chemical analysis and GC-MS analysis, the hydrocarbon compounds dominated the weathered palm stems (Table 1). There were ten dominant chemical compounds detected from GC-MS analysis. The highest chemical compounds obtained from the GC-MS analysis were decane compounds (Dodecane, 2-Cyclohexyl-, Decane, 2 Methyl-(CAS) 2-Methyldecane, Undecane, 2,3 Dimethyl-(CAS) 2,3-Dimethylundecane, Decane, 2,3,5,8-Tetramethyl-) and naphthalene compounds (Naphthalene, 2-Methyl- (CAS)2-Methylnaphthalene, 1-4Methanonapthalene, 1,4-Dihydro-(CAS) 5,6-Benzobicyclo(2,2,1) HE, Napthalene, 1,3-Dimethyl-(CAS) 1,3-Dimethylnapthalene). This decane compound is a hydrocarbon compound which has ten carbon and 22 hydrogen atoms which have covalently bonded to each other. Decane compounds 2, 3, 5, 8-Tetramethyl belong to the class of organic compounds known as acyclic alkanes. Mujiono et al. (2015) said that some decane compounds such as 2,3,5,8 tetramethyl include in the pheromone group. Furthermore, other decane derivatives such as 5-Butylnonane; Tetradecane; 1 Hexanol, 2-ethyl-2-propyl-; 1-Undecene, dodecane 4,6-dimethyl are compounds of the pheromone group. In the Lepidoptera, many of these compounds are as attractive as the *Spodoptera exigua* insect (Mujiono et al. 2015). Naphthalene compounds and their derivative compounds, according to El-Sayed (2018) are mostly kairomone. Yusuf et al. (2014) found naphthalene compounds and their derivatives in termites *Odontotermes* sp. It acts as kairomone so that predatory ants *Pachycondyla analysts* can more easily detect the presence of termites as their prey.

The compounds of this hydrocarbon group are compounds that are closely related to the life of the ant. Ants recognize colonies and their nests by the smell of hydrocarbon compounds found in the ant cuticle itself (Abril et al. 2018; Nowbahari 2007; Sharma et al. 2015). The results of Sharma et al. (2015) found that the ants could detect the number of hydrocarbon compounds present in the cuticle of the ant. It helps the ants to distinguish the castes in their colonies or from different territories. It is important to note that ants are able to identify small differences in the number of hydrocarbon compounds present between their colonies.

The results of GC-MS analysis for *O. rhinoceros* larvae obtained varied volatile compounds (Table 2). The most dominant volatile compound is the pyrindine. The function of these compounds varies between individuals. Kim et al. (2014) obtained 5H-1 pyrindine compound in the extraction of *Lilium longiflorum*. This volatile pyrindine compound is the only volatile odour that smells bad. This unpleasant odour also becomes a very noticeable odour released by *O. rhinoceros* larvae. Pyrindine is widely used in the pharmaceutical and agro-industry fields (Akalin & Karagoz 2011). Pyrindine and their its derivatives have been studied as anticancer drug discoveries, these derivatives have also been screened for various biological activities such as antitubercular, antioxidant, antimicrobial, anticancer, and anti-inflammatory activities (Sharma & Kumar 2014). Ara et al. (2012) explained that this pyrindine has been used as an antibiotic for bacteria that can cause disease in humans. Pyrindine with the chemical formula $C_8H_{10}N$ described by El-Sayed (2018) is function as kairomone in various types of insects. Meanwhile, compounds such as octadecenoic acid and ethyl octadic-9-enoate are volatile compounds commonly used by the group of *Bombus* sp. Bees as a chemical compound to recognize groups of colonies even similar groups that are not colonies originating from more distant regions (Bertsch & Schweer 2012). Besides El-Sayed (2018) also classified compounds such as octadecenoic acid and ethyl octadic-9-enoate as volatile compounds derived from the extraction of fat and skin of insects.

Ants widely use hydrocarbon compounds as compounds for communication (Martin & Drijfhout 2009; Van Wilgenburg et al. 2011). In the weathering process of wood, microorganisms such as fungi play an essential role in producing the carbon cycle. Mycelia-

mycelia fungi compete with each other to acquire their territory with antagonistic mechanisms to create several volatile chemical compounds (Hiscox et al. 2015). The compounds produced are mostly hydrocarbon compounds that function in ant communication systems. Besides, in the process of wood weathering, the process of breaking down cellulose into pure glucose compounds is necessary in the life of ants (Shafawati & Siddiquee 2013; Ulfa et al. 2014).

The results of the odours testing from oil palm trunk chips and *O. rhinoceros* larvae showed an increase in the number of ants that led to the Y tube arms that were scented (Figure 3). Ants which chose odors derived from oil palm trunk chips and *O. rhinoceros* larvae were approximately 7.8 individuals (78%), 0.4 individuals (4%) chose an arm that is not given a odour (control) and those that do not made a choice of 1.8 individuals (18%). Combining the volatile odors of oil palm trunk chips and the odors of *O. rhinoceros* larvae further increases the ant's choice to go to the specific arm. The compounds of the oil palm trunk chips are dominated by hydrocarbon compounds (Figure 1) which are indeed compounds that are widely used by ants to communicate. Ants use hydrocarbon compounds in conveying communication signals, including identifying their colony mates and identify their nests. Pieces of oil palm trunks which are the nests of *M. castanea* ants used in odour testing can be recognized by the smell of ants so that the number of ants that go to the smell reaches 54%. The research results of Wang et al. (2016) explained that *Iridomyrmex purpureus* worker ants were less aggressive when given the smell of hydrocarbon compounds that did not come from their nests. The volatile compounds from the extraction of *O. rhinoceros* larvae were dominated by pyrindine compounds (Figure 2), which had an unpleasant odour and were characteristic of the *O. rhinoceros* larvae. In addition, other volatile compounds are from the carboxylic acid and hydrocarbon groups, the carboxylic acid compounds obtained according to El-Sayed (2018) mainly function as kairomone and attractants.

CONCLUSION

Semiochemical compounds are essential elements in communication between insects, both intra-species and inter-species. Hydrocarbon compounds are the dominant volatile compounds obtained from GC-MS analysis results on *M. castanea* anthill wood dust. In contrast, pyrindine, carboxyclic acid and hydrocarbon compounds were the compounds obtained from GC-MS analysis results from *O. rhinoceros* larvae. Hydrocarbon compounds function as trace pheromones in ant colonies. The choice of *M. castanea* ants on the combined odors of oil palm trunk chip and *O. rhinoceros* larvae is higher (78%) compared to the choice only on oil palm trunk chip (54%) and only *O. rhinoceros* larvae (48%). The combination of oil palm trunk chip and *O. rhinoceros* larvae makes the volatile compound stronger those resulting in the ant’s preference towards the odours. Semiochemical compounds present in weathered oil palm trunks can be a medium of interaction between *M. castanea* ants as predators with *O. rhinoceros* larvae as their prey.

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