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Two Novel Volatile Compounds as the Key for Intraspecific Colony Recognition in *Macrotermes gilvus* (Isoptera: Termitidae)

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ABSTRACT

Factors that cause individuals to recognize nestmates and non-nestmates in the same termite species require ongoing research. Chemical communication between individuals is predicted to be a key factor in the recognition behavior. This study aims to determine the level of aggression intraspecies *Macrotermes gilvus* and identify chemical components that play a role in influencing the behavior. Agonistic test intra- and intercolony *M. gilvus* that separated distance far away and a GC-MS analysis to determine of volatile compounds which role communication among individuals *M. gilvus* have been done. The research results showed that no aggression behavior intraspecies *M. gilvus* both intra- and intercolony. Agonistic test on three termite colonies *M. gilvus* from Bandung, Bogor and Bandar Lampung within maximum distance 216 km showed no agonistic. Agonistic level in intracolony only happens antennation or examination, while in intercolony occurs antennation, examination, alarm behavior and avoidance one another. Aggressive behavior (as a marker of agonistic behavior) does not appear both inter- and intracolony. Identification of the volatile compounds that assumed to play a role as, a key of intraspecific colony recognition has been carried out using GC-MS analysis, the results are 22 volatile compounds of caste workers and soldiers (both minor- and major soldier). Two novel volatile compounds has been identified i.e., (Z)-6-octadecenoic acid and (E)-9-octadecenoic acid, respectively, as the key of individuals for recognizing both non- and nestmembers of *M. gilvus*. Those compounds are species-specific on *M. gilvus*.

Key words: Volatile compound, *Macrotermes gilvus*, agonistic, octadecenoic acid, Termitidae

INTRODUCTION

Profile of hydrocarbons has been stated as the species-specific and may explain the difference of intercolony and can be used to express a genetic relationships between individuals (Haverty *et al.*, 1988; Bagine *et al.*, 1994; Howard *et al.*, 1988; Haverty *et al.*, 1999, 2005; Batista-Pereira *et al.*, 2004). Cuticular hydrocarbons are prime candidates for recognition of the colony in social insects than morphological, genetic and geographic distances (Kaib *et al.*, 2004).

Cuticular hydrocarbon variation analysis, according to Kaib *et al.* (2004), in species *Macrotermes subhyalinus* in West Africa (Comoe National Park) on a short distance (<1 km) showed four chemically different phenotypes, also found a significant correlation between the differences hydrocarbons composition and intercolony aggression. The other side, Neoh *et al.* (2012)

stated that agonistic behavior does not occur in major workers individuals of *M. gilvus* despite coming from different colonies and distance apart (sites ranged from 6-18 km).

It is necessary to further research on the chemical components involved to explain the link between the different colonies (spatially) and a low aggression nature (even no aggression) intraspecies *M. gilvus* in the range colonies more distances (156-210 km). This research is done to determine the level of aggression behaviors both intra- and intercolony *M. gilvus* and identify the volatile components, which is the key recognition individuals intraspecies *M. gilvus*.

MATERIALS AND METHODS

Source colonies and maintenance in the laboratory: Three colonies of termites *M. gilvus* obtained from three different locations: Bandung, Bogor and Bandar Lampung, in July, 2014. Distance Bogor-Bandar Lampung is 156 km, Bandung-Bogor 164 km and Bandung-Bandar Lampung 210 km. Three nests of three colonies were moved and maintained to the laboratory for one week before bioassay. Nest consists some of combs and the royal chambers in which there are both caste workers, soldiers (minor soldier and major soldier) and reproductive (queen, king and alates). Maintenance termites using sealed plastic containers (volume 5.6 L), which filled moistened sand on the bottom and tissue (Tessa TP-01) as, a food source for termites. Maintenance is performed in the incubation chamber at temperature $28\pm 2^{\circ}\text{C}$ and humidity $>85\%$.

Intra and intercolony agonistic test: Individuals that are used to test agonistic are major workers, minor soldiers and major soldiers from those three colonies. The testing arena, modified by Wong and Lee (2010) and Neoh *et al.* (2012), using a filter paper disc (diameter 5 cm) moistened with distilled water was placed in a 5-cm-diameter plastic dish. The arena was equally divided into two areas by a plastic card. Termites in the ratio 1: 1 (five individuals from each colony) was tested with a combination of workers versus workers, workers versus minor soldiers, workers versus major soldiers, minor soldiers versus minor soldiers and minor soldiers versus major soldiers. Termites from one colony in a pair of workers versus workers, minor soldiers versus minor soldiers and major soldiers versus major soldiers were marked with a dot of white paint on the head capsule to distinguish them from the same individuals of the other colony. Termites were acclimatized in the test arena for 5 min before the separating plastic card was removed. The behaviors were video recorded during encounters (Ricoh WG-4) for 5 min. The videos were reviewed and analyzed for agonistic reactions. The aggression behaviors were leveled from 1-3 based on the definition described by Jmhasly and Leuthold (1999) with slight modifications of Neoh *et al.* (2012): (1) examination or antennation: contact between antenna, head or body (it means no agonistic behavior), (2) alarm reaction or avoidance, jerking, chasing and/or being chased (it means no agonistic behavior) and (3) aggression: mandibles open, seizing or biting (it means no agonistic behavior). The aggression behaviors that occur during interactions for each 10 sec intervals were recorded and a total of 30 observations for each test was made to scale and averaged from five replicates were there.

Chemical analysis of volatile compounds: For volatile sampling used live animals, such as; the method of Matsuura *et al.* (2010) and Himuro *et al.* (2011). A total of 20 individuals of major workers each colonies from Bogor (code W_B), Bandung (code W_D) and Bandar Lampung (code W_L), then 20 individuals of minor soldiers of Bandung's colony (code MS_D) and Bandar Lampung's colony (code MS_L), also 10 individuals of major soldiers from Bogor (code JS_B) and Bandar Lampung (code JS_L), those seven of the samples were washed for 15 min in 100 mL of n-hexane. Then 1 μL

sample leaching results were analyzed using gas chromatography. Gas Chromatography-Mass Spectrophotometer (GC-MS) analyzes of volatiles were performed on Hewlett Packard Agilent 5890, equipped with a capillary column HP-1 column (30 m long×250 µm phase thickness×0.25 µm diameter column). Column temperature was raised from 40°C (5 min hold) at 10°C min⁻¹ to 280°C. The injection was splitless, with helium as the carrier gas (1.5 mL min⁻¹). The MS data was obtained under the following conditions: ionization current, 100 µA; ionization energy, 70 eV; accelerating voltage, 1470.6V; scan range of 40-450 m/z. The gas chromatographic and mass spectrophotometer system were both controlled with a data processing system. Compounds were identified from comparisons of mass spectra with a National Institute of Standards and Technology (NIST) data library system.

Data analyses: Quantitative behavioral test with total 45 combination, i.e., 18 intracolony combinations and 27 intercolony combinations (Table 1), was executed in July, 2014. The aggression behaviors that occur during interactions, such as; examination and antennation (level 1), alarm reaction and avoidance (level 2) and aggression (level 3) were observed. If the behavior on each level occurred ≥80% in any combination then it is expressed as, + means existing and if, this is only ≤20% in any combination then expressed as means no existing.

RESULTS AND DISCUSSION

In general, no agonistic happened intraspecies *M. gilvus*. Caste differences both inter- and intracolony not indicated agonistic behavior, as well as the distance (ranged from 156-210 km) was not significantly to describe about intraspecific on *M. gilvus*. Combination treatment between workers versus workers or workers versus minor soldiers, or workers versus major soldiers, or minor soldier versus minor soldiers and minor soldier versus major soldier did not show any agonistic behavior in the species *M. gilvus*. *M. gilvus* termite colony from Bandung, Bogor, Bandar Lampung with the closest distance 0 km (intracolony) and the longest 210 km (intercolony) did not show any agonistic behavior. No agonistic behavior in *M. gilvus* presented in Table 1.

The scale of agonistic behavior only at the level of alarm/avoidance in the different colonies distance and at the level of examination/antennation on intracolony while the level of aggression, which is a sign of agonistic never occurred in all treatment combinations both intra- and intercolony in intraspecies *M. gilvus*. This is consistent with the results of research Neoh *et al.* (2012) that a combination of worker versus worker intra- and intercolony showed very low intraspecific aggression on *M. gilvus*, whereas in *M. carbonarius* showed little aggression towards non-nestmates. According Kaib *et al.* (2004), colony recognition in *Macrotermes* intraspecies not influenced by morphology, genetics, geography or distance significantly. This is also corroborated by Jmhasly and Leuthold (1999) that aggressive behavior and an examination on major worker *M. subhyalinus* and *M. bellicosus* not correlated with spatial distance intercolony, also not correlated with differences in the volume of the nest and colony age involved. Shelton and Grace (1997) also stated, the same thing that the geographic separation of termite colonies was not a factor in predicting agonism on *Coptotermes formosanus*. Low agonistic response not only happened in intraspecies *Macrotermes* spp. but also occurs on *Reticulitermes* spp. (Grace, 1996; Delphia *et al.*, 2003; Harahap *et al.*, 2005), on *Microcerotermes crassus* (Wong and Lee, 2010) and on *Coptotermes* spp. (Uchima and Grace, 2009; Shelton and Grace, 1997; Cornelius and Osbrink, 2003).

Table 1: Behavioral existence of major workers resulting from different colony combination in *Macrotermes gilvus*

Colony combination	Distances (km)	Behavioral		
		Examination/antennation	Alarm/avoidance	Aggression
W _B vs W _B	0	+	-	-
W _B vs W _D	164	+	+	-
W _B vs W _L	156	+	+	-
W _B vs MS _B	0	+	-	-
W _B vs MS _D	164	+	+	-
W _B vs MS _L	156	+	+	-
W _B vs JS _B	0	+	-	-
W _B vs JS _D	164	+	+	-
W _B vs JS _L	156	+	+	-
W _D vs W _D	0	+	-	-
W _D vs W _L	210	+	+	-
W _D vs MS _B	164	+	+	-
W _D vs MS _D	0	+	-	-
W _D vs MS _L	210	+	+	-
W _D vs JS _B	164	+	+	-
W _D vs JS _D	0	+	-	-
W _D vs JS _L	210	+	+	-
W _L vs W _L	0	+	-	-
W _L vs MS _B	156	+	+	-
W _L vs MS _D	210	+	+	-
W _L vs MS _L	0	+	-	-
W _L vs JS _B	156	+	+	-
W _L vs JS _D	210	+	+	-
W _L vs JS _L	0	+	-	-
MS _B vs MS _B	0	+	-	-
MS _B vs MS _D	164	+	+	-
MS _B vs MS _L	156	+	+	-
MS _B vs JS _B	0	+	-	-
MS _B vs JS _D	164	+	+	-
MS _B vs JS _L	156	+	+	-
MS _D vs MS _D	0	+	-	-
MS _D vs MS _L	210	+	+	-
MS _D vs JS _B	164	+	+	-
MS _D vs JS _D	0	+	-	-
MS _D vs JS _L	210	+	+	-
MS _L vs MS _L	0	+	-	-
MS _L vs JS _B	156	+	+	-
MS _L vs JS _D	210	+	+	-
MS _L vs JS _L	0	+	-	-
JS _B vs JS _B	0	+	-	-
JS _B vs JS _D	164	+	+	-
JS _B vs JS _L	156	+	+	-
JS _D vs JS _D	0	+	-	-
JS _D vs JS _L	210	+	+	-
JS _L vs JS _L	0	+	-	-

Of 22 volatile components were identified, it can be specified that the six main volatile components derived from termites workers *M. gilvus*, 11 main volatile components of minor soldier and 15 main volatile components of major soldier. Compounds detected by GC-MS analysis presented in Table 2.

There are two main volatile components are believed to play an important role in the non-agonistic behavior on *M. gilvus*, namely (Z)-6-octadecenoic acid and (E)-9-octadecenoic acid. Component (Z)-6-octadecenoic acid is contained in the worker caste of termites *M. gilvus* from all

Table 2: Compounds detected by GC-MS analysis from worker, minor soldier, major soldier of *M. gilvus*

Peaks	Compounds	CAS No.	Retention time (min)	Workers			Minorsoldiers		Majorsoldiers	
				W _B	W _D	W _L	MS _D	MS _L	JS _B	JS _L
1	2,6-Diisopropylnaphthalene	24157-81-1	17.60				+			
2	1-hexadecene	629-73-2	18.41				+			
3	n-hexadecanoic acid	57-10-3	20.53		+	+				+
4	Octadecane	593-45-3	20.65							+
5	Eicosane	112-95-8	21.80							+
6	(E)-9-octadecenoic acid	112-79-8	22.20				+	+	+	+
7	Oleic acid	112-80-1	22.26		+	+				
8	(E)-9-Eicosene	74685-29-3	22.45					+		
9	(Z)-6-Octadecenoic acid	593-39-5	22.47	+	+	+			+	
10	ethyl ester octadecanoic acid	111-61-5	22.73					+		
11	Heneicosane	629-94-7	23.40	+	+				+	
12	Docosane	629-97-0	23.71					+	+	+
13	1,1'-oxybis-dodecane	4542-57-8	24.30					+		
14	Heneicosane	629-94-7	24.55					+		+
15	Pentacosane	629-99-2	25.49				+		+	+
16	1-iodo-octadecane	629-93-6	26.64						+	+
17	1-iodo-hexadecane	544-77-4	27.03						+	+
18	Nonahexacontanoic acid	40710-32-5	27.51						+	+
19	Heptacosane	593-49-7	27.98	+				+		
20	Squalene	7683-64-9	27.99		+	+	+		+	
21	Tricosane	638-67-5	28.68						+	
22	Octacosane	630-02-4	29.90							+

W_B, JS_B: Colony from Bogor, W_D, MS_D: Colony from Bandung, W_L, MS_L, JS_L: Colony from bandar lampung, +: Identified, -: Not identified

three colonies and also at major soldiers of Bogor. While (E)-9-octadecenoic acid specifically found on the soldier caste both minor- and major soldiers in all colonies.

This is a new novelty and can explain about the components that play a role in nestmate and non-nestmate recognition intraspecies *M. gilvus*. A similar study of volatile compounds ever done Matsuura *et al.* (2010) and Himuro *et al.* (2011) on the species *Reticulitermes speratus* and *Nasutitermes takasagoensis*. Volatile compounds produced specifically queen on *Reticulitermes speratus* and *Nasutitermes takasagoensis* influence the behavior and physiology of colony members, these compounds have been identified by them i.e., n-butyl-n-butyrate and 2-methyl-1-butanol (in *R. speratus*) and phenylethanol (in *N. takasagoensis*), respectively.

Compounds (Z)-6-octadecenoic acid and (E)-9-octadecenoic acid has a formula C₁₈H₃₄O₂ with molecular weight 282.46 (Fig. 1 and 2). Both chemicals are thought to be a component of chemical messages in communication intra- and intercolony on termites *M. gilvus*. As explained Richard and Hunt (2013), also Trhlin and Rajchard (2011) that the chemical message is the primary mode of communication for the majority of intra-social insect species. Previous research as did by Stuart (1961), Smythe and Coppel (1966), Matsumura *et al.* (1968) and Tokoro *et al.* (1994) also began to assert that the pheromone trail markers on the various types of termites have been identified and played a major role in the introduction of the colony and kin recognition that influence the behavior of the colony in general in terms of sourcing food and defend the colony from predators. Pheromone trail marker or recruitment has been discovered in the 1960's, when three substances has been identified successfully. The first is the (E)-6-cembrene A of *Nasutitermes exitiosus* (Moore, 1966). The substance is not specific to the species because that is also found in *N. walker* and *N. graveolus* (Moore, 1966) and in *Trinervitermes bettonianus* (McDowell and Oloo, 1984). Then (3Z,6Z,8E)-3,6,8-dodecatrienol first identified as a pheromone trail marker of *Reticulitermes virginicus* (Matsumura *et al.*, 1968; Tai *et al.*, 1969).

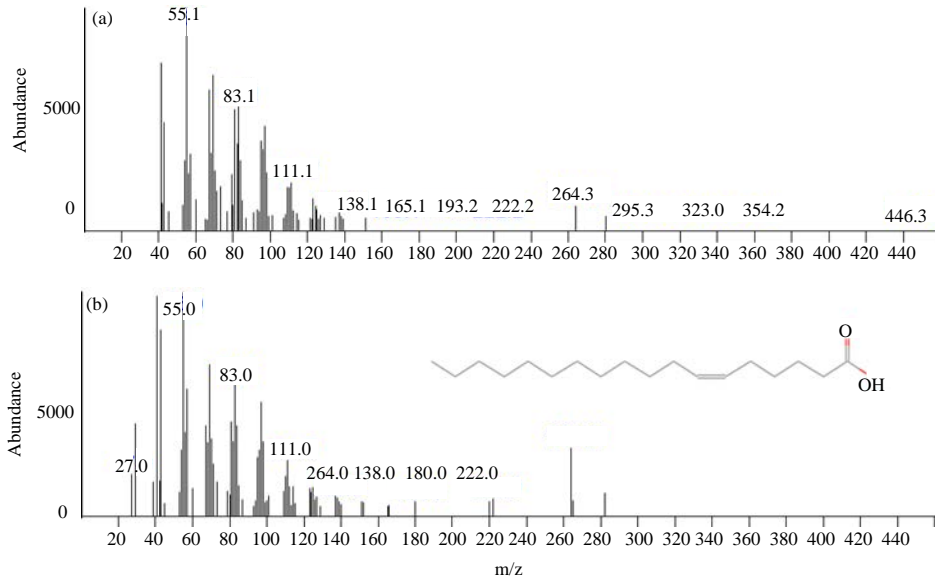


Fig. 1(a-b): Mass spectra of (Z)-6-Octadecenoic acid from workers and soldiers of *M. gilvus* (top) and from a commercial sources (bottom)

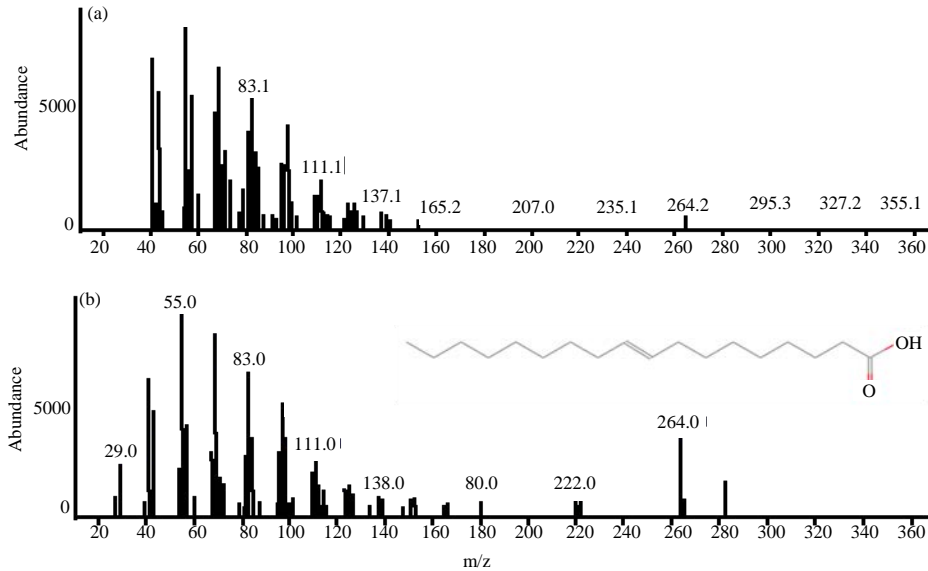


Fig. 2(a-b): Mass spectra of (E)-9-Octadecenoic acid from soldiers of *M. gilvus* (top) and from a commercial sources (bottom)

A novelty of volatile compounds in *M. gilvus* as an important chemical component in a chemical message intraspecies *M. gilvus* expected to expand further research on how to reset the definition of a colony in *M. gilvus* or may be in termite generally. Colony, which is known as a unique organization that does not apply to *M. gilvus* and perhaps more appropriately categorized as, a separate colony as supracolony, where a great distance but still occurs recognition colony members as well as, in intracolony.

CONCLUSION

Agonistic behavior is closely related to the individual chemical components issued in the colony to recognize members of intra- and intercolony intraspecific in *Macrotermes gilvus*. The absence of agonistic in *M. gilvus* both intra- and intercolony associated with volatile compounds, which are owned by the workers and soldiers. Both the main volatile compound is 6-octadecenoic acid, (Z) -and 9-octadecenoic acid, (E), respectively, which are owned by individual workers and soldiers of *M. gilvus* be key intra- and intercolony recognition in the same species.

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