

## Chemical Cues In Tritrophic Interactions On Biocontrol Of Insect Pest

Nurindah Nurindah<sup>1\*</sup>, Surjani Wonorahardjo<sup>2</sup>, Dwi Adi Sunarto<sup>1</sup>, Sujak Sujak<sup>1</sup>

<sup>1</sup> Indonesian Sweetener and Fiber Crops Research Institute, Jl. Raya Karangploso, PO Box 199 Malang, INDONESIA

<sup>2</sup> Department of Chemistry, Faculty of Mathematics and Science, Universitas Negeri Malang (UM), Jl. Semarang 5 Malang, INDONESIA

\*Corresponding authors: nurarindatta@gmail.com

Received 16 June 2016, Revised 31 January 2017, Accepted 8 March 2017

### ABSTRACT

Tritrophic interaction among host plant-herbivore-parasitoid involves chemical cues. The infested plant by herbivores has been reacted to produce volatiles which is a cue used by the herbivore parasitoids for host location. These volatiles can be developed to enhance natural control of insect pests, especially by optimally use of parasitoids. Egg parasitoids are biocontrol agents that play an important role in natural control of herbivores. This research used a tritrophic interaction model of rice plant-brown plant hopper (BPH)-egg parasitoid of BPH. Research on analysis of chemical cues in tritrophic interactions was aimed to identify volatiles that are used by the parasitoid to find its host. The volatiles that effectively affect the parasitoid orientation behavior could be developed into a parasitoid attractant. Extraction of volatiles as the egg parasitoid cues was done using soxhlet, and identification of the volatiles using Gas Chromatography-Mass Spectrometry (GC-MS). Bioassay of the volatiles on the BPH parasitoid orientation behavior was performed using Y-tube olfactometry. The volatiles that are used for host location cues by the parasitoid affect the parasitoid orientation behavior by showing the preference of the parasitoid females to the odor of volatile. Volatiles extracted from BPH-egg-infested plants and uninfested plants contain alcohol, hydrocarbon, and ester compounds. Based on the difference of the compound composition of both extractions, five compounds of long-chain hydrocarbon, both branched and unsaturated compounds are the main volatile components which caused positive orientation behavior of the egg parasitoid. The egg parasitoids showed positive behavior orientation toward the volatiles extracted from BPH-egg-infested plant. Those hydrocarbon compounds are potential materials to be developed into bio attractants of BPH egg parasitoid.

Keywords : plant volatile, hydrocarbon, *Nilavarpata lugens*, Gas Chromatography-Mass Spectrometry (GC-MS)

### INTRODUCTION

Plants have defense mechanism from herbivores infestation by producing volatile compounds to build up a resistant act against the herbivore feeding [1] or as indirect defense which can attract natural enemies [2,3]. Jasmonate acid (JA) and methyl jasmonate (MEJA) or are commonly called jasmonate (JAS) are compounds that have been intensively studied in related to the plant defense mechanism [4]. JA plays an important role as a signal from a plant as a result of plant hopper infestation [5,6,7]. Polyphenol oxidases are produced by a plant after treated with JA derivate [8]. Furthermore, Lu et. al. [9] reported that ethylene was

The journal homepage [www.jpacr.ub.ac.id](http://www.jpacr.ub.ac.id)  
p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

produced by plants after being infested by brown plant hopper (BPH) could attract the BPH egg parasitoid (Figure 1). These reports showed that chemical cues involve in tritrophic interactions.

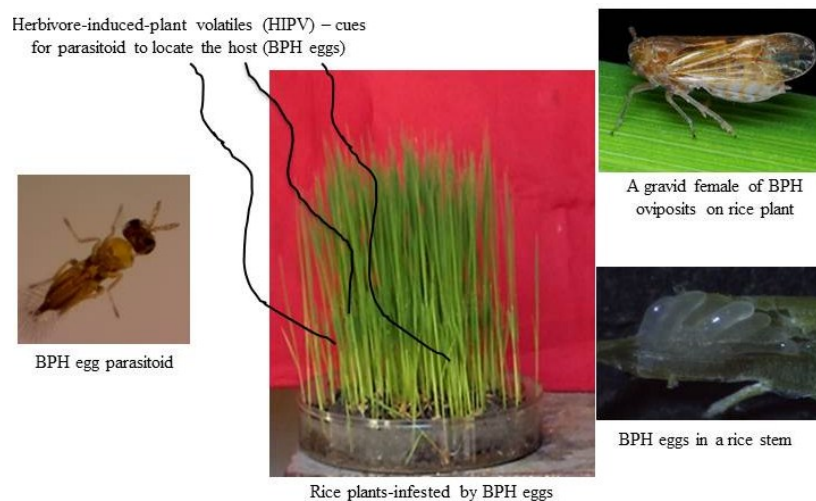


Figure 1. Tritrophic interactions: Rice plant-BPH eggs on rice plant-BPH egg parasitoid

Volatiles produced by plant as a plant reaction against herbivore infestation, and as a cue for the herbivore natural enemies, especially parasitoid, to locate their host, could be developed into an attractant for parasitoid. The attractant could be an environmentally friendly component in biological control of insect pest. A tritrophic interaction model of rice plant–BPH–egg parasitoid of BPH used to investigate host plant-herbivore-parasitoid involves chemical cues. Research on analysis of chemical cues in tritrophic interactions was aimed to identify volatiles that are used by the parasitoid to find its host. Once the volatiles are identified, developing a parasitoid attractant would be conducted. The attractant can be used as a component of biocontrol of the BPH population. The model of this attractant development also can be used for developing similar attractant for other pests of different plants.

This paper discuss about tritrophic interaction among host plant-herbivore-parasitoid *Nilaparvata lugens* involves chemical cues which used a tritrophic interaction model of rice plant-brown plant hopper (BPH)-egg parasitoid of BPH. Analysis of chemical cues in tritrophic interactions is important step to determine volatiles which is used by the parasitoid to be existent its host using Gas Chromatography-Mass Spectrometry (GC-MS).

## EXPERIMENT

### Materials Preparations

Materials preparation comprises of preparing uninfested and infested rice plants, BPH, egg parasitoid of BPH and chemicals for volatiles extraction that has been explained thoroughly in our previous report [10].

## Procedure

Extraction of volatiles for uninfested and the BPH-egg-infested rice plants (each of 100 g) was subjected to soxhlet extraction with 400 mL of methanol as the solvent. Extraction process was done in 10 cycles and then the afforded extract was concentrated using rotary evaporator (Buchi Rotavapor R-114) at 28<sup>o</sup> C for one hour. Initially, the original extract was dark green to brown color, but the final extract after evaporation was dark brown. The concentrated extracts (about 100 mL) were kept separately in a vial (Ø5 mm, h 25 mm) for GC-MS analysis and bioassay.

Identification for the volatile compounds was done by separating each component with gas chromatography and analysis of each component structure using mass spectroscopy. Helium was used as the carrier gas for chromatography with total speed 20 mL/min and column speed 0.5 mL/minutes. Temperature of column was programmed at 60<sup>o</sup>C for 5 minutes and gradually increased to 300<sup>o</sup>C for 41 minutes. Ion source and interface temperature was maintained at 305<sup>o</sup>C and 250<sup>o</sup>C, respectively, with cut time started at minute -3.75. The obtained chromatograms were called according to Library Wiley 8. Compounds with composition > 4% of the total area were categorized as dominant components (for example 3-Eicosyne had 6.95%), while those with similarity index (SI) above 90% for the first suggestions were determined as definite components (for example 3-Eicosyne matched 99% of the one in Wiley 8 database). Those compounds are listed in Table 1.

Bioassay of the BPH-egg parasitoid was conducted using Y-tube olfactometer according to Lou [11] which was modified by Meilin [12]. The olfactometer contains of Y-tube (Ø15 mm), cylinder glass (Ø100 mm, height 200 mm) for volatile source keeper, aerator with capacity of 1.5 mL/min, glass tube contains active carbon as air filter, cylinder glass with humidifier for keeping filtered air, flow meter (series RMA, Rate Master Flow meter, Dwyer Instrument Inc., USA) and 10 watt light source. Bioassays were maintained for 8-11 hr at 25 ± 2<sup>o</sup>C and RH 60-80%.

Validation of the olfactometer for the egg parasitoid orientation behavior to volatiles produced by the BPH-egg-infested plants was done by testing the parasitoids' response to the natural volatiles of BPH-egg-infested plants. The uninfested and infested plants were placed on each odor source. Three batches of 30 female parasitoids were used in this test. The parasitoids were introduced particularly into the Y-tube arm and the orientation of the parasitoid was observed. The observations were limited up to 5 minutes for each parasitoid. Parasitoids that indicated response were showing the orientation to each arm of Y-tube for at least 1 min. Parasitoids orientation toward the arm connected to the infected plant volatiles were categorized showing positive response (R+) and the uninfested plant were categorized showing negative response (R-). Those which did not show orientation to any arm were categorized as no response (NR). Bioassay of the extracted volatiles on the parasitoid orientation behavior was done as this validation test by changing the plants with the extract volatiles. Data analyses of bioassay observations were done using two ways Analysis of Variance, and post-hoc test by using Fisher's Least Significant Difference (LSD) test.

## RESULT AND DISCUSSION

The gas chromatography separation method for methanolic extract of both healthy and infested plant tissues confirmed the volatiles presence after being infested by brown plant hopper. Comparison of both compounds existing can be seen in Figure 2. Besides the biochemistry routes of compounds formations in the process after the stem wounding by the

BPH during oviposition, the extraction method and conditions plays role in obtaining the data.

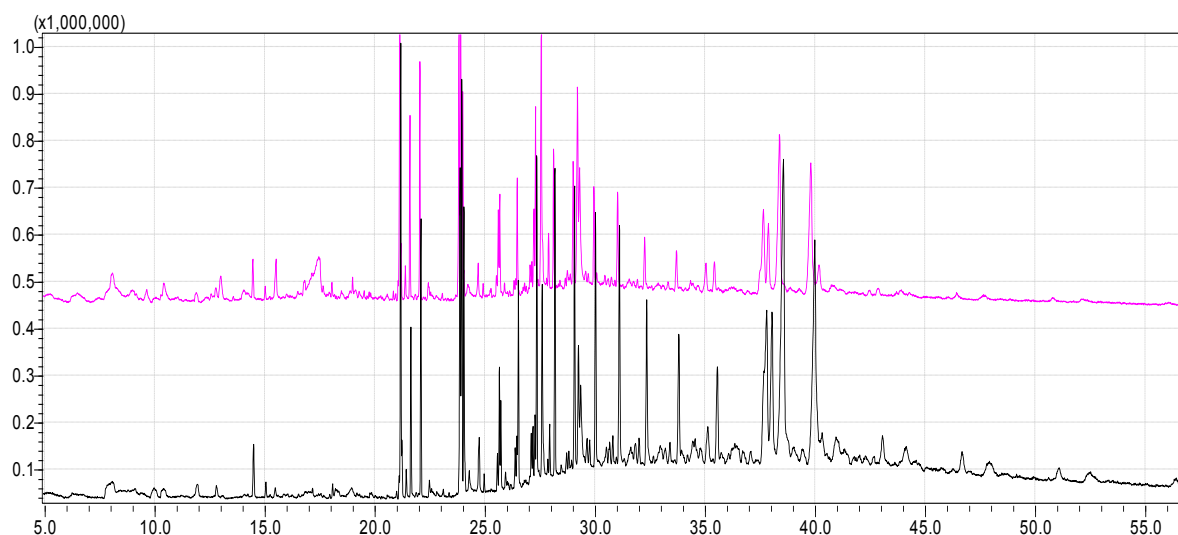


Figure 2. Comparison of chromatograms from healthy (pink curve) and infested paddy (black curve).

It can be seen clearly that the profile of both uninfested healthy and egg-infested paddy were similar to each other. Both of them have the same main volatiles, but the composition altered. The volatiles played important role as the blended substance that released from the surface of wounded stems. However, there was certain tendency to the groups of volatile compounds presence as chemical cues in the tritrophic interactions here.

The composition of volatiles according to Wiley 8 Library can be seen in Table 1 below. However, similarity index of the matching with database were not always high enough to make the convincing determination. There were some doubts left for further investigation of all existing compounds. Some might also be only transitional compounds trapped during extraction but some others could be the real usual components of the plants, such as stigmata components. There were also some compounds present in the healthy plants and disappeared after infestation, on the other side, some appeared in the infested extract. There were new formations in the circumstances after infestation.

Table 1. Composition of volatile compounds extracted from BPH-egg infested and uninfested plants.

Volatile compounds	Area (%)		Similarity Index* (%)	
	Un-infested	Infested	Un-infested	Infested
<b>Alcohol</b>				
stigmasta-5,22-dien-3-ol	5.45	0	98	-
stigmasta-5,24(28)-dien-3-ol, (3.β.,24e)-	5.45	0	98	-
2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	3.68	5.45	99	98
3-Furylmethanol	1.47	0	96	-
phenol, 2,6-dimethoxy-	0.23	3.68	96	96
phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	0.23	3.298	95	95
ethanol, 2-(dimethylamino)-	0	4.028	0	99

2-methoxy-4-vinylphenol	0	3.03	0	99
<b>Hydrocarbon</b>				
3-Eicosyne	6.95	3.99	99	99
eicosane, 7-hexyl-	0	3.50	0	99
2-Hexadecene	0.60	0		0
heptadecane, 3-methyl-	0	4.88	-	92
7-Hexyleicosane	3.54	0		-
1-decene, 8-methyl-	0.65	0	91	-
thanone, 1-(2-hydroxy-5-methylphenyl)-	0.91	0	99	-
docosane	1.28	1.66	92	92
dotriacontane	0	4.38	-	93
heptadecane	0	2.71	-	99
hexatriacontane	4.20	19.72	99	99
octacosane	1.85	4.88	99	99
hentriacontane	2.35	0	98	-
pentacosane	3.37	4.22	98	99
pentatriacontane	8.47	0	99	-
tricosane	6.80	2.71	99	98
tetratetracontane	0	3.42	-	99
<b>Ketone</b>				
17-(1,5-dimethyl-hexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-cyclopenta[a]phenanthren-4-one	6.47	0	98	-
3-hydroxypregn-5-en-20-one	2.98	0	98	-
ethanone, 1-(2-hydroxy-5-methylphenyl)-	0.91	0	98	-
<b>Aldehyde</b>				
benzaldehyde, 4-methyl-	0.61	0	93	-
<b>Ester</b>				
eicosanoic acid, methyl ester	5.24	3.42	99	99
9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-	4.15	2.93	99	98
hexadecanoic acid, 2,3-dihydroxypropyl ester	3.67	0	99	-
9,12-octadecadienoic acid, methyl ester, (e,e)-	3.6	2.35	99	99
9,12-octadecadienoic acid (z,z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.95	0	99	-
9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-	0	2.93	-	99
<b>Carboxylic Acid</b>				
methyl 5-oxo-2-pyrrolidinecarboxylate #	3.37	0	99	-
hexadecanoic acid	3.24	0	99	-
1-isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyloxy)disiloxanyltris(trimethylsilyloxy)orthosilicate #	1.50	0	99	-

SI based on the similarity with standard compounds in Wiley 8 Library

GC-MS analysis of the volatile extracts basically consists of six functional compounds, i.e. alcohol, hydrocarbon, ketone, ester, aldehyde, and carboxylate acids. Composition of compounds extracted from uninfested plants was different with that from the infested plants. Volatiles extracted from the infested plants did not contain ketone, aldehyde and carboxylate acid. The compounds composition of both extracts showed that long-chain hydrocarbon, both branched and unsaturated compounds are exist on volatiles extracted from infested plants (Figure 3). In general, volatiles extracted from infested plants contain new alcohol and hydrocarbon compounds. These new compounds could be a result of the heat, the existence of oxygen, or because of acid condition during extraction process. Lou et al., [11] reported

that n-heptadecane is a dominant component of infected plant by *N. lugens*. The proportion of this compound increased when the plant was infected by the plant hopper. Our chromatograph analysis showed the formation of 7-hexyl-eicosane, 3-methyl-heptadecane, dotriacontane, heptadecane and tetracontane after the plant being infected by the leaf hopper, however these compounds were not a predominant compound of the volatiles produced by the infected plant (Table 1). The plant produced a significant increase of hexatriacontane after being infected by the leaf hopper and this compound was a predominant of the volatiles produced by the infected plant. Naturally, rice contains alcohols, aldehydes, ketones, esters, hydrocarbons, acids, and heterocyclic volatile compounds and hexatriacontane was known as one of hydrocarbon compound found in Indica rice, but not in Japonica rice [13].

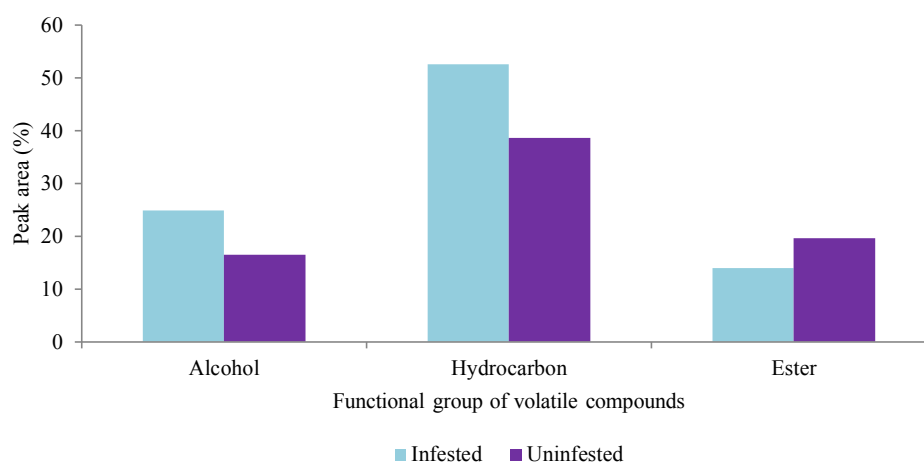


Figure 3. Composition of compounds according to the functional groups of volatiles extracted from uninfested and BPH-egg infested rice plants

Table 2. Orientation behavior of parasitoids toward BPH-eggs infested plants and extract on BPH-eggs infested plants in Y-tube olfactometry

Source of volatiles	Number of parasitoids showed orientation behavior		
	R+	R-	NR
Infested plant	21 c	9 b	0 a
Extract of infested plant	22 c	1 a	7 b

Notes: Values of parasitoid responses on the same row followed by a different letter showed a significant difference ( $P < 0.05$ , Fisher's LSD test) of parasitoid responses. R+: Parasitoids orientation showed positive response, R-: Parasitoids orientation showed negative response, NR: Parasitoids showed no response.

Table 2 also shows that parasitoids mostly oriented toward volatiles extracted from infested plants. This parasitoid orientation behavior showed that the parasitoid recognized the volatiles of infested plants as a cue for host location. This phenomenon has been reported by Lou & Cheng [14] that infested rice plant by BPH was very attracted for the parasitoids. However, the volatile compounds for parasitoid attractant have not been intensively studied.

The egg parasitoid of BPH showed positive orientation toward the volatiles extracted from BPH-egg infested plants. This means that the volatiles could be used as the parasitoid attractant. Rapusas et. al. [15] reported that *Cirtorhinus lividipennis*, the BPH predator was

more attracted to the volatiles of BPH-egg-infested plants instead of nymph-infested plants. This fact indicates that the parasitoid or predator as biocontrol agents use specific chemical cues for host location.

The use of volatiles compound extracted from plants for parasitoid attractant has not been intensively developed in pest management. Meanwhile, this previous research has proven that volatiles produced by a plant as its reaction to a specific way of herbivore infestation were used by specific parasitoids as a cue for host location. Therefore, the volatiles could be a high specificity cues for biocontrol agents. Developing such volatiles for parasitoid attractant would need an understanding both insects ecology as well as chemical ecology in tritrophic interactions. The volatiles for attractant could be developed by involving volatile adsorbent in the attractant formulation.

## CONCLUSION

Volatiles extracted from egg-infested plants were used as chemical cues by egg parasitoid to find its host. The volatiles compounds which mostly play an important role in tritrophic interaction among host plant-herbivore-parasitoid are long-chain hydrocarbon. The volatiles compound, then, could be developed into a parasitoid attractant that can be used as a tool in biocontrol of agricultural pest. The attractant would be a specific species, so that the model could be used for developing other attractants with specificity for other parasitoid species.

## ACKNOWLEDGMENT

This research was funded by Ministry of Research and Technology and Higher Education from National Research Innovation Award Year 2013-2015, batch: RT-2013-773, RT-2014-058, RT-2015-050.

## REFERENCES

- [1] Riyals, J., Uknes, S., Ward, E. *Plant Physiol.* **1994**, *104*, 1109–1112.
- [2] Green, T.R., Ryan, C. A. *Science.* **1972**, *175*, 776–777.
- [3] Paré, P. W., Tumlinson, J. . *Plant Physiol.* **1999**, *121*, 325–331.
- [4] Peña-Cortés H., P., B., F., D., Polanco, V., Sánchez, C., Sánchez, E., Ramírez, I. *J. Plant Growth Regul.* **2005**, *23*, 246–260.
- [5] Thaler, J. S., Stout, M. J., Karban, R., G., D. *Ecol. Entomol.* **2001**, *26*, 312–324.
- [6] Tebayashi, S. I., Horibata, Y., Mikagi, E., T Kashiwagi, Mekuria, D. B., Dekebo, A., Ishihara, A., Kim, C. S. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1521–1526.
- [7] Senthil-Nathan, S., Kalaivani, K., Man-Young, C., Chae-Hon, P. *Pestic. Biochem. Physiol.* **2009**, *95*, 77–84.
- [8] Constabel, C. A., Ryan, C. A. *Phytochemistry* **1998**, *47*, 507–511.
- [9] Lu, Y., Wang, X., Lou, Y., Cheng, J. *Chinese Sci. Bull.* **2006**, *51* (20), 2457–2465.
- [10] Wonorahardjo, S., Nurindah, Sunarto, D. A., Sujak, Zakia, N. *JEI*, **2015**, *12* (1), 48–57.
- [11] Lou, Y.-G., Ma, B., Cheng, J.-A. *J. Chem. Ecol.* **2005**, *31* (10), 2357–2372.
- [12] Meilin, A. Dampak Aplikasi Insektisida pada Parasitoid Telur Wereng Batang Coklat dan Deltametrin Konsentrasi sublethal terhadap *Anagrus nilaparvatae* (Hymenoptera: Mymaridae ), Disertasi, Universitas Gajah Mada, 2012.

- [13] Lin, J.-Y., Fan, W., Gao, Y.-N., Wu, S.-F., Wang, S.-X. *10th Int. Work. Conf. Stored Prod. Prot.* **2010**, 125–134.
- [14] Lou, Y., Cheng, J. *Entomol. J. East China* **1996**, 5, 60–64.
- [15] Rapusas, H. R., Bottrell, D. G., Coll, M. *Biol. Control* **1996**, 6, 394–400.