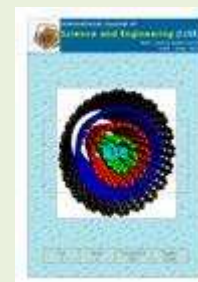




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The Sex Pheromone Content of The *Spodoptera Exigua* (Hubner) Under Artificial and Natural Diets

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Abstract—The control of the *Spodoptera exigua* Hubner (Lepidoptera: noctuidae) through sex pheromones is feasible by mass trapping, mating disruption, and population monitoring. Both synthetic sex pheromones and virgin females were used on the mass trapping and population monitoring methods as attractant source. The virgin females must be available through the mass rearing without affected on its pheromone content production. Therefore, a study on the response of the female's pheromone which reared by artificial and natural diets was important to be done. The GCMS analysis result had shown that female's pheromone glands extract which had reared by natural diets contained tetradecan-1-ol (0.23%) pheromone compounds and several other compounds, such as hexadecane (14.31%), heptadecane (0.42%), nonadecane (1.09%), and beta-caryophyllene (1.37%). Meanwhile, only tridecanol (3.39%), hexadecane (8.52%), nonadecane (0.23%), and trans-caryophyllene (3.11%) compounds had been found on the artificial ones. The cross-copulation test showed that both types of moths could do mating. The field trapping test showed that both extracts were attractive to males

Keywords: Sex pheromone, *Spodoptera exigua*, natural diet, artificial diet(;

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I. INTRODUCTION

Pheromone is a chemical substance extracted by living organisms in order to deliver messages or signals to other individual from similar species. The pheromones should be able to control the insects by disrupting their sex or mating behaviour. The insect's pheromone has also offered a unique opportunity to study the principle of the insect's communications. (Witzgall, 2004). The insect's sex pheromones has become an important pests control and has successfully identified and studied by many researchers. One of them was *S. exigua*. The sex pheromones of the *S. exigua* are Z9E12-14Ac, Z9-14OH, 14OH, E9-14OH, Z9E12-14OH, Z9Z12-14OH, 14Ac, Z9-14Ac, E9-14Ac, Z9Z12-14Ac, Z7-14Ac (Tumlinson, 1982); Z3-6Ac, 2-phenylacetaldehyde (Dong, 2004); Z11-16OH (Acin, et al., 2010).

Insect's controls by sex pheromones can be done either by synthetic pheromones or by the female moths directly. The uses of the female moths become an alternative pests control and has succeeded in the population forecasting and mating disruption in several countries. The efforts of the pheromone traps and female moths in monitoring the *S. exigua* population had been

done in North Florida (Mitchell, 1994), China (Xiang, et al., 2009), and Indonesia (Permana, 2006). The catch from using female moths *S. exigua* is not really different with the standard pheromone traps.

The use of female moths as attractant sources has become a promising prospect of the alternative pests' control. Meanwhile, the sufficient supply of the female moths is still become an obstacle so far. The supply of the female moths must be done through continuously mass rearing by artificial diets. In such a way, so that there's hesitancy whether the female's sex pheromone which reared continuously by artificial diets still get acquainted from the males or not.

II. RESEARCH METHOD

Larvae were field-collected from Yogyakarta, and reared in the laboratory under artificial diet at 12D:12L. This artificial diet was a composition of wheat bran, kidney bean, yeast (fermipan), ascorbate acid, methylparabenzoate (nipagin), L-cysteine, aquades, gelatine, formalin, and water (Singh and Moore, 1985). While the natural diets were 30 days fresh onion leaves. Sex separation was done on pupal stage, emerging adult were

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maintained in plastic jar and provided with dilute honey solution.

The extraction of pheromone glands had been done on the 3 days old virgin female moths at scotophase period. Abdominal tip had been excised, and soaked 1 ml hexane for 10 min. Then the extract had been filtered and concentrated, in order to obtain a concentration of 10 glands/ μ L. Furthermore, the vial had been stored in a refrigerator at -10 °C.

The GC-MS used was the GCMS-QP2010S SHIMAZDU with helium as carrier gas, Electron Ionisation (EI), Restek column RXi-5MS (30 m x 0.25 mm x 0.25 mm) with 0.52 mL/min current. Temperature in the oven column arranged at 50oC in the beginning and hold until 10 min. Next it increased 5oC/min continuously until 120oC. Then the temperature increased again 3oC/min until 190oC, and it hold for 30 min. It was detected by the Flame Thermionic Detector(FTD).

Laboratory tests had been conducted by cross copulation to both female. These tests aimed to know whether female' sex pheromone which reared by artificial diets still acquainted by the male which reared by natural diets or not.

The field observation tests conducted by installing traps which were given female pheromone glands extract reared with both natural diets and artificial diets. As the comparison tools, *S. exigua* synthetic sex pheromone compound had also used as the lure.

III. RESULT AND DISCUSSION

The Sex Pheromone Content:

Through the analysis of the sex pheromone glands of the *S. exigua* which reared by natural diets had shown there were some organic compounds from several group which had C12 until C26. As seen at Table 1. The total ion chromatogram and the mass compound spectrum found on the sex pheromone glands of the *S. exigua* which reared by natural diets can be seen at Table 1.

The *S. exigua* pheromone glands extract which reared with artificial diets contained primary alcohol compounds group and hydrocarbon. The total ion chromatogram and the mass spectrum compounds included in the *S. exigua*'s sex pheromone glands extract reared with artificial diets can be seen at the Figure 2.

Both total ion chromatogram's analysis result and the mass spectrum on the female's pheromone glands extract which reared with natural diets had shown one sex pheromone compound, which was tetradecane-1-ol [14OH] with concentration with 0.23%. On the other hand, on the female's pheromone glands extract which reared by artificial diets, the sex pheromone compound wasn't exist, Tumlinson (1981), found eleven sex pheromone on the *S. exigua*, one of the compounds was 14OH. Tetradecane-1-ol is a minor compound at the sex pheromone component of the *S. exigua*

Table 1. The organic compounds found on the sex pheromone glands of the *S. exigua* which reared by natural and artificial diets.

Compounds	Formula	Natural		Artificial	
		Retention times (minutes)	Area width (%)	Retention times (minutes)	Area width (%)
Naphthalene	C ₁₀ H ₈	15.875	16.55	15.952	37.49
5-Butylnonane	C ₁₃ H ₂₈	-	-	18.786	0.85
Tetradecane	C ₁₄ H ₃₀	18.967	0.02	-	-
11-(1-Ethylpropyl)heneicosane	C ₂₆ H ₅₄	19.120	0.14	-	-
1-Hexanol, 2-ethyl-2-propyl-	C ₁₁ H ₂₄ O	-	-	19.573	2.32
1-Undecene, 7-methyl-	C ₁₂ H ₂₄	19.759	2.89	19.844	2.48
Naphthalene, 2-methyl-	C ₁₁ H ₁₀	-	-	20.014	0.43
Nonane, 5-(2-methylpropyl)-	C ₁₃ H ₂₈	20.123	1.22	19.433	0.13
Nonadecane	C ₁₉ H ₄₀	20.457	1.09	20.775	0.23
alpha.-Copaene	C ₁₅ H ₂₄	21.836	0.63	-	-
Copaene	C ₁₅ H ₂₄	-	-	21.934	1.22
Heptadecane	C ₁₇ H ₃₆	22.642	0.42	-	-
alpha.-Gurjunene	C ₁₅ H ₂₄	-	-	23.059	0.84
beta.-Caryophyllene	C ₁₅ H ₂₄	23.306	1.37	-	-
trans-Caryophyllene	C ₁₅ H ₂₄	-	-	23.404	3.11
Acenaphthylene	C ₁₂ H ₈	24.529	1.53	24.603	0.52
Dodecane, 4,6-dimethyl-	C ₁₄ H ₃₀	24.708	1.38	20.210	0.84
Decane, 2,3,5,8-Tetramethyl-	C ₁₄ H ₃₀	-	-	25.452	1.83
delta.-Cadinene	C ₁₅ H ₂₄	27.065	1.42	27.156	1.15
2-Hexyl-1-octanol	C ₁₄ H ₃₀ O	27.242	6.15	27.972	6.34
Tridecanol	C ₁₃ H ₂₈ O	-	-	27.339	3.39
Hexadecane	C ₁₆ H ₃₄	27.517	14.31	27.559	8.52
Heneicosane, 11-(1-ethylpropyl)-	C ₂₆ H ₅₄	27.880	10.67	-	-
Cyclohexane, 1-ethyl-2-propyl-	C ₁₁ H ₂₂	28.262	5.80	28.355	3.63
Undecane, 3,8-dimethyl-	C ₁₃ H ₂₈	28.778	0.63	-	-
Tetradecane, 4-methyl-	C ₁₅ H ₃₂	29.226	0.75	28.892	0.50
Tetradecan-1-ol	C ₁₄ H ₃₀ O	29.458	0.23	-	-
Pentadecane	C ₁₅ H ₃₂	-	-	29.787	1.69

Other compounds found on the female's pheromone glands extract were known as the sex pheromone compounds and attractant on the lepidoptera ordo and other ordos, both natural and artificial reared.

Nonadecane [19Hy], hexadecane [16Hy], heptadecane [17Hy], and tridecanol [13OH] were known as the sex pheromone compounds and attractants on the lepidoptera ordo. They were the sex pheromone compounds on the Lepidoptera ordo, included noctuidea superfamily. 19Hy and 16Hy had been found at both extract types. On the natural reared female there were 1.09% 19Hy and 14.31% 16Hy found. On the other hand, the artificial reared female had less concentration for those two compounds 0.23% 19Hy and 8.52% 16Hy. The Heptadecane only found on the natural reared female's extract with 0.42% concentration. While the 13OH, only found on the artificial reared female extract with 3.39% concentration.

Based on the amount of the carbon atom and its structure, the compound 5-Butylnonane; Tetradecane; 1-Hexanol, 2-ethyl-2-propyl-; 1-Undecene, 7-methyl-; Nonane, 5-(2-methylpropyl)-; Nonadecane; Heptadecane; Decane, 2,3,5,8-Tetramethyl-; Dodecane, 4,6-dimethyl-; 2-Hexyl-1-octanol; Tridecanol; Hexadecane; Undecane, 3,8-dimethyl-; Tetradecane, 4-methyl-; Tetradecan-1-ol; and Pentadecane include as the sex pheromone compounds group, especially at the lepidoptera. These compounds suspected also had attractant features to the *S. exigua*.

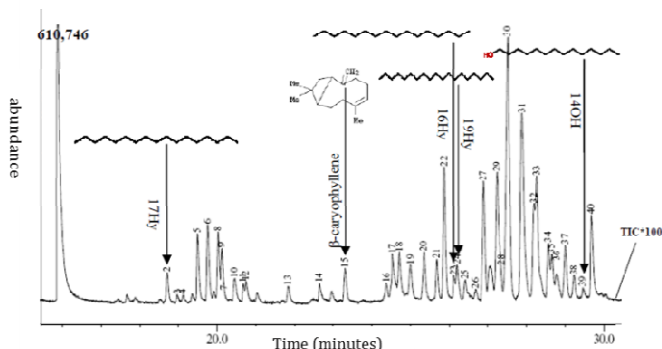


Figure 1. The Chromatogram profile of the total ion on the sex pheromone glands extract of the natural reared *S. Exigua*.

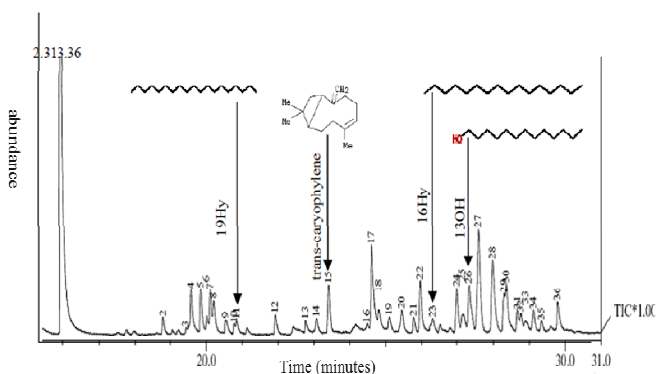


Figure 2. The Chromatogram profile of the total ion on the sex pheromone glands extract of the artificial reared *S. exigua*.

According to Ando et al. (2004), sex pheromone on the lepidoptera ordo based on two compound groups, 75% of the type 1 and 15% type 2 and other compounds 10%. The compound group type 1 based on primary alcohol, and its derivative generations (especially aldehyde and acetate) with straight chains and have 10 until 18 carbonic atoms (C10-C18), also have at least 0-3 double bounds or triple bounds. The compound group type 2 includes unsatiated hydrocarbon, and its derivative generations with straight chains and have 17 until 23 carbonic atoms (C17-C23).

17Hy, 16Hy, 19Hy and 13OH are known as pheromones compound of the lepidoptera. While the trans-caryophyllene has been known as attractant compound of the *S. exigua*. The chromatogram profile of the total ion on these compounds can be seen at Figure 3 and 5.

Nonadecane is a hydrocarbon compound. It has a long opened chain with 19 atoms C. Nonadecane has an important rule as the sex pheromone and allomone on several species and ordos. According to Schneider et al. (1992), nonadecane is pheromone on the *Chromolaena [Eupatorium] odoriferum* (Lepidoptera, Arctiidae). Not only as sex pheromone on the arctiidae family, nonadecane also has similar function to other families of the Lepidoptera ordo, such as: *Acrolepiopsis assectella*, *Acrolepiidae* (Thibout, et al., 1994); *Amauris niavius*, *Danaidae* (Schulz et al., 1993); *Alsophilapometaria*, *Geometridae* (Wong et al., 1984); *Orgyialeucostigma*, *Lymantriidae* (Grant, 1987).

Hexadecane is a hydrocarbon compound. It has a long opened chain with 16 atoms C. This compound is pheromone on the *Acrolepiopsis assectella* Zeller (Thibout et al., 1994), according to El-Sayed, (2011), hexadecane also ruled as pheromone on the hymenoptera: *Formicidae*, *Anthophoridae*; *Thysanoptera*: *Phlaeothripidae*; *Diptera*: *Sciaridae*; *Homoptera*: *Aphididae*, *Aphidinae*; *Coleoptera*: *Tenebrionidae*, *Tenebrioninae*, *Triboliini*; also allomone on the *Coleoptera*, *Carabidae*, *Metriinae*, *Metriini*.

Heptadecane is a hydrocarbon compound. It has a long opened chain with 17 atoms C. Heptadecane is pheromone on the leek moth *Acrolepiopsis assectella*, (*Lepidoptera*, *Acrolepiidae*) (Thibout et al., 1994), *Holomelina lamae* (*lepidoptera*, *Arctiidae*) (Schal et al., 1987), and *Amauris echeria* (*Lepidoptera*, *Danaidae*) (Schulz et al., 1993). According to Schneider et al. (1992) heptadecane and its isomers, like 2me-17Hy and 5me-17Hy, has rule as the sex pheromone on the *Pareuchates pseudoinsulata* Rego Barros (*lepidoptera*, *Arctiidae*). Heptadecane compound also ruled as the pheromone on other ordos such as *Diptera*: *Sciaridae* and *Ceratopogonidae*; *Trichoptera*: *Hydropsychidae*; *Homoptera*, *Aphididae*; *Hymenoptera*: *Colletidae*, *Andrenidae*, *Formicidae*, *Vespidae*, *Anthophoridae*, *Apidae*; *Thysanoptera*: *Phlaeothripidae*; and allomone pada *Coleoptera*, *Carabidae*; *Hymenoptera*, *Anthophoridae* (El-Sayed, 2011).

Tridecanol [13OH] is a primary alcohol compound; it has a long opened chain with 13 atoms C. According to Noguchi et al. (1985), 13OH is one of the sex pheromone

on the *Doxophyes orana fasciata* (Lepidoptera, Tortricidae). While, Srinivasan et al. (2006), *Solanum varium* dan *Licopersicum esculentum* produces 6-tridecanol and some of the volatile compounds which has attractant feature to the *Helicoverpa armigera*, especially when the imago had its oviposition.

Due to the GC-MS analysis result, it had shown the existence of the β -caryophyllene compound on the natural reared insect extract as well as the trans-caryophyllene on the artificial ones. These compound are two of the sesquiterpenoida groups which produced by some plants, suspected have also rule as the attractant to *S. exigua*. According to Haryadi (2009), trans-caryophyllene found on the onion leaves extract. And based on the experiment, tran-caryophyllene has been able to attract *S. exigua* both males and females. Meanwhile, according to Malvar et al. (2008), Terpenoid volatile compounds especially β -caryophyllene, (E)- α -bergamotene and (E)- β -farnesene are secondary metabolite compounds produced by plants in order to defence from herbivore attacks. According to Kollner et al. (2008), β -caryophyllene is quite attractive to parasite insect as well as entomopatogen nematode.

Naphthalene found on the female extract which reared by natural diets with 16.55% concentration and 37.49% concentration on the artificial diets ones. According to El-Sayed, (2011), naphthalene is also attractive to several insect ordos, such as: pheromone on the *Smicrideaannulicornis* (Trichoptera: Hydropsychidae), and *Apisdorsata* (Hymenoptera: Apidae), kairomone on the *Culexquinquefasciatus*, *C. tarsalis* (Diptera: Culicidae), and allomone on *Muscaautumnalis* (Diptera: Muscidae). According to De-Cristofaro, et al. (2000), *Ephestia kuehniella* Zeller (Mediterranean flour moth) gives response to the naphthalene compounds through Electroantennography (EAG) test.

Alpha-Gurjunene found on the female which reared by artificial diets with 0.84% concentration and 1.69% concentration on the natural diets ones. According to Carrasco, et al (2005), alpha-Gurjunene is attractive to Parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae).

Alpha-Copaene found on the female which reared by natural diets with 0.63% concentration. On the other hand, delta-Cadinene found on both extracts with concentration 1.42% and 1.15%. According to Light (1999), both compounds are bisex attractants to codling moth *Cydia pomonella* Tortricidae and several Lepidoptera species.

Nonane-5-Butyl; 11-(1-Ethylpropyl)heneicosane; 2-ethyl-2-propyl-1-Hexanol; 7-methyl-1-Undecene; 2-methyl-Naphthalene; Copaene; Acenaphthylene; 4,6-dimethyl-Dodecane; 2,3,5,8-Tetramethyl-Decane; 2-Hexyl-1-octanol; 1-ethyl-2-propyl-Cyclohexane; 3,8-dimethyl-Undecane; 4-methyl-Tetradecane. These compounds remain unknown for their attractiveness to insect yet. However, according to Ando et al. (2004), some of the lepidoptera family have pheromones which consist of hydrocarbon chains with methyl cluster branches, such as Geometridae, Noctuidae, Lymantriidae, and Arctiidae family. Male's Attraction to the Organic Compounds in the Extract.

The cross breeding test between imagos which reared by natural diets (F0) and the artificial ones (F9) had shown that copulation between both types could be done well, as seen at the Table 6. It indicated that the active sex pheromone compounds on both types had existed. And there were not any alteration happened on the sex pheromone composition, especially on the artificial diets type.

Table 6. The cross breeding test result between the natural diets reared imago (F0) and the artificial diets reared imago at the 9th generation (F9).

Treatment	Copulation	Laying Eggs	Hatch
♀ F9 > < ♂ F0	yes	yes	yes
♀ F0 > < ♂ F9	yes	yes	yes

The field trap test which had been done for three days used the delta trap type. It showed that the extract could attract the male *S. exigua*.

The GC-MS analysis result found that there were not any main sex pheromone components of the *S. exigua* found both with natural reared diets and the artificial ones. However, based on the males anxiety test by the use of the trap, it showed that the organic compounds in the female pheromone glands extract had been able to attract the male ones. Even they were different and had identified before, it also indicated that the existence of either sex pheromone compounds or the attractant compounds existed obviously.

The compounds of the hydrocarbon group which have attractant feature, estimated attractive to the *S. exigua* as well. According to Jurenka (2004), most of the sex pheromone on moth are straight chained compounds with 12, 14, and 16 atomic carbon, its functional compounds consists of alcohol, aldehyde and acetate ester. Due to its volatility, it also indicated that these compounds had also been able used as pheromone. Most of the moths' sex pheromone formed hydrocarbon straight chain and always have odd numbers of atomic carbon.

According to Ando et al. (2004), more than 528 noctuidea super family species have sex pheromone both males and females. The sex pheromone of these species generally contains primary alcohol and its derivative generations (especially aldehyde and acetate), unsaturated hydrocarbon and its derivative epoxy with even chains and a few odd chains. Some pheromones example with the odd chains such as; E4-13:OAc pada *Keiferia lycopersicella*, Gelechiidae, E3-13:OAc on the tobacco stem borer (*Scrobipalpa heliopa*) Gelechiidae, dan Z11-17:OAc on the cabbage armyworm (*Mamestra brassicae*) Noctuidae.

CONCLUSION

Artificial diets didn't have any effect to the sex pheromone content of the *S. Exigua*. The female's pheromone glands extract which reared both by natural diets and artificial diets until 10th generation (F₁₀) have similar lure to the male *S. exigua*.

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