Study on metabolic response of female and male *Trogoderma variabile* (Ballion) on different host grain using direct immersion solid-phase microextraction coupled with gas chromatography mass

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Abstract

The purpose of this study is to use the technique of gas chromatography coupled with mass spectrometry to study the metabolite profile of *Trogoderma variabile* using different host grains including canola, oats, wheat, and barley. Also, hydrocarbon profiling can be used as a chemo-taxonomical tool for insect species identification, especially for very morphologically similar species like *T. granarium*. For sample preparation insects were subjected to extraction with acetonitrile. Direct Immersion-Solid Phase Microextraction (DI-SPME) was employed, followed by Gas Chromatography-Mass Spectrometry analysis (GC-MS) for the collection, separation, and identification of compounds. Additionally, insect host grains have a significant effect on the insect chemicals that are identified from *T. variabile* adults such as fatty acid and hydrocarbons. Results showed that insect host grains have a significant influence on the chemical compounds that are identified in females and males. There were twenty-three compounds were identified from adults reared on canola and wheat. However, there were 26 and 28 compounds detected from adults reared on oats and barley respectively. Results also showed that 11-methylpentacosane; 13-methylheptacosane; heptacosane; docosane, 1-iodo- and nonacosane were the most significant compounds that identified form T. variabile male reared on different host grains. However, the main compounds identified from female cultured on different host grains include docosane, 1-iodo-; 1-butylamine, N-butyl-; oleic acid; heptacosane; 13-methylheptacosane; hexacosane; nonacosane; 2-methyloctacosane; n-hexadecanoic acid and docosane in the female samples.

Keywords: *Trogoderma variabile,* warehouse beetle, insect lipids, hydrocarbons fatty acids, Direct Immersion, metabolites grains.

Introduction

Trogoderma variabile (Ballion) or warehouse beetle, (Coleoptera: Dermestidae), is an internationally significant invasive pest that attacks a wide range of packed goods and stored grains [1]. Nowadays, *T. variabile* has been regarded as a persistent pest of grain storage and handling structures. Warehouse beetles are primary voracious feeders that infect variety of products such as cereal products, candy cocoa, corn, corn meal, dog food (dried and 'burgers'), fishmeal, flour, oatmeal, milk powder, spaghetti, spices, peas, wheat, barley and pollen. In grains, they can't feed on whole grain, but can feed on broken kernels that are usually present in the store [2]. Larvaes of *T. variabile* can infest 119 of different kinds of commodities [3].

Lipids are compounds that are naturally excreted in animals and plants [4]. The significance of lipids is not only in their role as a main source of energy but also as an essential part of the cell membrane [5]. Lipids composition occurs naturally for performing an essential role in the metabolism of insects and plants [4,6]. Insects commonly contain a high content of lipids, making up 50-75% of the dry weight in some insects [7,8]. Studies mentioned that the season of field collection, geographical origin of strain, genetic background, and number of generations has an effect on lipid content of lesser grain borer, *Rhyzopertha dominica* [9]. These factors affect the composition of different types of compounds, such as long chain hydrocarbons, waxes, alcohols, aldehydes and free fatty acids. Lipid types and content in insects vary according to the life stages and insect species. Total lipid content for grasshoppers and other related species (Orthoptera) is a relatively low; ranging from 3.8 g to 5.3 g/100 g fresh insects. In contrast, caterpillars (Lepidoptera) ranges from 8.6 to 15.2 g/100 g fresh [10]. Other studies observed that the fat content of yellow mealworms was strongly affected by the different protein and starch content of their diets, suggesting that larvae fed with a low nutritional quality diet probably use fat reserves for energy, thereby reducing fat content [11,12]. Long chain fatty acids, such as palmitoleic, palmitic, stearic, linoleic, and oleic acids have been found in the cuticular extracts and exocrine secretions of many insects [13].

The development of analytical technology with powerful qualitative and quantitative capabilities, as well as high specificity, are essential for the study of metabolic samples. Previous studies showed that Solid-Phase Microextraction (SPME) coupled with GC has been used because it provides an efficient method to detect chemicals [14,16]. Proving that SPME technique is a cheaper, easier and faster, so it can be used as an alternative extraction method [17]. Also, SPME has been used to extract cuticular hydrocarbons from ants [18]. The SPME technique coupled with GC-MS has also been used to detect long-chain free fatty acids from insect exocrine glands [19].

This study investigates the feasibility of using high-resolution DI-SPME coupled with GC-MS for profiling of *T. variabile* adults. DI-SPME is more sensitive compared with HS-SPME, and it is the method of choice for the analysis of clean aqueous samples [20]. The two extraction modes were evaluated and, despite being less sensitive

than HS-SPME in the case of the more volatile compounds, DI-SPME mode successfully extracted 16 pesticides, compared to HS-SPME which was able to extract only 12 compounds [21]. In previous studies, eight solvents were used to extract lipids *Tribolium castaneum* and *Rhyzopertha dominica* and acetonitrile extract showed the highest peak numbers with 41 compounds; including some of the fatty acids and hydrocarbon waxes [22].

Numerous tools have been used to identify *Trogoderma spp*. such as genetic tools, morphological and taxonomic keys. However, these methods are expensive and inefficient because it takes time for identification and need professional taxonomic staff. Also, insect hydrocarbons could be used as an alternative method when the taxonomical identification of the insect is not feasible due to its damaged condition or if its DNA is too degraded [23].

The aim of this paper is to use the technique of gas chromatography coupled to mass spectrometry (GC–MS) to study the metabolism of *T. variabile* that reared on different host grains including canola, oats, wheat, and barley grains and use the hydrocarbons chemicals for insect identification.

Materials and Methods

Insect culture

Trogoderma variabile was obtained from the Post-Harvest Plant Biosecurity and Food Safety laboratory, School of Science, Health, Engineering and Education, Murdoch University, Murdoch, Western Australia, Australia. To get adult females and males of *T. variabile*, 150 adults were added into 1L plastic jars containing 450g of sterilized canola, oats, wheat, and barley separately and then the jars were covered with a meshed lid. Prior to usage the insect food was sterilized by keeping it at -20°C for five days using 4L glass jars and then maintained the jars at 4°C until used. Before using the insect food for culture it was thawed at room temperature. The insects were reared in a controlled room with 29 ± 2°C and 70 ± 2% relative humidity .

Apparatus and equipment

Gas chromatography GC-MS 7890B equipped with a 5977B MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The Agilent HP-5MS column (30m, 0.25mm, 0.25µm film thickness) were used in the experiments. Helium was used as a carrier gas with 99.99% v/v purity (BOC, Sydney, Australia). GC-MS operation conditions were as follows: injector port temperature was 270°C. The initial oven temperature was 60 °C with an increase to 270 °C (increasement of 5°C/min). MS Quad at 150°C; MS source at 230°C; pressure at 10.2 psi. The flow rate of the column was 1:1 ml/min, while the split less was 30 ml/min at 1.2 min. The total run time of GC-MS was 54 min .

The extraction and analysis method

Adults of *T. variabile* reared on different grains (canola, oats, wheat, and barley) were used in the trials. One adult male or female from each host grains was separately transferred into 2mL plastic microtube (Benchmark Scientific, From Sigma-Aldrich,

lot no.3110, USA). Then, two milling balls were added. After that, 200µL of acetonitrile ≥ 99.9 v/v (HPLC grade, fisher chemical scientific, Glee, Belgium) was added to the microtube using micropipette and homogenized for two minutes using BeadBug microtube homogenizer. The extract was centrifuged at 8150× g for three minutes by Dynamica mini centrifuge (Model no. velocity 13µ), and transferred to 300µl insert glass (Thermo scientific micro-insert, 31x6mm clear glass, 15mm top) placed into 2000µL clear screw HPLC vial (Agilent Technology, China) using micropipette. Finally, theSPME fibre 50/30µm with 2cm DVB/ CAR/ PDMS coating (Sigma-Aldrich, Bellefonte, PA, USA) was inserted into extracted samples for 16 hours in the room temperature 25 ± 2°C. After that, the fibre was withdrawn and removed from the vial and immediately introduced into the GC-MS injector port for thermal desorption .

Data collection and analysis

The GC–MS signals were collected by the Mass Hunter Acquisition software (Agilent Technologies, Santa Clara, CA, USA). The National Institute of Standards and Technology (NIST) mass spectra library was used to identify chemical compounds. The retention index was used to assist identification. The experiment was repeated three times to confirm the chemicals. The area, which represents each peak in the chromatogram, was extracted using Mass Hunter Acquisition software (quantitative analysis) B.06.00 (Agilent Technology, USA). After selecting the compounds, peak area of each compounds was generated to Microsoft Excel 2016, which was also used for data arrangement and sorting. Data were statistically analyzed using MetaboAnalyst version 4
https://www.metaboanalyst.ca/MetaboAnalyst/upload/StatUploadView.xhtml

Results and Discussion

Effect of insect gender of *T. variabile* on the compound production

Results in Table 1 showed that *T. variabile* cultured on canola produced overall 23 compounds from female and male, also, differences in the number of compounds are gender specific. Female yielded 20 compounds while male yielded 22 compounds. Sixteen compounds showed a significant difference which were 1,2-benzisothiazole; 2-decenal, (E)-; heptadecane; methoxyacetic acid, 2-tridecyl ester; 1-decanol, 2-hexyl-; n-hexadecanoic acid; oleic acid; docosane; tetracosane; heptadecane, 9-octyl-; pentacosane; 11-methylpentacosane; 2-methylhexacosane; hexacosane; heptacosane; docosane, 1-iodo-; 13-methylheptacosane; 2-methyloctacosane and nonacosane. Some compounds were only detected in male including heptadecane; methoxyacetic acid, 2-tridecyl ester and docosane, 1-iodo.-

Furthermore, results in table 1 showed that rearing insects on oats affected the quantity, quality, and number of the compounds produced by female and male. DI-SPME and GC-MS method extracted and detected overall 26 compounds from both genders. Results showed that 22 and 23 compounds were identified from the female and male respectively. Statistical analysis revealed that there were significant differences in the GC-MS response (peak areas) using insect samples collected from oat such as nonanal;

decanal; 2-decenal, (E)-; 2-undecenal; dodecanal; caryophyllene; 1-decanol, 2-hexyl-; pentadecanoic acid; oleic acid; docosane; heptadecane, 9-hexyl-; tetracosane; heptadecane, 9-octyl-; pentacosane; 11-methylpentacosane; hexacosane; heptacosane; 13-methylheptacosane; 2-methyloctacosane and nonacosane. However, some compounds were identified from male which were 2-decenal, (E)-; 2-undecenal; dodecanal and pentacosane while nonanal and decanal were only detected from female reared on oats and not from male (table 1).

There were 23 compounds obtained from both female and male reared on wheat. Fourteen compounds were significantly different between these two genders including tetradecanoic acid; n-hexadecanoic acid; nonadecanoic acid; oleic acid; tricosane, 2-methyl-; tetracosane; 11-methylpentacosane; 2-methylhexacosane; hexacosane; heptacosane; docosane, 1-iodo-; 13-methylheptacosane; 2-methyloctacosane and nonacosane (table).

In the case of female and male reared on barley, results in table 1 showed that there were differences among compounds for each gender. Some of the compounds detected in female, were found to be absent in male. From 28 compounds in total detected from T. variabile adults reared on barley, 23 compounds produced by the female. Many compounds were detected in male but not in female and these included hexadecanes; decanoic acid, hexyl ester; 2-hexadecanol; heptadecane and 1-decanol, 2-hexyl-. However, 22 compounds showed a significant difference such as 1-butanamine, N-butyl-; 2-decenal, (E)-; hexadecane; decanoic acid, hexyl ester; 2-hexadecanol; heptadecane; 1-decanol, 2-hexyl-; pentadecanoic acid; nonadecanoic acid; oleic acid; docosane; heptadecane, 9-hexyl-; tricosane, 2-methyl-; heptadecane, 9-octyl-; pentacosane; 11-methylpentacosane; 2-methylhexacosane; hexacosane; docosane, 1-iodo-; 13-methylheptacosane; 2-methyloctacosane and nonacosane. This study has focused on the metabolism of T. varibile adults, which reared on different host grains including canola, oats, wheat and barley grains .

The results also showed that there were three compounds identified from male and not detectd in female cultured on canola including heptadecane; methoxyacetic acid, 2-tridecyl ester and docosane, 1-iodo- while1-decanol, 2-hexyl- were identified from female . In case of male reared on oats, nonanal; decanal and caryophyllene identified from male only compared with 2-decenal, (E)-; 2-undecenal; Dodecanal and pentacosane were identified from female only. However, five compounds were detected from male cultured on barley which is hexadecane; decanoic acid, hexyl ester; 2-hexadecanol; heptadecane and 1-decanol, 2-hexyl- (table 1).

Our results confirmed that there was a significant difference in the chemical compounds between female and male. This finding was agreed with data that collected by Howard (1992) [24] where there study confirmed that there were significant differences between T. variabile genders lipids content. Furthermore, differences in lipid content were found between adult males and females when species were separated by sex [25].

The current study showed that male tend to produce more compounds than female. Results showed that 22, 23, 23, 28 compounds were detected from male reared on canola, oats, wheat and barley respectively. Our data is incosistent with Kinn et al. (1994) [26] study where they found that females of Dendroctonus frontalis were heavier, had more lipid. Where Kinn et al. (1994) [26] confirmed that lipids content between genders varied based on their activity such as flying. Beetles that tend to fly have more lipids compared with others lowest lipids content [27].

Our data showed that chemical compounds identified from female and male were qualitatively similar, while showing appreciable quantitative differences between them. Previous studies marked that females and males, had similar chemicals components but in different proportions [28]. In addition, insects lipid allocation was varied between female and male and that agreed with the results collected by Lease and wolf (2011) [25]. Furthermore, the chemicals components profiles especially hydrocarbon of male and female Bagrada hilaris were qualitatively equal but marked sex-specific quantitative differences were observed for some of the linear alkanes [29].

As hydrocarbons used in many previous studies as a reliable chemotaxonomically tool for classification of insect species [30,31]. Therefore itproposes that the results of chemical compounds that identified in this study especially hydrocarbons might be useful as a taxonomy tool between T. variabile and other species like T. granarium. However, no data areavailable for comparison because of unavailability of T. granarium culture in Australia .

The identification of the insect's species according to their hydrocarbon composition demonstrates that is a highly reliable tool in insect taxonomy and play an important role in chemotaxonomy [32,33]. The lipids considered a successful diagnostic tool for the identification of insect, especially hydrocarbons which are biochemical characteristics and chemotaxonomic tools for identification of insects [13, 23, 34, 35]. Soares et al. (2017) [36] investigated that some compounds were identified in three species of Mischocyttarus (Hymenoptera: Vespidae) Mischocyttarus consimilis, M. bertonii, and M. latior and these compounds include heneicosane, docosane, pentacosane, octacosane, hexacosane, 2-methylhexacosane, 2-methyloctacosane. The compounds of henicosane, oleic acid, docosane, tricosane, tetracosane, pentacosane, hexacosane, octacosane, 2-methylhexacosane, 13-methylheptacosane and nonacosane were reported in Tribolium castaneum (Herbst) and Rhyzopertha dominica [22]. Oleic acid was also identfied to be the primary fatty acid in the larvae of Oryctes rhinoceros, Imbrasia belina, and Rhynchophorus phoenicis [37,38].

Table (1): showed the peak areas (10^{-5}) of compounds that detected from *T. variabile* male and female reared on canola, oats, wheat, and barley

compounds	Features	KI NIST	KI	Canola		Oats		Wheat		Barley	
				Female	Male	Female	Male	Female	Male	Female	Male
1-butanamine, N-butyl-	7.59_129.15	1015	948.6	51.86±20.49	50.32±1.16	77.68±14.93	55.12±13.99	37.00±7.97	43.03±5.32	292.29±0.00	50.11±6.56*
nonanal	15.72_142.13	1104	117.7	n.d.	n.d.	4.46±0.93	n.d.*	n.d.	n.d.	2.37±0.23	1.87±0.12*
decanal	19.58_156.15	1204	1164.2	4.92±0.61	1.98±1.13*	3.49±0.45	n.d.*	n.d.	n.d.	n.d.	n.d.
1,2-benzisothiazole	20.21_135.01	1208	1200.4	2.04±0.73	6.41±1.19*	2.50±0.24	4.35±1.14	n.d.	n.d.	n.d.	n.d.
2-decenal, (E)-	21.53_154.25	1212	1202.1	n.d.	n.d.	n.d.	4.46±0.61*	n.d.	n.d.	7.98±0.15	0.77±0.20*
2-undecenal	24.42_168.15	1311	1325.8	n.d.	n.d.	n.d.	2.50±0.07*	n.d.	n.d.	n.d.	n.d.
dodecanal	25.77_184.18	1402	1408.8	n.d.	n.d.	n.d.	1.91±0.35*	n.d.	n.d.	n.d.	n.d.
caryophyllene	25.95_204.18	1494	1489.7	n.d.	n.d.	1.86±0.58	n.d.*	3.34±0.61	2.32±0.80	2.30±0.59	2.00±0.87
hexadecane	30.38_226.26	1612	1560.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.24±0.16*
tetradecanal	30.67_212.21	1601	1601.3	4.52±0.62	2.72±1.17	n.d.	n.d.	4.33±1.00	3.03±0.21	n.d.	n.d.
decanoic acid, hexyl ester	31.27_256.24	1779	1629.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.60±0.28*
2-hexadecanol	32.11_242.26	1774	1704	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.72±0.06*
heptadecane	32.65_240.28	1711	1669.4	n.d.	1.35±0.11*	n.d.	n.d.	n.d.	n.d.	n.d.	2.27±0.18*
tetradecanoic acid	34.05_282.20	1769	1778.8	n.d.	n.d.	17.89±11.38	4.80±0.25	6.97±0.69	2.21±0.02*	4.72±1.02	2.55±1.25
methoxyacetic acid, 2-tridecyl ester	34.69_272.23	1791	1780.3	n.d.	2.17±0.56*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-decanol, 2-hexyl-	34.85_242.26	1790	1854.1	2.67±0.97	n.d.*	1.98±1.15	5.16±0.56*	5.98±1.72	4.32±0.65	n.d.	4.04±0.28*
pentadecanoic acid	36.16_242.22	1869	1890.3	n.d.	n.d.	36.19±6.10	11.19±4.27*	4.58±1.45	4.18±0.31	9.27±0.69	1.22±0.06*
n-hexadecanoic acid	38.24_256.24	1968	2012.3	27.6±5.53	32.77±5.96	136.64±17.45	123.27±28.93	118.54±13.51	91.26±4.32*	130.46±27.33	73.33±2.42*
nonadecanoic acid	40.14_188.22	2266	2209.9	n.d.	n.d.	n.d.	n.d.	5.20±2.49	25.31±3.82*	1.74±0.25	4.98±4.40
oleic acid	41.58_282.25	2175	2171.9	5.20±0.76	34.38±1.65*	273.15±11.20	21.40±7.94*	57.02±3.32	41.62±0.54*	44.31±3.00	28.04±5.68*
docosane	44.07_310.35	2228	2230.2	57.2±7.49	89.62±9.38*	27.49±2.31	138.22±1.31*	183.01±32.86	226.47±9.73	65.55±9.92	181.15±7.82*
heptadecane, 9-hexyl-	44.76_324.37	2413	2308	69.3±8.18	62.54±2.55	37.93±8.43	180.55±14.86*	158.62±22.55	130.85±16.11	135.41±17.92	214.12±3.40*
tricosane, 2-methyl-	45.35_338.39	2343	2398.7	n.d.	n.d.	n.d.	n.d.	28.05±2.19	6.60±3.73*	26.77±3.41	18.58±4.25
tetracosane	45.77_338.39	2407	2412.6	10.6±0.66	20.46±3.11*	28.20±2.21	19.01±2.75*	31.24±5.77	27.27±2.75	33.39±1.98	38.64±1.26*
heptadecane, 9-octyl-	46.22_352.40	2442	2449.9	20.4±3.54	22.83±0.64	10.36±6.20	56.43±7.38*	37.71±10.26	52.16±8.11	30.41±1.67	89.77±4.93*

pentacosane	47.17_352.40	2506	2501.6	17.2±4.84	103.99±2.68*	n.d.	165.10±26.26*	44.56±12.62	187.61±10.4*	41.52±5.36	209.31±9.82*
11-methylpentacosane	47.85_366.42	2542	2533.7	72.5±10.6	585.63±13.16*	59.56±8.55	5.71±0.17*	222.97±8.95	1353.±28.4*	171.41±7.45	931.22±15.7*
2-methylhexacosane	48.42_380.43	2641	2566.3	51.9±11.8	9.46±2.90*	13.58±4.41	20.37±12.44	154.95±22.33	39.15±12.5*	118.15±16.60	25.11±2.40*
hexacosane	48.98_366.42	2606	2610.4	427.±26.4	48.71±21.85*	282.85±18.33	108.90±3.54*	745.58±22.50	83.89±6.87*	597.08±15.63	117.95±11.6*
heptacosane	50.16_380.43	2705	2666	179.±7.28	130.61±11.37*	94.72±1.53	119.56±6.89*	439.06±25.03	661.69±5.26*	329.46±25.75	343.69±17.7
docosane, 1-iodo-	50.25_436.25	2622	2611.5	n.d.	217.95±11.37*	112.64±34.64	132.84±9.18	412.46±20.80	127.75±2.26*	172.70±12.53	272.96±15.8*
13-methylheptacosane	50.67_394.45	2740	2692.5	147.±27.9	76.67±11.44*	9.00±0.55	540.74±14.85*	385.43±9.02	351.7±18.3*	170.53±20.57	235.55±11.52*
octacosane	51.13_394.45	2840	2718.6	54.20±14.44	57.21±8.73	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-methyloctacosane	51.31_408.46	2840	2723.6	76.1±10.6	54.55±10.13	47.45±6.47	97.60±14.70*	177.90±19.17	132.77±14.65*	195.80±18.67	77.75±15.4*
nonacosane	52.91_408.46	2904	2846.1	123.±10.7	78.90±2.06*	42.01±5.31	364.20±4.86*	249.27±14.01	155.79±8.16*	198.61±3.39	43.67±13.2*

*Means significant different between male and female in each host grain. Feature ID includes retention time (min) and m/z ratio; RI NIST is retention index from National Institute of Standards and Technology database (NIST); RI is retention index calculated by running n-alkane standard C7-C40; n.d is not detected.

Effect of host grains (canola, oats, wheat and barley) on the compound production

The principle components analysis (PCAs) showed the effect of host type on quality and quantity of the chemical compounds (figures 1 and 2). The PCAs in score plot describe the differentiation among the host grain (figures 1a and 2a). According to the graph, the separation was obvious among all the diet types. However, the most intensive differentiation in female and male samples was between oats and other grain types. The loading plots in figures 1b and 2b showed the most important compounds that significantly participated in the differentiation among the diet types. Results showed that docosane, 1-iodo-; 1-butanamine, N-butyl-; oleic acid; heptacosane; 13-methylheptacosane; hexacosane; nonacosane; 2-methyloctacosane; n-hexadecanoic acid and docosane in the female samples (figure 1b). While 11-methylpentacosane; 13-methylheptacosane; heptacosane; docosane, 1-iodo- and nonacosane were the most significant compounds that identified form T. variabile male (figure 2b). The results showed that insect host grains have a significant effect on the chemical compounds such as fatty acid and hydrocarbons. The number of extracted compounds from different host grains varied, whereas barley produced the highest compound number compared to the other host grains. In addition, the host grains influenced the peak area of some compounds .

Results in table 2 showed that the number of compounds detected from female and male reread on different diets. The female results showed that there were 15 compounds detected in all kind of host grains from both genders 1-butanamine, N-butyl-; n-hexadecanoic acid; oleic acid; docosane; heptadecane, 9-hexyl-; tetracosane; heptadecane, 9-octyl-;11-methylpentacosane; 2-methylhexacosane; hexacosane; heptacosane; docosane, 1-iodo-; 13-methylheptacosane; 2-methyloctacosane and nonacosane. The results showed that octacosane and methoxyacetic acid, 2-tridecyl ester were idenfied from the T. variabile reared on canola compared with two compounds detected from oats that include 2-undecenal and dodecanal. Futhermore, there were four comounds identified from T. variabile reared on barley such as nonanal; hexadecane; decanoic acid, hexyl ester; 2-hexadecanol.

However, three compounds were detected in canola which is not detected in other grains, such as decanal; methoxyacetic acid, 2-tridecyl ester and octacosane while two detected in oats, for example: 2-2-undecenal and dodecanal. In case of barely, our results in table 2 showed that three compounds were detected in barley including Hexadecane; decanoic acid, hexyl ester and 2-hexadecanol.

Our findings consistent with the data that collected in previous studies that showed the significant effect of different host grains on the lipids content of T. garanarium larvae [39]. Also, our results agreed with other previous studies where the extracted lipids of insects strongly affected by their vary host grains [40,41]. The diet of insects is mainly responsible for the variations in the lipids and Fatty Acids (FAs) composition of the insects [30,42]. Other studies showed that diet appears to be another factor that influences the fat content of insects. A comparison of the fat content of the wild orthopteran Heteracris littoralis, at 8.2%, with captive-bred orthopterans (Acheta

domestica, Gryllus assimilis and Locusta migratoria), with a higher proportion of fat, suggests that diet could affect lipid content [30,40]. The data obtained in this experiment agree with (Justi et al., 2003) [43] who showed that fatty acids content of insects is more dependent on diet. Other studies showed that different diet can lead to differences in lipids profile in some species [44,45].

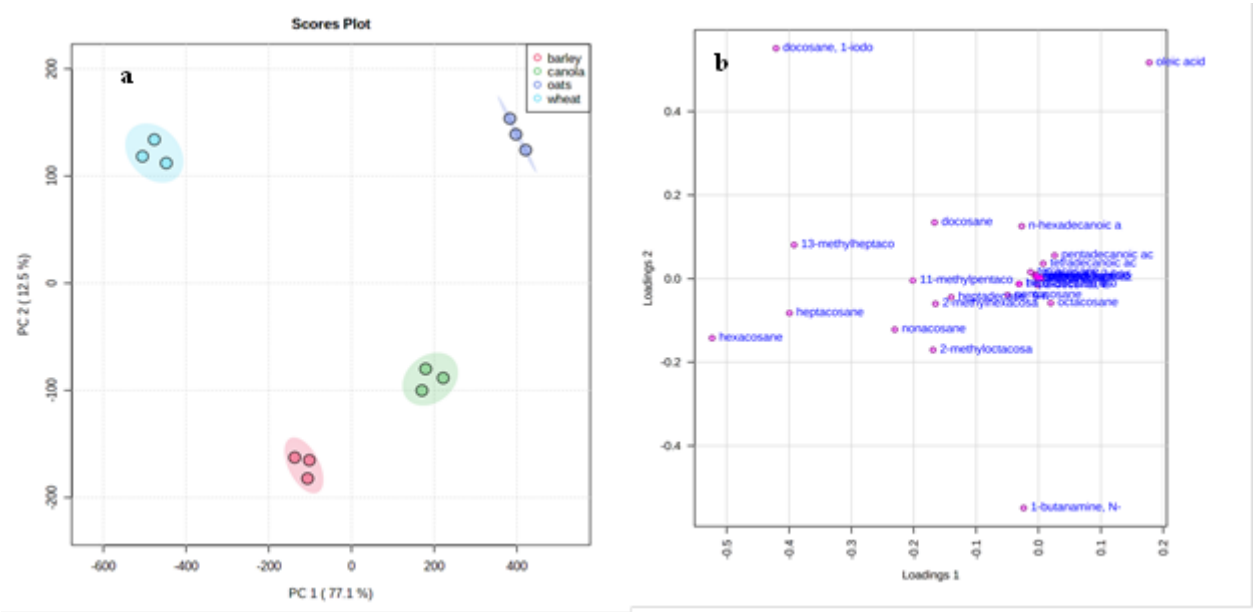

Figure (1a): Score plot of thePCA for chemical compounds obtained from *T. variabile* female reared on different host grains (canola, oats, wheat and barley), 1b. loading plot shows the most significant compounds that participated in the differentiation

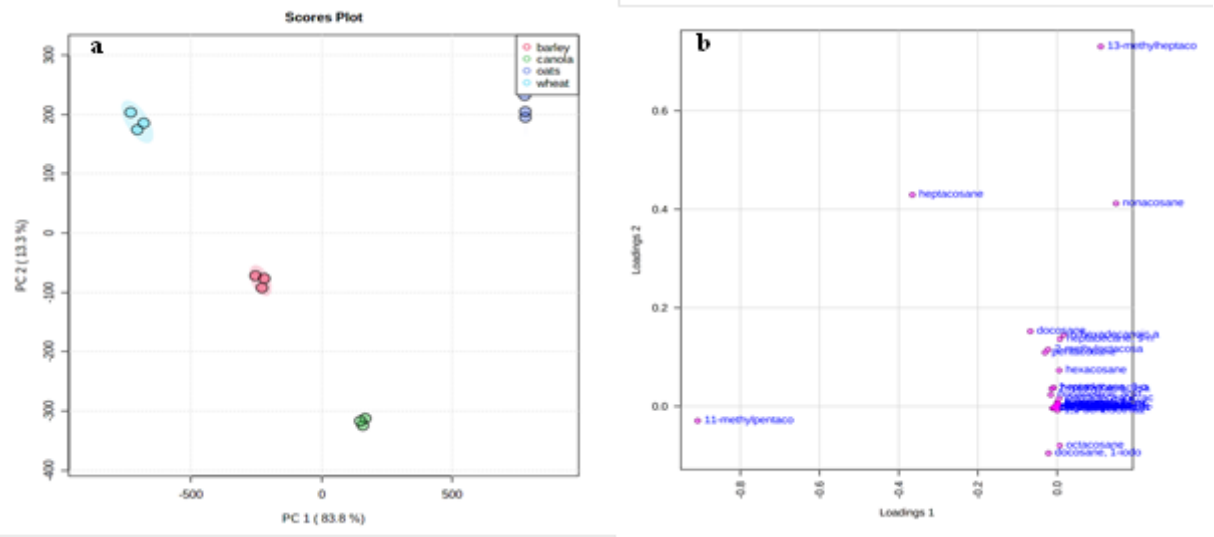

Figure (2a): Score plot of thePCA for chemical compounds obtained from T. variabile male reared on different host grains (canola, oats, wheat and barley), 2b. loading plot shows the most significant compounds that participated in the differentiation.

Table (2): showed the number of compounds that detected and not detected from *T. variabile* female and male reared on canola, oats, wheat and barley

	Female				Male			
Chemical compounds	Canola	Oats	Wheat	Barley	Canola	Oats	Wheat	Barley
1-butanamine, N-butyl-	+	+	+	+	+	+	+	+
nonanal	-	+	-	+	-	-	-	+
decanal	+	+	-	-	+	-	-	-
1,2-benzisothiazole	+	+	-	-	+	+	-	-
2-decenal, (E)-	-	-	-	+	-	+	-	+
2-undecenal	-	-	-	-	-	+	-	-
dodecanal	-	-	-	-	-	+	-	-
caryophyllene	-	+	+	+	-	-	+	+
hexadecane	-	-	-	-	-	-	-	+
tetradecanal	+	-	+	-	+	-	+	-
decanoic acid, hexyl ester	-	-	-	-	-	-	-	+
2-hexadecanol	-	-	-	-	-	-	-	+
heptadecane	-	-	-	-	+	-	-	+
tetrdecanoic acid	-	+	+	+	-	+	+	-
methoxyacetic acid, 2-tridecyl ester	-	-	-	-	+	-	-	-
1-decanol, 2-hexyl-	+	+	+	-	-	+	+	+
pentadecanoic acid	-	+	+	+	-	+	+	+
n-hexadecanoic acid	+	+	+	+	+	+	+	+
nonadecanoic acid	-	-	+	+	-	-	+	+
oleic acid	+	+	+	+	+	+	+	+
docosane	+	+	+	+	+	+	+	+
heptadecane, 9-hexyl-	+	+	+	+	+	+	+	+
tricosane, 2-methyl-	-	-	+	+	-	-	+	+
tetracosane	+	+	+	+	+	+	+	
heptadecane, 9-octyl-	+	+	+	+	+	+	+	+
pentacosane	+	-	+	+	+	+	+	+
11-methylpentacosane	+	+	+	+	+	+	+	+
2-methylhexacosane	+	+	+	+	+	+	+	+
hexacosane	+	+	+	+	+	+	+	+
heptacosane	+	+	+	+	+	+	+	+
docosane, 1-iodo-	-	+	+	+	+	+	+	+
13-methylheptacosane	+	+	+	+	+	+	+	-
octacosane	+	-	-	-	+	-	-	-
2-methyloctacosane	+	+	+	+	+	+	+	+
nonacosane	+	+	+	+	+	+	+	+

+ is detected compounds

-is not detected compounds

In this study, identified chemicals were used to study T. variabile adult's metabolism. As hypothesized there should be difference between the gender of T. variabile and the commodity the insects were reared upon. This difference can be used as developing future diagnostic methods. The results from this study support this hypothesis. DI-SPME coupled with GC-MS could be performed successfully to identify lipids from T. variabile female and male. Also, results showed that there were a significant difference between adults fed on four different host grains. Thus, the chemical hydrocarbons could be used for comparison as taxonomic tool to identify different T. variabile adults including female and male from other Trogoderma sp .

Author Contributions: T.A., M.A. and A.L. provided methodology and experiment design. T.A., M.T.. and P.K.. performed all experiment procedures. T.A. and A.L. did the data analysis. T.A., M.A., P.K.., M.T. did the original draft preparation. All authors edited and accepted the final manuscript .

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