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New Diagnostic and Bio-control tool for *Trogoderma variabile* (Ballion) larvae using (DI-SPME) Coupled (GC-MS)

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ABSTRACT

The objective of this study is to analyze *Trogoderma variabile* metabolism fed on various host grains (Rice, maize, wheat, oats, barley and canola) using GC-MS. Also, hydrocarbons profiling is suggested as a chemo-taxonomical marker/ tool to identify species of insect and especially when they are morphological quite similar like *T. granarium*. Six different commodities were the rearing substrates of *T. variabile.* To prepare the samples, insects were treated with acetonitrile. A method called DI-SPME was utilized, followed by GC-MS for analyzing and categorizing compounds. Furthermore, the specific type of grain that the insects fed on had a notable impact on the chemical compounds detected in *Trogoderma variabile* different larvae stages, including fatty acids and hydrocarbons. However, there were 17,16 , 14,16,14 and 13 compounds extracted form third larvae instar reared on barley, wheat,canola, maize, rice and oats respectively. in comparison to sixth larvae instar, results showed that there were 13,13,21,21,15 and 14 compounds from the larvae reared on oats and barley, wheat,canola, maize, rice and oats respectively
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استخدام طريقة حديثة لمكافحة وتشخيص حشرة *Trogoderma variabile* Ballion باستعمال تقنية DI-SPME مع تقنية جهاز الكروماتوغرافيا الغازي

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الخلاصة

الهدف من هذه الدراسة هو تحليل التمثيل الغذائي لحشرة *Trogoderma variabile* التي يتغذى على الحبوب المختلفة (الأرز والذرة والقمح والشوفان والشعير والكانولا) باستخدام GC-MS. أيضًا، يُقترح تحديد خصائص الهيدروكربونات كأداة تصنيفية كيميائية لتحديد أنواع الحشرات وخاصة عندما تكون متشابهة شكليًا تمامًا مثل *T. granarium*. تم تربية الحشرة على ستة محاصيل مختلفة . لتحضير العينات، عولجت الحشرات بالأسيتونيتريل. تم استخدام طريقة تسمى DI-SPME، تليها GC-MS لتحليل وتصنيف المركبات. علاوة على ذلك، فإن نوع الغذاء كان له تأثير ملحوظ على المركبات الكيميائية المكتشفة يرقات االحشرة ، بما في ذلك الأحماض الدهنية والهيدروكربونات. أشارت النتائج إلى وجود اختلاف كبير في المركبات الكيميائية بين الاطوار المختلفة من اليرقات. وعلى وجه التحديد، تم التعرف على ثلاثة وعشرين مركبًا من يرقات العمر الثالث التي تربى على القمح والكانولا. ومع ذلك، تم استخراج 16،17، 14، 16، 14 و 13 مركباً من الطور اليرقي الثالث المربى على الشعير، القمح، الكانولا، الذرة، الأرز والشوفان على التوالي. بالمقارنة مع العمر السادس لليرقات أظهرت النتائج وجود 15،21،21،13،13 و14 مركب من اليرقات المرباة على الشوفان والشعير والقمح والكانولا والذرة والأرز والشوفان على التوالي.الكلمات الافتتاحية:حشرة *Trogoderma variabile* ،جهاز الكروماتوغرافي الغازي ;تقنية SPME

INTROUCTION

A highly invasive worldwide pest, the *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), feeds on a broad range organic material and commodities like grains in store (Castalanelli et al. 2011). Now,this insect has been reported as a stored-grain pest in nature. It is also reported the primary voracious feeder warehouse beetle infects different products such as candy, cereal, corn, cocoa, corn meal, dog food (dried and 'burgers'), fishmeal, flour (wheat etc.), oatmeal, milk powder (kitten replacement milk etc.), spaghetti, spices (oregano etc.), peas dry beans barleyand meat scraps; Pollen entire colonies have been lost whether prepared or raw pollen has been stored carefully. They cannot feed on whole grains, but may eat fragmented kernels that are often seen in stores (Mason, 2003). *T. variabile* larvae may infest 119 distinct commodities (Hagstrum et al., 2013). *Trogoderma spp.* have been identified using a variety of methods, including genetic, morphological, and taxonomy keys. However, these methods are expensive and inefficient (Favret, 2005; Khidher, 2024).

Also, some methods are time-consuming as they might need overnight incubation, in addition to use toxic or corrosive chemicals which increase the risk of losing DNA by isopropanol or ethanol precipitation. Several of studies has focused on the identification of fatty acid from insects, but different GC method and various solvents were used. Fatty acids are carboxylic acids characterized by hydrocarbon chains typically containing 4 to 28 carbon atoms. The most common chain lengths range from 10 to 22 carbon atoms, predominantly even-numbered, and natural fatty acids usually exhibit straight chains whether saturated or unsaturated (Gunstone & Norris, 2013). Due to their long aliphatic hydrocarbon chains, most fatty acids are insoluble in polar solvents like water; however, very short fatty acids are soluble in water but insoluble in nonpolar solvents (Akoh & Min, 2008). Youn *et al*. (2012) had identified fatty acid from *Allomyrina dichotoma* larvae using GC-MS technique and ethanol as a solvent. Wathne et al. (2018) also used GC-MS to identify fatty acid in *T. viridissima*, *C. biguttulus*, and *C. brunneus* and chloroform as a solvent to extract lipids. Some studies had used dichloromethane, hexane, chloroform, and methanol as solvent and GC-FID to extract fatty acid (Said et al. 2005 and Nelsen et al. 2000). A studies had used a mixture of solvents, for instance, acetonitrile with water and methanol acetonitrile-water to extract fatty acid from Mediterranean fruit fly *Ceratitis capitate* (Al-khshemawee et al. 2018).

Solid-phase microextraction (SPME) is utilized for rapid sample preparation and is an efficient method for detecting chemicals (Arthur and Pawliszyn., 1990; Al-khshemawee et al., 2017; Najafian and Rowshan ., 2012; Bicchi *et al.*, 2000). According to Aulakh et al. (2005) and Malik et al. (2006), one of the most effective techniques for extracting waxes is direct immersion SPME (DI-SPME), wherein the SPME fiber is directly immersed in a small volume of the liquid-extracted sample. The aim of this study is develop new diagnostic and bio-control tool to identify *Trogoderma variabile* Ballion (male, female, and larvae) feeds on six different commodities (Canola, wheat, oats, rice, barley and maize) and to study of *T. variabile* metabolism.

MATERIAL AND METHODS

Insect culture

Specimens of *Trogoderma variabile* were obtained from the Department of Plant Biosecurity, School of Science, Health, Engineering, and Education at Murdoch University in Murdoch, Western Australia, Australia. In order to collect *T. variabile* larvae, 150 adult insects were placed in 1 L plastic containers with 450 g of sterilized canola, maize, oats, wheat, barley, and rice individually. The containers were sealed with meshed lids. The insect food was sterilized by first storing it at -20°C for five days in four-liter glass jars, followed by an additional two days at 4°C before being used in the experiments. The insects were bred in a controlled environment at 29°C and 70% relative humidity. The containers were kept in the culture room for 3-4 months to reach the fourth generation of adult insects and larvae, which were utilized in our research.

Apparatus and equipment.

In this study, a solid phase microextraction (SPME) fiber (50/30µm) DVB/CAR/PDMS was utilized to gather compounds. The analysis was conducted using a GC system 7890A (serial number CN14272038) equipped with an HP-5MS nonpolar column (30 m × 0.25 µm, film thickness 0.25 µm, RESTEK, catalog number 13423). The carrier gas employed was 99.999% helium (BOC, Sydney, Australia). The GC-MS parameters were set as follows: the injector port temperature was maintained at 325°C, the initial oven temperature was set to 50°C and then increased at a rate of 5°C/min until it reached 325°C. The MS quad temperature was 150°C, the MS source temperature was 230°C, and the column pressure was kept at 10.629 psi. The column flow rate was 1 ml/min, with a splitless flow of 20 ml/min for 1.5 minutes. The total runtime for the GC-MS analysis was 53 minutes. The experiment included three replicates for two different Instar. Compound peaks were analyzed using MassHunter Acquisition software (Agilent Technologies, USA) quantitative analysis B.06.00. The experiment was repeated three times to verify the identification of the chemicals.

The extraction and analysis method

Each third and sixth instar larva of *Trogoderma variabile* was placed into a 0.1 ml vial (Thermo Scientific 0.1 ml micro-insert, 31x6mm clear glass, 15 mm top), which was then situated inside a 2 ml microtube (Benchmark Scientific, from Sigma-Aldrich, lot no. 3110, USA). Two milling balls were added and manually shaken, and the sample was ground using a tissuelyser for one minute at 270 rpm. Following this, 200µl of acetonitrile (HPLC grade, Fisher Chemical, USA) was added to the microtube using a micropipette and homogenized for two minutes with a BedBug microtube homogenizer (Model no. D1030-F). The extract was then transferred to a 0.1 ml vial (Thermo, 0.1 ml Micro-Insert, 31x6mm, clear glass, 15mm top) placed inside a 2 ml clear screw HPLC vial (Lot no. 17036152, Agilent Technology, China) using a micropipette. The extract was centrifuged for three minutes using a Dynamica mini centrifuge (Model no. 13µl). The supernatant was transferred into a 2 ml HPLC vial. A Solid Phase Microextraction (SPME) fiber (50/30 µm, DVB/CAR/PDMS coating, Sigma-Aldrich, Bellefonte, PA, USA) was then inserted into the vial and left for 16 hours at room temperature (25 ± 5°C). Finally, the fiber was removed from the vial and immediately introduced into the GC injector port for thermal desorption.

Data analysis

Mass Hunter Acquisition software (B.06.00, Agilent Technology, USA) were used to extract peak areas from each chromatogram. After selecting compounds, the peak area data was transferred to Excel for organization. Analysis was then conducted using IBM SPSS Statistics (University edition), where one-way ANOVA followed by Tukey's was used. Statistical significance was set at $P \leq 0.05$.

RESULTS AND DISSCUSION

The data that was obtained from biological samples of *Trogoderma variabile* larvae using direct immersion solid phase micro extraction method coupled with gas chromatography mass spectrometry was statistically tested using analysis of variation to determine the analytical variability of the data generated from two different stages (third and sixth instars). Results showed that feeding on six different types of diet (barley, wheat, canola, maize, rice, and oats) affected the quantity, quality, and number of the compounds produced by the larvae. Overall, DI-SPME detected 25 and 40 compounds from third and sixth instar larvae, respectively. In the first stages, 17, 16, 14, 16, 14 and 13 compounds were identified from the third stages larvae that feed on barley, wheat, canola, maize, rice, and oats respectively. In the sixth larvae stages, 17, 14, 24, 21, 15 and 14 compounds were identified from the larvae samples fed on barley, wheat, canola, maize, rice, and oats, respectively. Results showed that there was a significant difference observed among samples collected from different grains. In the third instar, 17 compounds were significantly different among the different grains including 1-butanamine, N-butyl-, nonanal, decanal, 2-trifluoromethylbenzoic acid, 2-ethylhexyl ester, pentadecanoic acid, palmitoleic acid, n-hexadecanoic acid, oleic acid, docosane, tetracosane, pentacosane, 11-methylpentacosane, hexacosane, heptacosane, 13-methylheptacosane, octacosane, and nonacosane (Table 1). Also, the results showed only 9 compounds were detected from the third larvae stage collected from different grains.

In the sixth instar , 19 compounds were significantly different which were benzoic acid, 2-amino-4-methyl-, 1-butanamine, N,N-diethyl-, nonanal, caryophyllene, 2-Hexadecanol, tetradecanoic acid,1-Decanol, 2-hexyl-,pentadecanoic acid, palmitoleic acid, n-Hexadecanoic acid, 9-Octadecenoic acid, (E)-,9,12-octadecadienoic acid (Z,Z)-, oleic acid, octadecanoic acid, cis-13-eicosenoic acid, heptadecane, 9-octyl-, pentacosane,11-Methylpentacosane, docosane, 11-butyl-, hexacosane, heptacosane, 13-methylheptacosane, and 2-methyloctacosane (Table 2).

DI-SPME)combined with GC-MS was employed to identify fatty acids and hydrocarbons from various stages of Trogoderma variabile (larvae and adults). Malosse et al. (1995) demonstrated that SPME is cost-effective, user-friendly, rapid, and highly reproducible. SPME has also been used for analysing ant cuticular hydrocarbons (Monnin et al., 1997). The solid phase microextraction approach, along with gas chromatography mass spectrometery has also been utilized to identify long-chain free fatty acids in insect exocrine glands (Maile et al. 1995). In their study, Filho et al. (2010) demonstrated that DI-SPME exhibits greater sensitivity compared to Headspace Solid Phase Microextraction (HS-SPME). DI-SPME was found to be particularly advantageous for analyzing clean aqueous samples. Although HS-SPME showed higher sensitivity for more volatile compounds, DI-SPME successfully extracted a broader range of pesticides, detecting 16 compounds compared to HS-SPME's extraction of only 12 compounds. This highlights DI-SPME as the preferred method for comprehensive pesticide analysis in such samples.

Long-chain fatty acids such as stearic, linoleic, oleic, palmitic, and palmitoleic acids have been reported in the exocrine secretions and cuticular extracts of many insect species (Lockey, 1988).

These compounds are crucial in various biological functions, including communication and protection against desiccation. Researchers have employed DI-SPME (Direct Immersion Solid-Phase Microextraction) using solvents such as hexane or pentane to analyze signaling chemicals and long-chain hydrocarbons extracted from different anatomical parts of wasps (Moneti et al., 1997). The results indicated differences in larvae stages feeding on different commodities. The biosynthesis and accumulation of fatty acids in insects can be directly affected by their habitat and environmental conditions (Chakravorty et al., 2011; Fast, 1971; Fontaneto et al., 2011).

Fatty acids are typically found in many insect species (Buckner, 1993, Lockey, 1988). The fatty acid composition will vary with individual species, their development phase, diet, and environmental factors (Paul et al. 2016). Oleic acid was identified as the major fatty component in Trogoderma molitor larvae, while linoleic acid predominated in Acridotheres domesticus lipids. These findings are consistent with previous studies by Tzompa-Sosa et al. (2014) and Ravzanaadii et al. (2012). Van Broekhoven et al. (2015) found that the diets' varying protein and starch contents had a significant impact on the fat content of yellow mealworms. This suggests that larvae fed a diet low in nutritional quality likely use their fat reserves as energy, which lowers their fat content (Arrese and Soulages,2010). The fatty acids identified in the cuticular lipids of Apis mellifera ranged from 16 to 36 carbon atoms in the alkyl chain and consisted exclusively of saturated entities (Blomquist et al., 1980). Major fatty acids in the cuticular lipids included tetracosanoic (29%), hexacosanoic (12%), and octacosanoic (11%) acids (Cerkowniak et al., 2013).

Table (1) GCMS response (peak area) for compounds detected and identified at third larvae stages feed on six different commodities by DI-SPME-GC-MS

Compound name	RT	RI NIST	RI	GCMS response (10^5) ±SE (n=3)						P.VALUE
				BARLEY	WHEAT	CANOLA	MAZIE	RICE	OATS	
1-Butanamine, N-butyl-	7.59	1015	948.6	6.18±0.48	15.80±2.93	14.30±0.49	51.88±2.27	168.22±2.75	53.32±3.41	0
Nonanal	15.64	1104	1117.7	N.D	5.70±0.07	2.81±0.44	N.D	N.D	N.D	0.003
Decanal	19.58	1204	1164.2	N.D	1.39±0.05	14.30±0.49	N.D	N.D	N.D	0
2-Undecenal	24.51	1311	1325.8	N.D	N.D	N.D	N.D	N.D	20.37±1.36	
Dodecane, 5,8-diethyl-	27.88	1483	1490.9	6.63±0.47	N.D	N.D	N.D	N.D	N.D	
2-Trifluoromethylbenzoic acid, 2-ethylhexyl ester	31.24	1639	1629.3	1.48±0.27	19.62±3.70	N.D	N.D	N.D	N.D	0.008
Oxirane, tetradecyl-	32.62	1702	1669.8	5.81±0.87	N.D	N.D	N.D	N.D	N.D	
Tetradecanoic acid	34.19	1769	1778.8	4.01±0.77	4.82±0.78	N.D	8.81±3.99	6.86±0.72	N.D	0.424
Pentadecanoic acid	36.21	1869	1890.3	5.27±0.20	2.28±0.35	2.60±0.10	N.D	61.29±1.57	N.D	0
Nonadecane	36.29	1910	1910.8	N.D	N.D	N.D	251.42±20.35	N.D	N.D	
Palmitoleic acid	37.81	1976	1982.4	N.D	6.75±0.66	9.70±0.41	11.08±0.46	16.08±3.12	6.10±0.25	0.004
n-Hexadecanoic acid	38.24	1968	2012.3	141.04±22.53	87.03±3.09	71.50±9.00	139.12±15.28	81.93±1.65	59.50±6.24	0.001
Eicosane	38.47	2009	2022.6	N.D	N.D	N.D	246.35±20.13	N.D	N.D	
Oleic Acid	41.58	2175	2171.9	46.93±12.02	5.33±0.22	12.60±0.86	27.20±8.60	9.26±0.12	85.31±12.02	0
Docosane	42.26	2228	2230.2	30.40±4.06	N.D	N.D	155.47±17.41	N.D	N.D	0.002
Tricosane, 2-methyl-	45.01	2343	2381.6	N.D	N.D	N.D	N.D	N.D	22.65±6.92	
Tetracosane	45.61	2407	2412.2	34.91±3.73	50.02±2.37	N.D	14.48±5.55	N.D	N.D	0
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	46.17	2413	2443.3	N.D	N.D	N.D	N.D	37.90±0.06	N.D	
Pentacosane	47.17	2506	2501.6	298.34±20.61	N.D	15.07±0.35	28.64±3.10	14.34±0.23	22.28±2.92	0
11-Methylpentacosane	47.85	2542	2533.7	17.98±1.51	319.67±1.09	160.17±9.08	287.27±20.00	261.95±13.98	227.03±15.02	0
Hexacosane	48.98	2606	2610.4	118.48±9.07	111.73±6.28	15.65±1.91	81.23±0.63	69.92±5.11	18.55±0.63	0
Heptacosane	50.1	2705	2666	168.55±24.90	257.94±3.58	141.08±1.03	257.07±15.44	68.73±3.58	115.60±7.15	0
13-Methylheptacosane	50.73	2740	2692.5	155.93±14.30	154.23±9.06	89.87±2.45	136.18±3.82	135.09±7.41	112.30±14.30	0.001
Octacosane	51.13	2804	2715.5	134.51±13.41	83.94±3.07	58.50±2.95	114.69±2.95	109.17±6.89	22.76±2.50	0
Nonacosane	52.91	2904	2846.1	42.48±3.52	35.79±1.61	76.93±1.68	15.35±1.99	56.13±4.46	49.73±7.36	0

RT is retention time; RI NIST is retention index from NIST; RI is retention index calculated by running n-alkane standard C7-C40; N.D is not detected

Table (2) GCMS response (peak area) for compounds detected and identified at sixth larvae instar fed on six different commodities by DI-SPME-GC-MS

Compound name	RT	RI NIEST	RI	GCMS response (10^5) ±SE (n=3)						P. value
				BARLEY	WHEAT	CANOLA	MAIZE	RICE	OATS	
Benzoic acid, 2-amino-4-methyl-	5.57	949	910.2	7.54±0.07	N.D	3.14±0.82	N.D	N.D	N.D	0.006
1-Butanamine, N-butyl-	7.59	1015	948.6	N.D	27.58±0.90	25.26±7.43	129.71±4.05	129.35±9.50	20.37±6.95	0
Nonanal	15.64	1104	1117.7	N.D	6.05±0.67	2.07±0.19	N.D	4.35±0.32	N.D	0.002
Decanal	19.58	1204	1164.2	N.D	3.97±0.70	2.58±0.78	N.D	N.D	N.D	0.261
1,2-Benzisothiazole	20.2	1208	1200.4	N.D	N.D	N.D	2.61±0.26	N.D	N.D	
Tridecane	21.91	1313	1258.6	N.D	N.D	0.56±0.06	n	N.D	N.D	
2-Undecenal, E-	24.51	1311	1325.8	N.D	N.D	N.D	2.51±1.04	N.D	N.D	
Dodecanal	25.62	1402	1408.8	N.D	N.D	1.71±0.32	N.D	N.D	N.D	
Caryophyllene	25.95	1494	1489.7	7.05±0.92	3.47±1.05	2.96±0.24	N.D	N.D	N.D	0.024
Tetradecane, 2,6,10-trimethyl-	28.93	1519	1492	N.D	N.D	1.59±0.09	4.86±2.46	N.D	N.D	0.256
Hexadecane	30.34	1612	1560.8	N.D	N.D	4.16±0.62	N.D	N.D	N.D	
E-2-Tetradecen-1-ol	30.67	1664	1667.8	N.D	N.D	0.55±0.19	N.D	N.D	N.D	
Heptadecane	32.63	1711	1669.4	N.D	1.99±0.41	N.D	N.D	N.D	N.D	
2-Hexadecanol	33.8	1774	1704	4.39±1.02	N.D	N.D	20.29±2.96	N.D	N.D	0.007
Tetradecanoic acid	34.191	1769	1778.8	3.49±0.19	2.95±0.33	4.09±1.24	3.91±0.92	4.83±0.96	7.42±1.66	0.05
1-Decanol, 2-hexyl-	34.78	1790	1854.1	4.56±0.33	N.D	N.D	2.96±0.51	N.D	N.D	0.06
Pentadecanoic acid	36.211	1869	1890.3	N.D	5.64±0.62	1.35±0.10	2.45±0.15	6.85±1.29	N.D	0.002
2-Heptadecanone	36.92	1847	1933.1	N.D	N.D	N.D	3.20±0.77	N.D	N.D	
Palmitoleic acid	37.813	1976	1982.4	N.D	N.D	8.43±0.26	N.D	17.13±1.63	N.D	0.006
n-Hexadecanoic acid	38.24	1968	2012.3	126.28±14.07	172.50±3.91	43.20±8.08	236.04±3.05	131.28±12.07	124.58±11.73	0
9-Octadecenoic acid, (E)-	40.14	2175	2116	N.D	N.D	2.30±0.60	N.D	N.D	55.04±18.76	0.048
9,12-Octadecadienoic acid (Z,Z)-	41.15	2183	2169	4.60±0.39	6.82±0.87	N.D	N.D	N.D	N.D	0.082
Oleic Acid	41.58	2175	2171.9	8.13±0.64	N.D	17.08±0.56	177.45±18.15	45.06±22.95	64.97±17.72	0
Octadecanoic acid	41.95	2167	2210.4	N.D	N.D	N.D	25.36±4.51	8.14±0.67	21.74±5.11	0.048
5-Methyl-Z-5-docosene	42.32	2292	2234.5	N.D	N.D	2.95±0.24	N.D	N.D	N.D	
Undec-10-ynoic acid, decyl ester	43.26	2274	2284.4	8.03±0.36	N.D	N.D	10.22±2.19	N.D	N.D	0.381
1-Bromoeicosane	44.07	2395	2329.3	4.40±0.07	N.D	N.D	N.D	7.76±1.68	25.94±8.33	0.43

cis-13-Eicosenoic acid	44.30	2374		3.38±0.10	5.13±0.06	N.D	6.88±1.27	N.D	N.D	0.042
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	44.59	2336	2356.4	N.D	71.62±15.11	N.D	N.D	N.D	N.D	
Heptadecane, 9-hexyl-	44.76	2413	2308	N.D	4.21±0.46	3.70±0.42	N.D	N.D	N.D	0.465
Heneicosyl acetate	45.87	2475	2427.7	3.46±0.05	N.D	N.D	N.D	N.D	N.D	
Heptadecane, 9-octyl-	46.29	2442	2449.9	N.D	N.D	3.70±0.42	8.02±0.20	N.D	N.D	0.001
Pentacosane	47.17	2506	2501.6	198.32±3.21	N.D	19.14±0.99	n	37.14±8.19	26.85±3.21	0
11-Methylpentacosane	47.85	2542	2533.7	N.D	184.68±27.42	310.83±4.77	33.14±8.19	285.6±16.25	217.05±4.15	0
Docosane, 11-butyl-	48.33	2542	2561.3	7.67±0.33	N.D	N.D	N.D	23.40±4.63	25.35±6.75	0.073
Hexacosane	48.98	2606	2610.4	16.04±0.46	N.D	N.D	29.60±3.69	N.D	N.D	0.022
Heptacosane	50.1	2705	2666	68.03±4.57	18.61±2.49	115.63±3.86	83.00±5.04	121.61±16.88	74.22±6.63	0
13-Methylheptacosane	50.73	2740	2692.5	53.55±7.46	N.D	152.50±7.52	117.71±5.53	152.22±8.67	91.61±2.20	0
2-methyloctacosane	51.31	2840	2723.6	N.D	N.D	N.D	24.89±1.69	86.45±5.52	72.68±9.56	0.001
Nonacosane	52.91	2904	2846.1	N.D	N.D	190.22±10.21	150.89±1.95	N.D	185.18±23.47	0.205

RT is retention time; RI NIST is retention index from NIST; RI is retention index calculated by running n-alkane standard C7-C40; N.D is not detected .

CONCLUSION

In this study, identified chemicals were utilized to investigate the metabolism of *T. variabile* adults. It was hypothesized that there would be differences between the genders of *T. variabile* and the commodities on which the insects were reared. This difference can be instrumental in developing future diagnostic methods. The results from this study support this hypothesis. DI-SPME coupled with GC-MS was successfully performed to identify lipids in *T. variabile* larvae. Additionally, the results showed a significant difference between adults fed on four different host grains. Therefore, the chemical hydrocarbons could be used as a taxonomic tool to differentiate *T. variabile* larvae, from other *Trogoderma* species.

CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this manuscript.

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