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Application of Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry in the Study of Volatile Chemicals from *Solanum viarum* Dunal

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Headspace solid-phase microextraction (HS-SPME) and gas chromatography with ion trap mass spectrometric detection were used to investigate the volatile chemicals profile from *Solanum viarum*, a perennial Solanaceae plant reported to have attracted and stimulated oviposition of *Helicoverpa armigera*, which is one of the most economically-damaging pests in tropical and subtropical regions. SPME fibers coated with 100 μm polydimethylsiloxane (PDMS) and 75 μm Carboxen (CAR)-PDMS were tested. The latter was chosen for optimizing extraction procedures. Differences in volatile chemicals composition were observed in several compounds, depending on the coating characteristics of the fiber used. The partition coefficient, K_1, between the headspace phase and SPME polymeric coating, the relative standard deviation and the concentrations of the volatile chemicals from the leaves of *S. viarum* were investigated. The SPME method showed efficiency in extracting low molecular weight compounds from the leaves of *S. viarum*.

Key Words: Solid phase microextraction, Tropical soda apple, *Helicoverpa armigera*.

INTRODUCTION

Tomato fruitworm, *Helicoverpa armigera*, is a serious pest of cotton, pigeon pea, chickpea, sunflower and tomato, distributed throughout Africa, the middle east, southern Europe, India, central and southeastern Asia and many eastern Pacific Islands[1,2]. Due to its wide host range, multiple generations, migratory behaviour, high fecundity and existing insecticidal resistance[2], *H. armigera* became a difficult pest to control. Alternative methods of control using semiochemicals have been investigated in past[3-5].

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In 1988, the Asian Vegetable Research and Development Center (AVRDC) found a heavy infestation of *H. armigera* larvae feeding voraciously on *Solanum viarum* Dunal[6]. *S. viarum*, commonly known as tropical soda apple (TSA) in the USA, is a perennial Solanaceae plant native to southeastern Brazil, northeastern Argentina, Paraguay and Uruguay[7]. Several studies showed the preference of *H. armigera* female to lay eggs on *S. viarum* leaves over its natural host plant tomato[6,8]. The presence of oviposition and larval feeding stimulants from leaf extracts of *S. viarum* was also detected[9-11]. Such finding provided the possibility of using *S. viarum* as a trap crop for the control of *H. armigera* in the field. Since trap crops may occupy a lot of surface area, identification and synthesis of volatile chemicals as an ovitrap to enhance oviposition of the female *H. armigera* could be of practical use in crop protection. However, to formulate an effective ovitrap requires an adequate knowledge on the composition of the volatile chemicals emitted from *S. viarum*.

The methods currently available for extracting the plant compounds may involve the use of solvents, which is time consuming. Their results may depend on such factors as the type of solvent used and or its polarity[12]. Solvent extraction may also result in extracting such compounds with higher molecular weight as cuticle lipids and chlorophyllous substances[13] or unwanted contaminants derived from sample preparation (Huang, pers. comm.). Adequate sampling methods for different kinds of compounds have not yet been developed, but entrainments in porous polymers and solid phase microextraction (SPME) have been reported as suitable techniques[13,14]. SPME, used in this study to investigate the profile volatile chemicals from *S. viarum* leaves was reported as an efficient method in sampling plant volatiles in ecological chemical studies[15,16]. It does not require solvents in extracting a wide variety of analytes. Therefore, it has recently become a popular sample preparation technique alternative to classical extraction and liquid injection methods.

EXPERIMENTAL

Headspace SPME collection: Extraction by SPME was performed with a manual SPME fiber holder and SPME fibers with 2 different coatings; an absorbent non-polar poly(dimethylsiloxane) (PDMS, 100 μm; volume of coating = 0.612 μL) and an absorbent bipolar carboxen-PDMS (CAR-PDMS, 75 μm film thickness; volume of coating = 0.436 μL)[17]. The two fibers, considered to be efficient in the extraction of plant compounds[18], were selected. Before sampling, each of the two fibers was reconditioned for 0.5 h in the GC injection port at 250°C. The fiber was introduced in a 22 mL headspace vial that contained 1 g of *S. viarum* leaves of vegetative stage. The plant material was submerged in 11 mL of distillated water.

Volatile chemicals were extracted after an optimization was completed in extraction time, pH, salt concentration and injection in the gas chromatography (GC) inlet.

Partition coefficient calculation: With the HS-SPME sampling, we measured the partition coefficient, K_1, between the headspace gaseous phase and SPME polymeric coating. Solutions of 0.5 µg/mL of the standard volatile chemicals were used and the areas were quantitatively compared as described by Bicchi *et al.*[18]. According to Zhang and Pawliszyn[19], the amount of analyte concentrated through HS-SPME in a fiber was the result of two different equilibria; one is the matrix/headspace equilibrium responsible for the headspace composition, which is dependant upon the volatility of the analyte and the physical characteristics of the matrix and the other is the headspace/polymeric fiber coating equilibrium, which depends on the diffusion of the analyte from the vapour phase to the fiber coating and the analyte interaction with the polymeric coating. In HS-SPME, the total recovery of an analyte from a solid or liquid matrix was related to the overall partition coefficient, K, of the analyte between the SPME fiber coating and the matrix itself. K can be obtained from the expression: $K= K_1 K_2$, where K_1 is the analyte partition coefficient between SPME fiber coating and sample headspace and K_2 is the partition coefficient between headspace and sample matrix[18,19]. K_1 can, therefore, be assumed as a parameter representative of the recovery process of an analyte from the headspace of a sample onto the polymeric coating of a fiber and can be calculated[18] as follows:

$$K_1^i = (A_f V_g)/(A_g V_f)$$

K_1^i is the partition coefficient for the analyte i; A_f is the area of analyte i on the SPME fiber; V_g is the volume of the gas sample injected; A_g is the area of analyte i in the headspace and V_f is the volume of the fiber polymeric coating.

Gas chromatography-mass spectrometry: Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a Hewlett Packard 5890 series II gas chromatograph equipped with a split-splitless injector coupled to an ion-trap 5972 Mass Selective Detector (MSD) system using 70 eV electron impact ionization with the GC capillary column connected directly to the ion source. The mass range was 1-30000 molecular weights. The ion source temperature was 187°C, 54 m torr for a total acquisition time of 69 min. The operating parameters were controlled by an HP series G1701BA computer version B.01.00-ChemStation. Chromatography was performed using a non-polar capillary column HP-5MS (60 m length × 0.25 mm i.d. × 0.25 µm film thickness). Helium was used as the carrier gas at a linear flow rate of 1.39 mL/min. The samples were heated in the injection port at 250ºC. The oven temperature program was set at 50°C for 2

min, followed by 5°C/min to 250°C for 1 min, then 2°C/min to 280°C for 50 min. Detection was made by comparing their mass spectra with library spectra. Chemical having a match quality of ≥ 80 % in the GC-MS library were chosen and confirmed by comparing EI-MS and GC retention indices with synthetic standards under the same operating condition[20].

RESULTS AND DISCUSSION

The results were obtained by comparing the areas of characteristic volatile chemicals sampled in the headspace by the two fibers used. Because of the reference was not available in the use of SPME to extract *S. viarum* volatile chemicals, the effect of the most important parameters (*e.g.*, extraction time, ionic strength and pH) influencing the extraction efficiency[16] and the desorption time were optimized. The same sampling parameters were used for both fibers to facilitate comparison. Samples were continuously vibrated to enhance the analytes equilibration and the process between the headspace vapour phase and fiber coating[18].

Extraction time: Extraction time profile was investigated in order to establish a sufficient extraction time for equilibrium. SPME extraction is a dynamic partitioning process of the target compounds between the SPME fiber and the sample solution[21]. In present experiment, the extraction equilibrium was established within the range of 5 to 40 min at 24 ± 2ºC. The stirring at 400 rpm during the absorption period, the equilibrium time was reached at 0.5 h (Fig. 1). This extraction time was, therefore, used throughout the rest of the experiments.

Effect of ionic strength: According to Saraji[22], the addition of salt to the sample matrix decreases the solubility of the analytes in the sample matrix, allowing more analytes to move to the sample headspace and enhancing the extraction efficiency. Therefore, the effect of salt on the extraction efficiency was investigated by adding different amounts of NaCl ranging 5-30 % (w/v) (Fig. 2). It was found that, according to the GC area, the concentration at 10 % NaCl enhanced the efficiency and extracted more analytes; therefore, such concentration parameter was set for all the experiments followed.

Effect of pH: The effect of pH indicated that the acidic pH value (pH 2) had higher extraction efficiency than the pH values of 4, 6, 8, 10 and 12. However it is assumed that at pH higher than 7, it is expected that the polymeric −OH groups are ionized to the ionic; thus increasing the affinity of the analytes[23]. This explained the reasons of extracting a number of compounds at pH 10 and 12 but the number was lesser that at pH 2. On the basis of the results on the number of compounds extracted (Fig. 3), pH 2 was chosen as an optimal pH for the rest of the experiments.

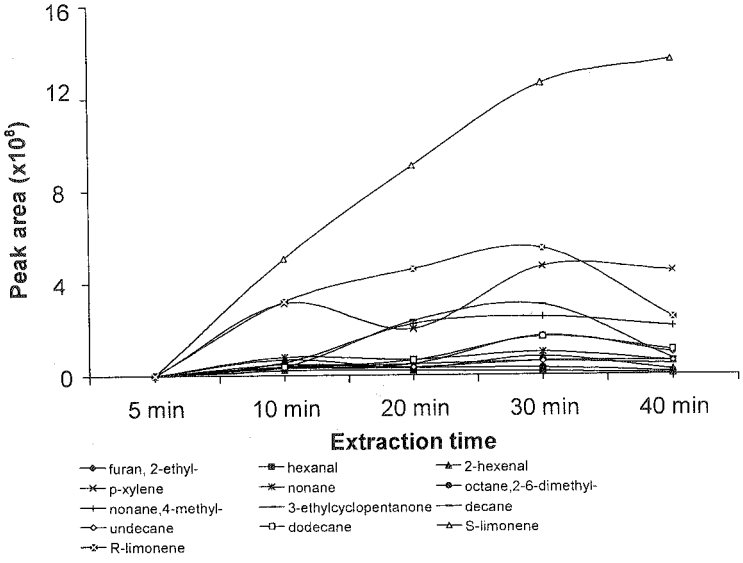

Fig. 1. Effect of extraction time on desorbed volatiles

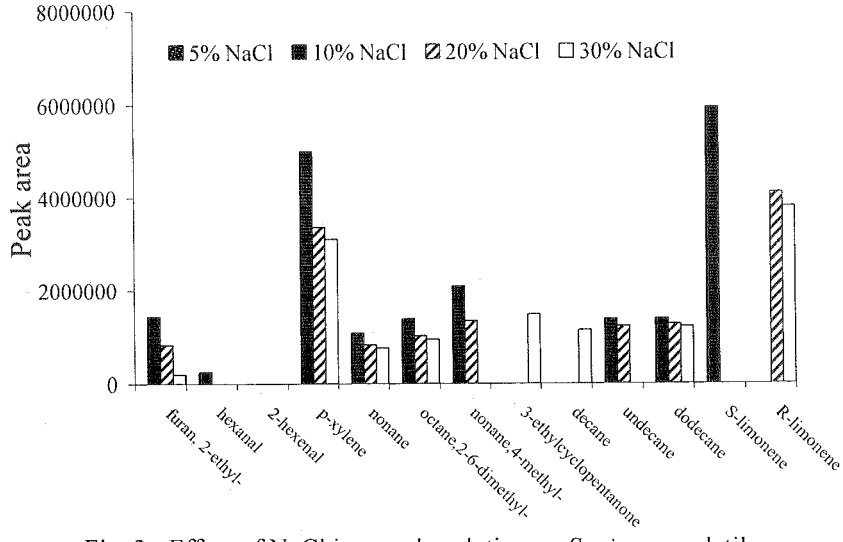

Fig. 2. Effect of NaCl in sample solution on *S. viarum* volatiles

Desorption time: There are many factors that can effect desorption profile of analytes, including boiling point and partition constant of the analytes, thickness of the stationary phase and desorption temperature. It was found that analytes can be desorbed more completely with a longer desorption time, *e.g.*, 5 min. However the stability of the fiber used can be affected by prolonging the desoption time; thus the analytes might be decomposed if the desorption temperature was too high[21]. Therefore,

desorption time of 4 min, which extracted a similar amount of analytes as at 5 min (Fig. 4.) was chosen as an optimal desorption time in all of the present experiments.

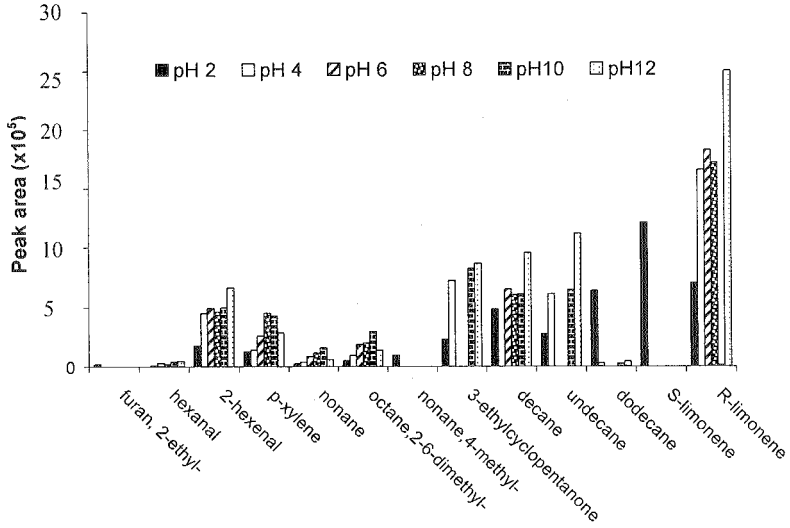

Fig. 3. Effect of pH in sample solution on *S. viarum* volatiles

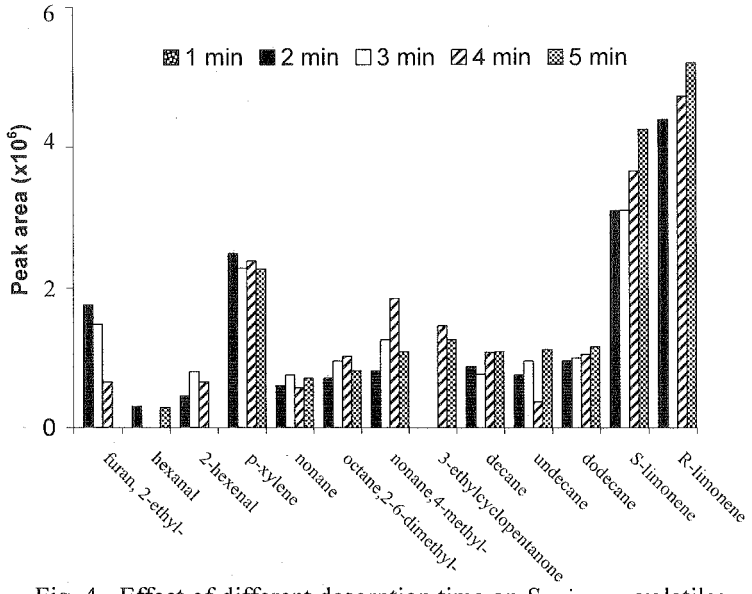

Fig. 4. Effect of different desorption time on *S. viarum* volatiles

Extraction efficiency: The results presented in Tables 1 and 2 reported K_1^1, the relative standard deviations (RSD), the mean concentration and the mass spectral data for the volatile chemicals obtained with the 75 μm CAR-PDMS and the 100 μm fibers, respectively. The approach

involved adopting same analysis conditions in both fibers so that analyte areas may be assumed to be influenced only by the concentration capability of the fiber[18]. K_1 for the 75 µm CAR-PDMS fiber was in the range of 10^2-10^6, while that for the 100 µm PDMS was in the range of 10^2-10^6. The reproducibility for the two fibers investigated, calculated over four analyses, varied from 4.1 to 10.5 % for the 75µm CAR-PDMS fiber and from 4.5 to 9.0 % for the 100 µm PDMS fiber. The mean concentration of the volatile chemicals varied from 0.29 % (naphthalene) to 31.44 % [(E)-2-hexenal] extracted from the 75 µm CAR-PDMS fiber. For the concentration of the 100 µm PDMS fiber, it varied from 0.041 % for 1-pentene,3,3-dimethyl- to 35.33% for (Z)-9-octadecenamide. The three volatile chemicals, (Z)-(E)-2-hexenal, 9-octadecenamide, and naphthalene were commonly found in both fibers. (Z)-(E)-2-hexenal and 9-octadecenamide had a high K_1 value $\geq 10^5$, which indicated that they had a better recovery rate in both fibers. The K_1 range (at 10^2) for naphthalene was similar in both fibers. The RSD values for both fibers were ≤ 10.5. The number of volatile chemicals extracted was 16 for the 75 µm CAR-PDMS fiber and 18 for the 100 µm PDMS fiber (Figs. 5 and 6).

A total of 31 volatile chemicals were identified in the study (Figs. 5 and 6). The method showed good reproducibility, which were calculated over 4 analytes with the RSD variations ≤ 10 % in both fibers. The optimal K_1 value in our extraction was $\leq 10^6$, with a good stability in the 100 µm PDMS fiber which K_1 ranged between 10^5 to 10^6. The mean concentration of the analytes from the 75 µm CAR-PDMS fiber was between 0.29 to 31.44%, while it was between 0.041 to 35.33% for the 100 µm PDMS fiber. The 100 µm PDMS fiber showed higher concentration for the fatty amide (Z)-9-octadecenamide, (Mean %: 35.33) and better recovery (K_1: $7.1.10^5$). However, we can not ascertain from such finding a specificity of the PDMS fiber to the fatty amid class regarding the lesser number of such chemical class identified. Similarly, the 75 µm CAR-PDMS fiber showed higher affinity to (E)-2-hexenal (Mean %: 31.44, K_1: 8.0×10^6). The K_1 value between the two isomers S-(-) and R-(+)-limonene, did not differ to much, there were in the same range at 10^5 but the concentration was higher in S-(-)- limonene (7.13%). It was reported that, the concentration factor of an analyte depends on its structure and volatility, physico-chemical characteristics of the absorbing fiber and analyte/fiber affinity[18]. In addition, it also depends on some physical factors such as matrix, agitation, headspace equilibration, temperature, analyte diffusion rate from the vapour phase to the fiber surface and the class of compounds present in the leaves[18] reported that higher recovery of monoterpene hydrocarbons was obtained with 75 µm CAR-PDMS for rosemary and thyme while sesquiterpene hydrocarbons from thyme were better recovered with 100 µm PDMS.

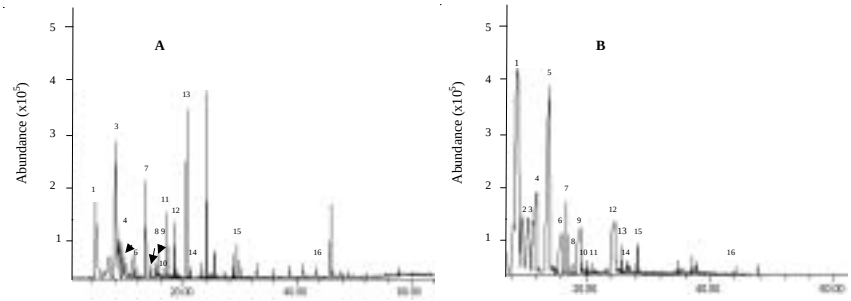

Fig. 5. SPME-GC-MS (Total ion currents, TIC) of the 75 µm CAR-PDMS volatile extracts (A) compared to its standard (B) spiked at 10 µg/µL

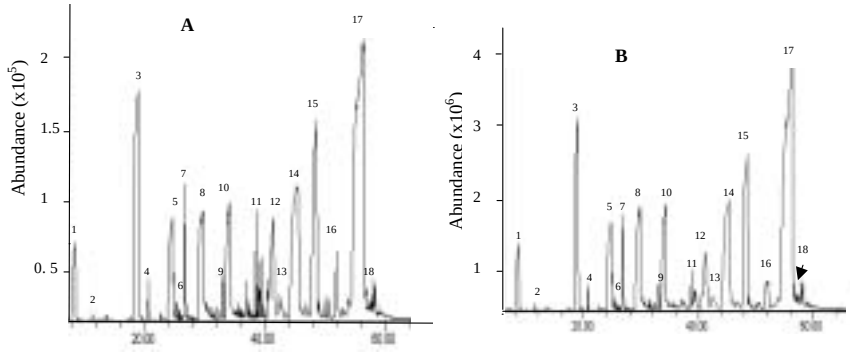

Fig. 6 SPME-GC-MS (Total ion currents, TIC) of the 100 µm PDMS volatile extracts (A) compared to its standard (B) spiked at 10 µg/µL

The present results showed that the quality of extracts was influenced by such factors, as fiber coating, addition of NaCl to the sample solution, extraction time, pH value and desorption time. In these study, increasing salt content at 10% yielded more detectable compounds. Modifications of the combination of salt and pH have been reported to enhance the extraction of analytes from headspace[17]. The addition of NaCl to the sample solution has been described by Pawliszyn[24] as a mean to increase the extraction recovery and decrease the solubility of hydrophilic compounds in the aqueous phase. However, according to Rodriguez *et al.*[25], addition of NaCl increased the strength of the aqueous solution and decreased the affinity of both species for the fiber coating. As such, it may affect negatively the extraction efficiency of some chemicals. This has prompted the extraction of anti-inflammatory drugs without the addition of salt by Rodriguez *et al.*[25].

When comparing the types of fiber coating, the non-polar 100 µm PDMS fiber, which had been reported as suitable for the extraction of volatiles and semivolatiles compounds[17], extracted compounds with higher

TABLE-1
CHARACTERISTIC MS IONS AND PERCENTAGES OF VOLATILE CHEMICALS EXTRACTED BY 75 µm CAR-PDMS FIBER AND IDENTIFIED BY GC-MS

Peak No.	Volatile chemicals	K_1^i	RSD (%)	Mean[a] (%)	Characteristics MS ions[b]
1	2-Ethyl furan	5.1×10^3	6.5	0.43	81(100), 96(55), 39(11), 53(20), 67(10)
2	Hexanal	1.3×10^3	5.8	2.16	44(100), 39(26), 56(98), 67(20), 72(26), 100(25)
3	E-2-Hexenal	8.0×10^6	9.1	31.44	41(100), 55(80), 69(98), 83(55), 98(25)
4	*p*-xylene	1.8×10^2	7.1	0.96	91(100), 41(32), 55(30), 69(30), 77(11), 83(24), 106(65)
5	Nonane	4.5×10^3	4.2	1.87	57(100), 43(90), 71(27), 85(50), 99(13)111(2), 128(13)
6	2, 6-Dimethyl octane	6.1×10^3	6.3	1.37	57(100), 41(60), 71(75), 86(18), 113(20), 126(4), 142(2)
7	3-Ethyl cyclopentanone	3.3×10^3	4.1	1.76	57(100), 43(42), 71(73), 83(80), 97(25), 112(30)
8	4-Methyl nonane	5.9×10^3	4.6	1.72	57(100), 43(75), 71(40), 85(13), 98(32), 142(4)
9	*S*-(-) limonene	2.4×10^5	8.9	7.13	57(100), 68(80), 71(95), 83(50), 93(60), 136(20)
10	*R*-(+) limonene	5.0×10^5	7.4	0.44	57(100), 68(80), 71(95), 83(50), 93(60), 136(20)
11	Decane	3.1×10^5	5.8	6.24	57(100), 43(75), 71(40), 85(35)142(10)
12	Undecane	1.6×10^2	5.8	1.49	57(100), 43(74), 71(56), 85(48), 98(9), 127(3), 156(10)
13	Dodecane	1.4×10^2	7.6	0.71	57(100), 43(75), 71(72), 85(49), 98(9), 127(6), 170(8)
14	Naphthalene	1.5×10^2	6.8	0.29	128(100), 51(7), 63(7), 69(34), 77(5), 128(7)
15	Butyrolactone	7.2×10^4	7.2	8.88	42(100), 56(38), 67(5), 81(8), 86(60)
16	(Z)-9-Octadecen-amide	6.9×10^5	10.5	5.69	59(100), 72(60), 41(38), 83(15), 98(15), 126(10), 281(2)

[a]Mean concentration of volatile chemicals, [b]Characteristics MS ions data consisted of mass-to charge ratios (relative intensity)

molecular weight than that extracted by the 75 µm fiber as well as longer retention time, mostly starting from 20 min. The bipolar 75 µm CAR-PDMS fiber extracted majority of the compounds with a retention time up to 18.98 min with lower molecular weight up to 281 and which mostly fell between 96 to 170. This difference in elution time showed that the profiles of the volatile chemicals extracted from the 2 fibers were different. Therefore, the selectivity of the fiber can be altered by the type of polymer coating

TABLE-2
CHARACTERISTIC MS IONS AND PERCENTAGES OF VOLATILE CHEMICALS EXTRACTED BY 100 μm PDMS FIBER AND IDENTIFIED BY GC-MS

Peak No.	Volatile chemicals	K_1^i	RSD (%)	Mean[a] (%)	Characteristics MS ions[b]
1	4-Methyl-2-pentene	3.6×10^2	6.8	3.12	25(25), 42(100), 68(99), 85(25)
2	2-Ethoxyethanol	2.1×10^2	7.8	0.96	59(100), 45(30), 72(28), 87(10)
3	3, 3-Dimethyl-1-pentene	1.1×10^2	8.1	0.041	69(100), 41(70), 57(54), 83(40), 98(15), 131(28), 219(40
4	E-2-Hexenal	7.4×10^5	5.9	8.71	41(100), 55(80), 69(98), 83(55), 98(25)
5	5-Heptyldihydro-2(3H)-furanone	6.1×10^4	9.0	9.68	80(100), 120(10), (170(13), 210(13), 246(8)
6	Naphthalene	1.2×10^2	7.6	1.19	128(100), 51(7), 63(7), 69(34), 77(5), 102(7), 128(0)
7	Tetradecane	4.8×10^2	6.3	2.77	71(100), 85(75), 98(20), 198(10)
8	Butylated hydroxytoluene	4.8×10^5	7.3	8.02	69(30), 105(45), 161(35), 205(100), 226(20)
9	Dodriacontane	3.4×10^3	6.2	4.23	71(100), 85(60), 99(10), 219(10), 263(5)
10	Hexadecane	1.4×10^3	4.5	1.59	71(100), 85(70), 99(22), 113(10), 226(5)
11	Methyl ester-propanoic acid	3.1×10^4	6.2	4.64	71(100), 43(50), 56(8), 111(8), 159(5), 219(8), 274(3)
12	Octadecane	1.6×10^2	9.4	0.81	71(100), 85(55), 97(25), 131(12), 219(30), 254(10)
13	Palmitamide	5.7×10^3	7.8	6.24	59(100), 43(24), 72(40), 86(8), 128(8), 255(3)
14	(Z)-9-Octadecen-amide	7.1×10^5	4.8	35.33	59(100), 72(60), 41(38), 83(15), 98(15), 126(10), 281(2)
15	Chrysene-d12	3.8×10^3	5.4	4.16	240(100), 45(8), 59(6), 92(6), 106(13), 120(15)
16	Hexatriacontane	1.5×10^2	9.5	0.94	71(100), 85(60), 97(30), 131(25), 219(32), 263(5)
17	Squalene	5.9×10^3	6.9	14.9	70(100), 80(50), 90(10), 120(8), 220(3), 410(0)
18	Heptacosane	1.2×10^2	4.9	0.71	66(100), 82(50), 142(50), 220(45), 280(50), 380(0)

[a]Mean concentration of volatile chemicals, [b]Characteristics MS ions data consisted of mass-to charge ratios (relative intensity)

used on the fibers and the coating thickness. The adsorption of the compounds to polymers could also be affected by the physical and chemical parameters of compounds and the adsorbents[26-28].

The present results are in agreement with the characteristics of the fiber itself as described by Supelco[17] that the CAR-PDMS fiber was composed of a porous carbon with a surface area of 720 m^2/g, optimal for small molecules. It is suggested that micropores of the CAR-PDMS fibre retain

smaller analyte then do other fibers. We found that the fiber (PDMS) was more suitable for higher molecular weight compounds. These results demonstrated that both of the 2 fibers showed efficiency and were complementary in extracting volatile chemicals from *S. viarum* leaves. Bicchi *et al.*[18] reported that the liquid phase polydimethylsiloxane and the porous solid carboxen and/or divinylbenzene were the most effective fibers in headspace SPME-gas chromatographic analysis of aromatic and medicinal plants. In present study, sampling time has been previously set after a preliminary optimization test at 0.5 h and temperature at 30°C as optimal conditions for the extraction of the volatiles. The same temperature at 30°C has been reported as optimal to avoid artifacts and Maillard reactions[24]. Most chemicals extracted in both fibers belonged to the class of aromatic hydrocarbons, Jasmones (cyclopentanones such as; 3-ethylcyclopentanone), aldehyde hydrocarbons including common wound chemical, the oxygenated aliphatic compounds (E)-2-hexenal and hexanal, alkane, alphatic lactones (bytyrolactone), isoprenoids (limonene isomers), structural furan class for the chemical 2-ethyl furan, which is also found in tomato and fatty acids such as (Z)-9-octadecenamide.

Several volatile chemicals detected in present study were previously described as semiochemicals which elicited responses from insects. These chemicals included butyrolactone or γ-butyrolactone (GBL) with the chemical name of 2(3H)-furanone dihydro. This compound was detected from the extraction of the volatile chemical emitted by *S. viarum* leaves by Georgievska[8] using microwave extraction method. This finding confirms that the chemical furanone could be a stable chemical in *S. viarum* leaves as it was found in both microwave assisted extraction and SPME extract. Furanone has been described as an insect repellent[29]. The volatile chemical butylated hydroxytoluene, mainly used in the formulation of insect pheromone is an antioxidant in food, animal feed, animal and vegetable oils. Some studies indicated that butylated hydroxytoluene had some antiviral activity[30] and antimicrobial properties[31]. Hexanal, (E)-2-hexenal and limonene were found to elicit electroantennographic (EAG) responses in *H. armigera*[3]. Limonene, reported to be the most abundant orange-emitted volatiles, induced actively EAG responses of *Ceratitis capitata*[32]. Francis *et al.*[33] reported that most of the terpenes are infochemicals for herbivore species when emitted from the related host plant. *Diaphania nitidalis* (Stoll.) (Lepidoptera, Pyralidae) is attracted by R-, S-limonene and from *Cucurbita pepo* leaves. It was also reported that (E)-2-hexenal elicited behavioral responses in other insect species[34-39].

In summary, SPME method was a fast method to extract the volatile chemicals from *S. viarum* leaves. The fibers 75 μm CAR-PDMS and 100 μm PDMS were complementary in the extraction of *S. viarum* volatile

chemicals. The SPME method has also been shown as accurate and stable with high efficiency. Thus, SPME can be used as a convenient alternative method to classical solvent extraction because of its elimination of the risk of artifact development and the use of toxic organic solvent. However, finding the physiological and behavioral responses to those volatiles either as an attractant for egg-laying or repellent of *H. armigera* could lead to the formulation of the concerned semiochemicals for their potential testing in the field.

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