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Original article (Orijinal araştırma)

Comparative study of the sex pheromone of carob moth, *Apomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) from four regions of Iran using headspace solid phase micro extraction - gas chromatography/mass spectrometry

İran'ın dört bölgesinden toplanan Harnup güvesi *Apomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae)'nde eşeysel feromonun üst katman katı faz mikro ekstraksiyonu - gaz kromatografisi / kütle spektrometresi kullanarak karşılaştırılması

Saeede NOORBAKHSH[1*] **Babak Heidary ALIZADEH[2*]**

Hossein FARAZMAND[2] **Moosa SABER[3]**

Summary

Carob moth, *Apomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) is the most important pest of pomegranate in Iran as well as in most other countries. There is no suitable chemical method available for controlling this pest. The sex pheromone components, emitted by virgin female of *A. ceratoniae* were characterized by headspace solid phase micro extraction (HS-SPME) and subsequently analyzed by gas chromatography/mass spectrometry. The low rate of release of pheromone from the gland, common to the most of the lepidopteran insects, is one of the limiting factors in pheromone research studies. As a result, sex pheromone components of the insect from four different geographical regions of Iran were analyzed comparatively by HS-SPME in 2015. The major component, (*Z,E*)-9,11, 13-tetradecatrienal, and minor components, (*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal, were identified and compared to reference samples. Compared to gland extraction, the simplicity of HS-SPME technique revealed its suitability for identification of the pheromone components.

Keywords: *Apomyelois ceratoniae,* GC/MS, micro extraction, (*Z,E*)-9,11-tetradecadienal

Özet

Harnup güvesi, *Apomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) diğer ülkelerde olduğu gibi İran'da da narın en önemli zararlısıdır. Zararlıların mücadelesi için uygun herhangi bir kimyasal yöntem bulunmamaktadır. *Apomyelois ceratoniae*'nin çiftleşmemiş dişisi tarafından yayılan eşeysel feromon bileşenleri, üst katman katı fazlı mikro ekstraksiyon (HS-SPME) yöntemi ile karakterize edilmiş ve daha sonra gaz kromatografisi / kütle spektrometresi ile analiz edilmiştir. Lepidopterlerin çoğunda olduğu gibi feromon araştırmalarında sınırlayıcı faktörlerden biri, salgı bezinden feromon salım oranının düşük olmasıdır. Sonuç olarak, İran'ın dört farklı coğrafi bölgesindeki böceklerin cinsiyet feromon bileşenleri HS-SPME yöntemiyle karşılaştırmalı olarak 2015 yılında analiz edilmiştir. Ana bileşen olan (*Z,E*)-9,11,13-tetradekatriyenal ve iz bileşenler, (*Z,E*)-9,11-tetradekadienal ve (*Z*)-9-tetradekenal tanımlanmış ve referans örneklerle karşılaştırılmıştır. Bez çıkarma ile karşılaştırıldığında, HS-SPME tekniğinin basitliği, feromon bileşenlerin tanımlanması için uygunluğunu ortaya koymuştur.

Anahtar sözcükler: *Apomyelois ceratoniae,* GC/MS, mikro ekstraksiyon, (*Z,E*)-9,11-tetradekadienal

[1] Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Maragheh, Iran
[2] Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
[3] Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
* Corresponding author (Sorumlu yazar) e-mail: alizadehbh18@gmail.com
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Introduction

Insect pest management, monitoring and control programs, utilizing sex pheromones as behavior modifying chemicals, have become important for several insect groups, particularly moths. Established methods of analyzing insect pheromones involve extraction by solvents. The low rate of release from the glands, common to most of the Lepidoptera, is one of the limiting factors. These methods often require tedious and solvent consumptive procedures. Also, before analytical studies can be undertaken, large quantities of insects are needed for extraction of pheromone. Recently, solid phase micro extraction (SPME) has been used widely for the analysis of traces of organic compounds by trapping or rubbing insect glands. SPME is a viable alternative to solvent extraction and offers a convenient, solvent-free and time saving method (Miklas et al., 2000; Gago et al., 2013; Kühbandner & Ruther, 2015). An optimized SPME method coupled with gas chromatography/mass spectrometry (GC/MS) has been developed for the determination of the sex pheromone of *Eucosma notanthes* Meyrick, 1936 (Lepidoptera: Tortricidae). Compared to solvent extraction methods, the optimized SPME method is easier, faster and more efficient. Moreover, it consumes no solvent, and is less prone to contamination from living tissues (Chu et al., 2005). Frerot et al. (1997) used this method to extract (*Z*)-11-hexadecan-1-ol pheromone by rubbing SPME fiber on the gland surface and gland washes. SPME trapped a larger amount of each identified pheromone components than gland washes; about 120 ng per gland with SPME compared to 60 ng per gland by the wash technique. In addition, it is worth to noting that the isolation of the *Thyrinteina arnobia* Stoll, 1782 (Lepidoptera: Geometridae) pheromone components has also been achieved by two different techniques: gland extract and SPME of virgin females (Heath et al., 1983; Delisle & Royer 1994; Jardel et al., 2013).

The carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae), is a worldwide pest of several nuts and fruits including carobs, almonds, and dates. Its range has been expanding to the new parts of the world. For instance, in the USA, this species is a primary pest of dates in the desert valleys of southern California and has the potential to expand north to threaten vast almond and pistachio groves of the San Joaquin Valley (Gothilf 1984; Warner 1988; Cosse et al., 1994). Three pheromone components including (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal, (*Z*)-9-tetradecenal have been extracted by solvent (Baker et al., 1991; Todd et al., 1992). In Iran, pomegranates are grown in several climate regions and the carob moth is the most important pest, causing serious damage to fruit. However, no chemical method is available to control this key pest. Furthermore, the commercial pheromone lure did not attract any male insects in field tests (Farazmand, 2011). In order to evaluate the pheromone of carob moth for pest control in Iran, SPME was used to compare pheromone from Iranian populations of the moth with that reported by Baker (1991). Populations of *A. ceratoniae* were sampled form four regions of Iran, viz. Isfahan, Sistan, Lorestan and South Khorasan, and volatile substances emitted by virgin females collected, analyzed and compared them to a reference samples (Millar, 1990, Baker et al., 1991).

Materials and Methods

Insect culture: Infested pomegranates, containing the pupal stage of *A. ceratoniae*, were collected from Isfahan, Sistan, Lorestan, and South Khorasan in 2015. Fruits were kept at room temperature (25-28°C). Moths were allowed to emerge under a 14L:10D h photoperiod regime and female moths sexed while in pupal stage.

Collection of pheromones by dynamic SPME: Headspace solid phase micro extraction (SPME; Supelco, Sigma-Aldrich Corporation, St. Louis, MO, United States) was used to collect pheromone compounds emitted by virgin female carob moths. The SPME fiber (70 µm polydimethylsiloxane coating) was conditioned in a GC injector at 250°C for 10 min before use. Five virgin female moths were placed in a vial (3.0 × 2.5 cm) sealed with a cap, and left under laboratory conditions at 25±1°C. The SPME fiber was located in the outlet tube of the vial to collect the emitted volatiles for several days, then the loaded fiber was immediately analyzed by coupled GC/MS.

Chemical analysis: All samples were analyzed by GC/MS on a fused capillary column HP5-MS (30 m × 0.25 mm I.D., 0.25 µm film thickness, Agilent, Technologies, Palo Alto, CA, USA) in an Agilent mod. The 6890 chromatograph was equipped with the mass selective detector Agilent 5973 under the following conditions: the injector temperature was held at 250°C; it as carrier gas at l ml/min.; the sample was injected in the split less mode, oven temperature program: 5 min isotherm at 45°C followed by a linear temperature increase of 4ºC/min up to 300°C, which was held for 10 min. All chemicals and reagents were obtained from Merck (Kenilworth, NJ, USA) and Sigma-Aldrich. ^{1}H-NMR spectra were measured using a Bruker 500 MHz spectrometer (Bruker, Rheinstetten, Germany), and chemical shifts were expressed as δ (ppm) with tetramethylsilane as internal standard. The infrared (IR) spectra were obtained on a Shimadzu IRPrestige-21 (Tokyo, Japan). The purity of all compounds was confirmed by the thin-layer chromatography (TLC) using different mobile phases. The elemental analysis was performed with an Elementar Analysensysteme GmbH (Langenselbold, Germany) VarioEL in CHNS mode, which was within 0.4% of theoretical values for C, H and N.

Chemicals

(*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal were obtained from Agrisense-BCS Ltd (Pontypridd, UK). (*Z,E*)-9,11,13-tetradecatrienal was synthesized (Figure 1) by modification of the method of Millar (1990). Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under N_2. Prepared solutions were dried over anhydrous Na_2SO_4, and concentrated by rotary evaporation under reduced pressure. Crude products were purified by flash or vacuum flash chromatography on silica gel (230-400 mesh). Reactions with air- or water-sensitive reagents were done in dried glassware under N_2 atmosphere.

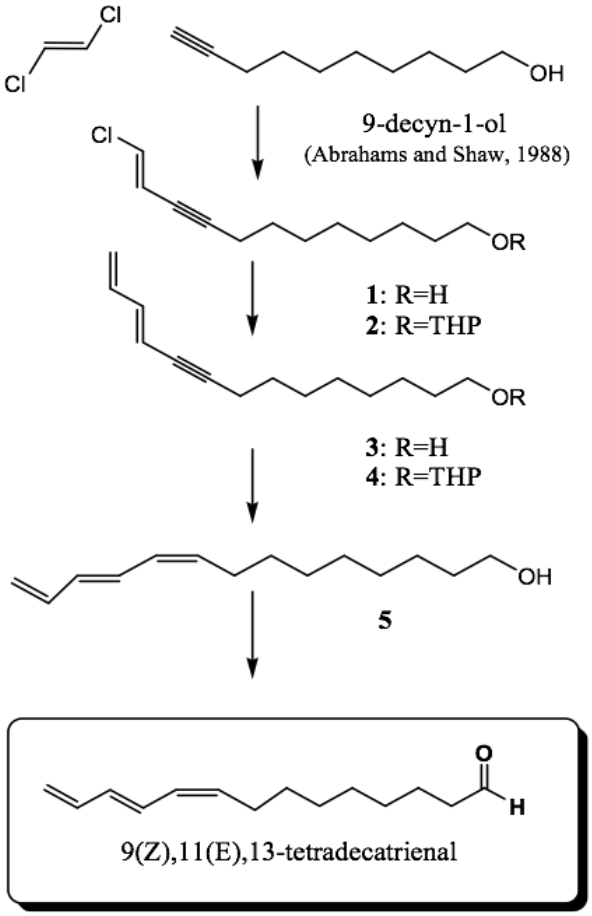

Figure 1. Synthesis of (*Z,E*)-9,11,13-tetradecatrienal.

Synthesis of (*Z,E*)-9,11,13-tetradecatrienal as the major sex pheromone component:

1. (*E*)-1-chloro-dodec-l-en-3-yn-12-ol (1)

A dry 50-ml flask was loaded with bis (triphenylphosphine) palladium (II) chloride (300 mg, 0.45 mmol) and CuI (160 mg, 0.8 mmol) flash with Ar. 9-Decyn-1-ol (1 g, 7.5 mmol) prepared by Zipper reaction (Abrahams & Shaw, 1988), trans-1,2-dichloroethylene (0.13 ml, 1.7 mmol) and THF (3 ml) were added to the reaction flask. Diisopropylamine (0.18 ml, 1.3 mmol) was added dropwise to the stirred mixture, and the initially pale yellow solution rapidly turned into brown then black. The mixture was stirred up at room temperature for 1 day. Hexane (20 ml) was then added, and the mixture filtered. The filtrate was extracted by saturated aqueous NH_4Cl (2× 3 ml), dried and passed through a column of silica gel, and eluted with 20% EtOAc in hexane. The eluate was concentrated, yielding 1.2 mg, 5.6 mmol (85% yield) of chloroalcohol (1). ^{1}NMR δ*:* 1.21-1.44 (m, 8H, H7, 8, 9, 10), 1.45-1.6 (m, 5H, H6, 11, OH), 2.28 (td, 2H, *J* = 6.8, 2.2 Hz, H5), 3.65 (t, 2H, *J* = 6.7 Hz*,* H12), 5.92 (dt, 1H, *J* = 13.5, 2.3 Hz, H2), 643 (d, 1H*, J* = 13.5 Hz, H1). MS *m/z:* 179 (4, M-Cl), 114 (37), 105 (39), 91 (70), 79 (100), 67 (40), 55 (52). *Anal.* Calcd for $C_{17}H_{27}ClO_2$: C, 68.32; H, 9.11, Found: C, 68.12; H, 9.32.

2. THP ether of (*E*)-l-chloro-dodec-l-en-3-yn-12-ol (2)

Compound 1 (1.2 g, 5.4 mmol) was protected as the THP ether by treatment with dihydropyran (1 ml) and a few crystals of p-toluenesulfonic acid in the ether overnight. The mixture was prepared by extraction with sat. aq. $NaHCO_3$ and brine, dried, concentrated and removed solvent traces under vacuum. The protected alcohol 2 (1.3 g, 4.4 mmol) 80% gave one spot on TLC (5% EtOAc in hexane) and was used for next reaction without further purification. ^{1}HNMR δ*:* 1.25-1.45 (m, 8H, CH_2), 1.45-1.65 (m, 8H, CH_2), 1.65-1.9 (m, 2H, H12), 2.28 (td, 2H, *J* = 6.8*,* 2.3 Hz, H5), 3.38 (dt, 1H, *J* = 9.6, 6.7 Hz, H12), 3.46-3.54 (m, 1H, THP), 3.73 (dt, 1H, *J* = 9.6*,* 6.9 Hz, H12'), 3.84-3.91 (m, 1H, THP), 4.57 (br. t, 1H, *J* = 2.7 Hz*,* THP), 5.90 (dt, 1H, *J* = 13.6, 2.3 Hz, H2), 6.43 (d, IH, *J* = 13.6 Hz, H1). IR cm^{-1}: 3075 (w), 2915 (s). MS *m/z:* 299 (M^+), 101 (14), 85 (100), 79 (16), 55 (20). *Anal.* Calcd for $C_{17}H_{26}ClO_2$: C, 68.32; H, 9.11. Found: C, 68.47; H, 9.01.

3. 11(*E*),13-tetradecadien-9-yn-1-ol (4)

Tetrakis (triphenylphosphine) palladium (0.3 g, 0.26 mmol) and chloride 2 (1.3 g, 4.4 mmol) were added to 10 ml of toluene under N_2 at room temperature. The mixture was cooled in an ice bath and vinyl magnesium bromide (10 ml of a 1 M solution in THF) was added dropwise over 5 min. The mixture warmed to room temperature while being stirred overnight. The reaction mixture was poured into hexane (30 ml), extracted thoroughly with 2 M NH_4Cl and brine, dried and concentrated. The residue (**3**) was dissolved in MeOH (10 ml) and catalytic amount of *p*-toluenesulfonic acid, and stirred at room temperature. After the completion of the reaction (checked by TLC), $NaHCO_3$ (0.3 g) was added and the mixture concentrated on a rotary evaporator to remove MeOH. The residue was partitioned between water and hexane (30 ml each). The hexane layer was washed with brine, dried and partially fractioned by passing through a column of silica gel, eluting with 20% EtOAc in hexane. The fraction containing the purified product was concentrated and pumped under vacuum, giving dienynol 4 as a yellow oil (0.7 g, 3.3 mmol) 76% yielded over 3 steps. ^{1}NMR δ*:* 1.24-1.46 (m, 8H, H3, 4,5,6),1.46-1.65 (m, 5H, H2, 7, OH), 2.33 (td, 2H, *J* = 6.9*,* 2.2 Hz, H8), 3.65 (t, 2H, *J* = 6.6 Hz*,* H1), 5.12 (d, 1H, *J* = 9.7 Hz*,* H14), 5.25 (br.d, 1H, *J* = 16.5 Hz*,* H14), 5.62 (dt, IH, *J* = 15.6*,* 2.0 Hz, H11), 6.35 (dt, 1H, *J* = 16.5*,* 10.0 Hz, H13), 6.51 (dd, 1H, *J* = 10.9*,*

15.4 Hz). IR cm^{-1}: 3333 (s, br.), 2932 (s). MS *m/z:* 206 (2, M^+), 105 (29), 91 (100), 79 (37), 65 (31), 41 (37). *Anal.* Calcd for $C_{14}H_{22}O$: C, 80.50; H, 10.75. Found: C, 80.77; H, 10.86.

4. (*Z,E*)-9,11,13-tetradecatrienol (5)

Zinc dust (2.39 g, 46.50 mmol) was stirred with 6 ml × 3 HCl (3%) for 2 min under N_2. The acid was decanted and the zinc was rinsed twice with distilled water twice. Cu(II)OAc (0.2 g, 1.02 mmol) in hot water (2.6 ml) was slowly added, then $AgNO_3$ (0.24 g, 1.41 mmol) in H_2O (2.6 ml) was added to the solution and it was stirred for 15 min. The mixture was filtered and the filtrate was added into the flask containing MeOH (5 ml) and H_2O (7 ml), followed by the solution of dienynol 4 (0.3 g, 1.45 mmol) in 2 ml MeOH. The mixture was stirred for 11 h at 50°C under N_2, then filtered, and washed with MeOH 3 ml, MeOH (10 ml) and HCl 10% (1.2 ml). The filtrate was concentrated and the residue was extracted with Et_2O: hexane (1:1), aq. Sat. NH_4Cl, which was dried over $MgSO_4$, and concentrated, and purified by vacuum flash chromatography on silica gel (hexane: EtOAc, 95:5) gave 0.16 g (0.32 g, 1.45 mmol, quantitative yield) of olefin 5 as a colorless oil. ^{1}NMR (Figure 2) *δ*: 1.2-1.45 (m, 8H, H3, 4, 5, 6), 1.5-1.65 (m, 4H, H2, 7), 2.19 (br. quartet, 2H, *J* = 6.8 Hz*,* H8), 2.37 (s, lH, OH), 3.64 (t, 2H, *J* = 6.6 Hz*,* Hl), 5.08 (d, lH, *J* = 10.2 Hz, H14), 5.21 (d, lH, *J* = 15.6 Hz, Hl4'), 5.48 (dt, lH, *J* = 10.7, 7.7 Hz, H9), 6.02 (br. t, lH, *J* = 11.0 Hz, H10), 6.20 (dd, lH, *J* = 14.9, 10.7 Hz, H12), 6.41 (dt, lH, *J* = 16.8,10.3 Hz, H13), 6.51 (dd, lH, *J* = 14.8*,* 11.3 Hz, H11). IR cm^{-1}: 3345 (s), 2940 (s), 1005 (s). MS *m/z:* 208 (25, M^+), 107 (13), 91 (51), 79 (100), 67 (25). *Anal.* Calcd for $C_{14}H_{24}O$: C, 80.71; H, 11.61. Found: C, 80.87; H, 11.53.

5. (*Z,E*)-9,11,13-tetradecatrienal

The trienal alcohol 5 was converted to corresponding aldehyde by pyridinium chlorochromate (PCC) oxidation (Moreira 2006). Flask was charged with CH_2Cl_2 (10 ml) and PCC (0.36 g, 1.68 mmol), and powdered molecular sieve. Alcohol (0.18 g, 0.84 mmol) in CH_2Cl_2 (2 ml) was added to this solution, and it was stirred up for 3 h. Furthermore, hexane was added, and the mixture was stirred up for 10 min and then filtered. As the filtrate was dried, concentrated and then flash chromatogramed (SiO_2), it gave 9(*Z*),11(*E*),13-tetradecatrienal as a colorless oil (0.16 g, 0.77 mmol, 91%). ^{1}H-NMR (500 MHz, CDCl3, Figure 2) δ: 1.24- 1.31 (m, 8H, H4, 5, 6, 7), 1.55- 1.62 (m, 2H, H3), 2.18 (brq, 2H, *J* = 6.8 Hz, H8), 2.42 (td, 2H, *J* = 7.0, 2.0 Hz, H2), 5.09 (d, 1H, *J* = 10.0 Hz, H14), 5.22 (d, 1H, *J* = 16.8 Hz, H14`), 5.47 (dt, 1H, *J* = 10.8, 7.6 Hz, H9), 6.02 (brt, 1H, *J* = 10.8 Hz, H10), 6.20 (dd, 1H, *J* = 15.2, 10.8 Hz, H12), 6.41 (dt, 1H, *J* = 16.8, 10.4 Hz, H13), 6.41 (dt, 1H, *J* = 16.8, 10.4 Hz, H13), 6.50 (dd, 1H, *J* = 15.2, 11.6 Hz, H11), 9.78 (t, 1H, *J* = 2 Hz, aldehyde H). 3040 (w), 2940 (s). IR cm^{-1}: 2747 (m), 1730 (s), 1008 (s), 945 (m). MS m/z: 207 (13), 206 (M^+, 86), 178 (4), 135 (7) 121 (5), 107 (15), 94 (20), 93 (52), 91 (65), 79 (100), 77 (60), 67 (22), 41 (22). *Anal*. Calcd for $C_{14}H_{22}O$: C, 81.50; H, 10.75 Found: C, 81.66; H, 10.56.

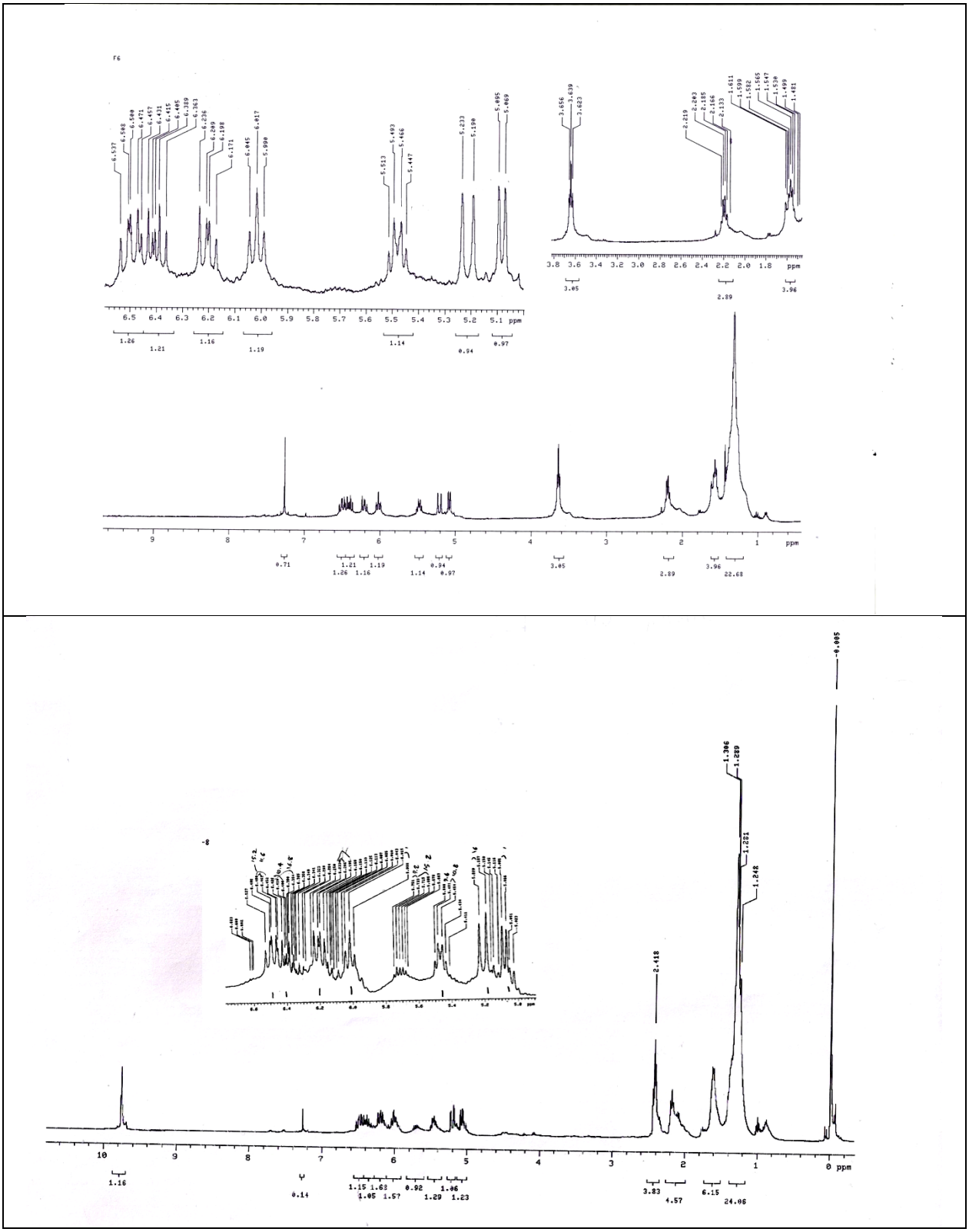

Figure 2. H-NMR of alcohol **5** (top), (Z,E)-9,11,13-tetradecatrienal (bottom).

Results and Discussion

In the present study, four pomegranate regions with different climate conditions were selected for the isolation of the volatile compounds of the sex pheromone of carob moth. This solvent-free technique was reliable, greatly sensitive and fast. Also, it did not damage insects and was applied on the same sample over several consecutive days. Isolation of the pheromone components was carried out using solid phase micro extraction (SPME) of virgin females. (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal were assigned according to mass analytical data and retention time (RT) standard. On the basis of careful comparison of synthetic compounds from the Isfahan, Sistan, Lorestan, and South Khorasan samples with the MS data from the literature and the RT, three sex pheromone components were assigned in Iran (Figure 3). Comparison of diagnostic ions (79, 67, 55, 206, and 91) with each other indicated the peak at RT =12.56± 0.01, belonging to (*Z*,*E*)-9,11,13-tetradecatrienal (Figures 3, 4). In addition, comparison of the ions 67 and 55, as the base peak, for two other components resulted in the assignment of two peaks at RT =11.45±0.01 and 10.86±0.01, for compounds (*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal, respectively (Figure 6). As well, Frerot et al. (1997) isolated three components which were similar to those of *A. ceratoniae* from *Sesamia nonagrioides* (Lefebvre, 1827), including *Z* (11)-hexadecen-1-ol, acetate form and 16:OAc by rubbing SPME on the gland surfaces. The consequence revealed that, compared to gland washes, the SPME technique appeared to be more efficient in trapping both synthetic and natural pheromone. Baker et al. (1991) isolated three components from date fruits which included (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal carob moth sex pheromone in the ratio of 10:1:1. As shown in Table 1, the ratio of three sex pheromone components in four regions of Iran are different from each other; that is, while this ratio was 10:0.45:0.43 in Isfahan sample, it was 10:1.1:0.9 and 10:0.9:0.9 in South Khorasan and Sistan, respectively. This is similar to the data ratio of Baker et al. (1991). Also, the Lorestan sample had a different ratio of 10:2.5:2.1. Considering the discrepancies in these ratios, it seems that there might be a conspecific relationship between carob moth species in Iran. Moreover, in an unpublished field study, a trap cage with a virgin female was utilized, and notably, it revealed that only males form the same regional population were captured; that is, males from the other regions were not captured. Furthermore, commercial pheromone of carob moth lure did not attract male insects. It seems that there is regional variability among the sexual behaviors of carob moths in different climatic areas of Iran. Similar sexual behaviors were also reported in the males of *Nezara viridula* (L., 1758) (Heteroptera: Pentatomidae). It is worth noting that the sexually mature males specifically release a pheromone which is attractive in the field to conspecific females. As the author explained, the pheromone strains of *N. viridula* from Florida were different from those form Hawaii (Aldrich et al., 1987).

Four chromatograms of volatile compounds of carob moth were extracted by SPME (Figures 3, 4) in Isfahan, Sistan, Lorestan and South Khorasan. Ions 79, 67, 55, 206 and 91 of the peak at (RT) =12.56± 0.01 belong to (*Z*,*E*)-9,11,13-tetradecatrienal (Figure 5). Ions 67 and 55 as base peaks at (RT) =11.45 ± 0.01 and 10.86 45 ± 0.01, belong to (*Z*,*E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal, respectively (Figure 6). Baker (1989) isolated CS_2 extraction solvent and identified sex pheromone components of the carob moth by solvent extraction method and upon comparison with a synthetic samples, GC/MS analysis, three fragment ions: M^+ = 79, 91, 206 for (*Z*,*E*)-9,11,13-tetradecatrienal. Rodstein et al. (2009) identified 3,5,9-trimethylundecanoic acid as Females sex pheromone of cerambycid beetle by SPME technique and characterized the fragment ions of the mass spectrum, a base peak at m/z 74, m/z 143 and 185, which was the same fragment ions analogous to the synthetic compound and also with the same retention time.

The retention times and ratios of the sex pheromone components of *A. ceratoniae* in Isfahan, Sistan, Lorestan and South Khorasan are shown in Table 1. The RT for (*Z*,*E*)-9,11,13-tetradecatrienal, (*Z*,*E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal were 12.56±0.01, 11.45±0.01 10.86 45 ± 0.01, respectively. Baker et al. (1991) reported Rt for these three components of 8.56, 8.93, and 9.28 min, respectively, with GC/EAG with 30-m DB-1 column.

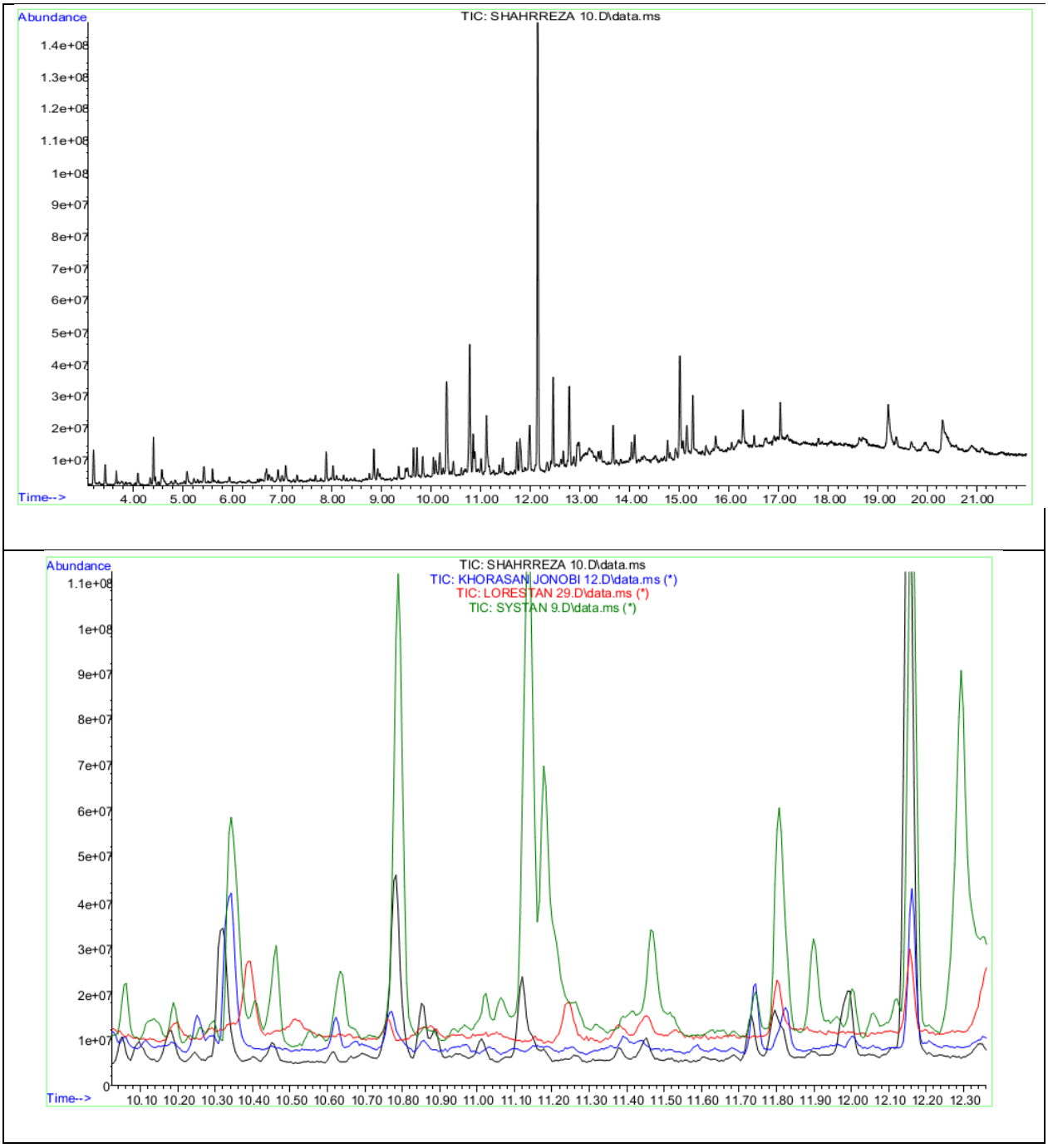

Figure 3. Chromatogram obtained by SPME on pheromone glands of *Apomyelois ceratoniae* in Isfahan, Iran (top), and chromatogram expansion at 10.60-12.20 min of four regions (bottom).

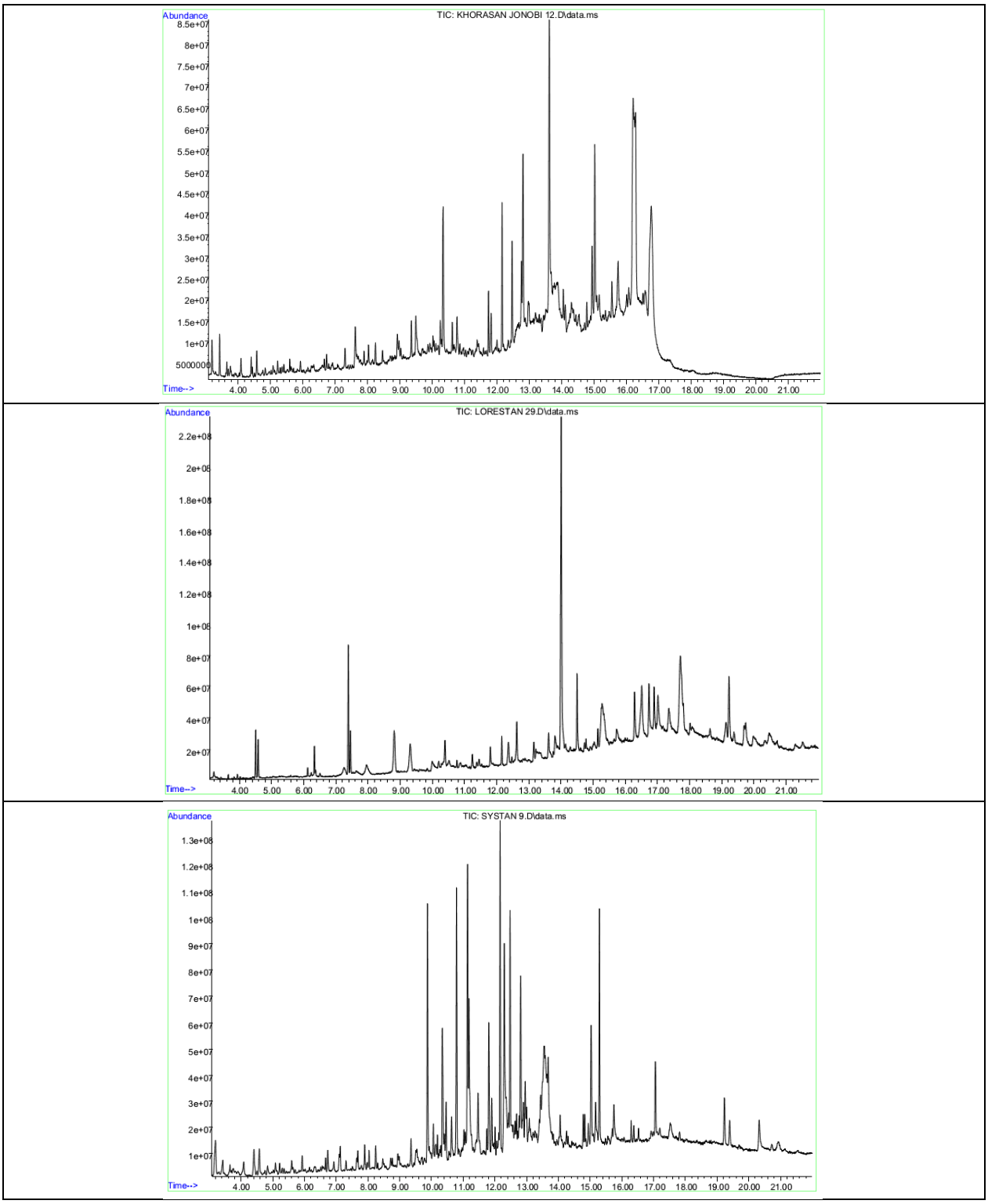

Figure 4. Chromatogram obtained by SPME on pheromone glands of *Apomyelois ceratoniae* in Khorasan, Lorestan and Sistan province.

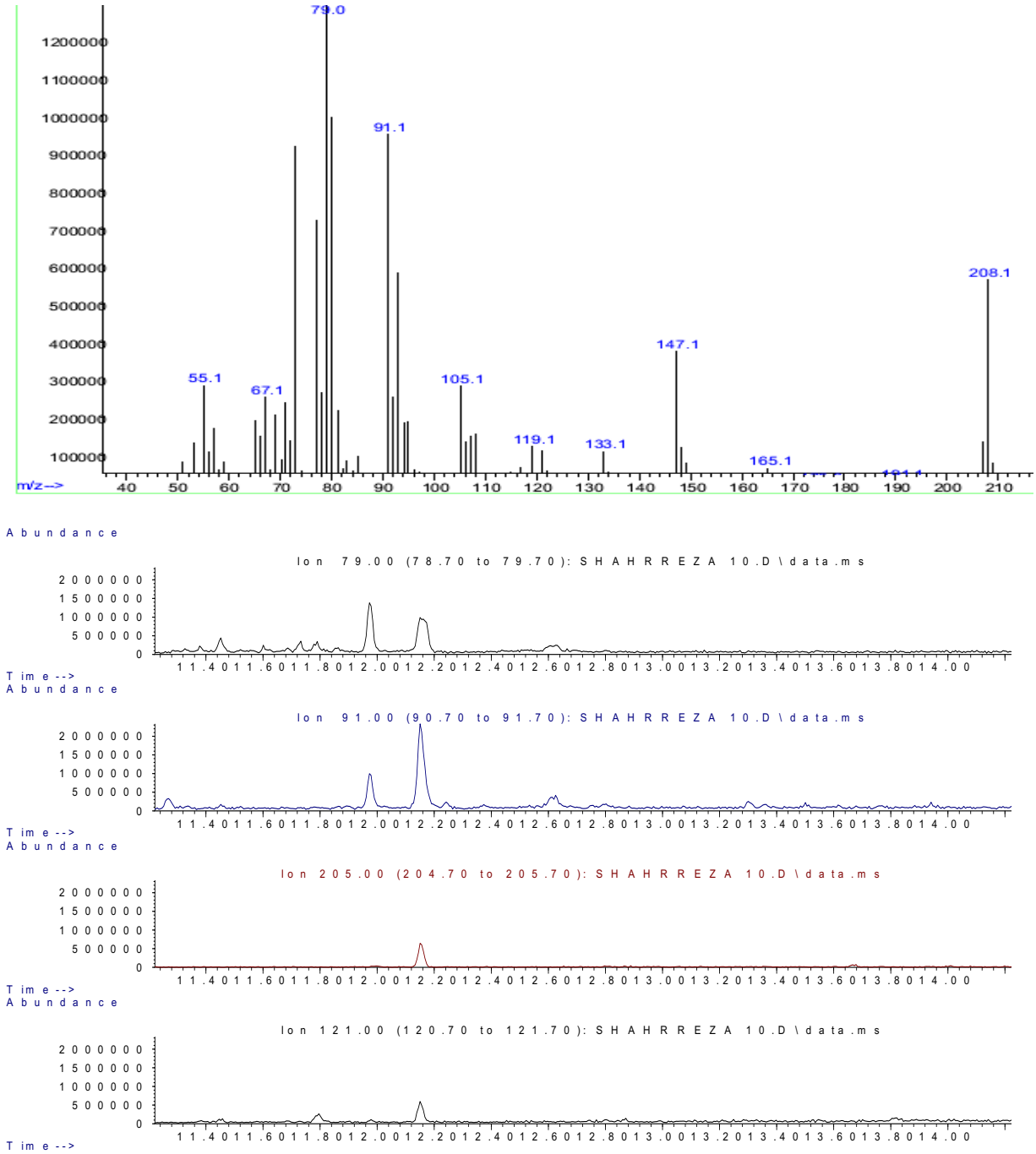

Figure 5. Results of the GC/MS of component (*Z*,*E*)-9,11,13-tetradecatrienal (top) and extracted ion chromatograms with diagnostic ions: i.e., 79, 91, 121 and 206 (bottom).

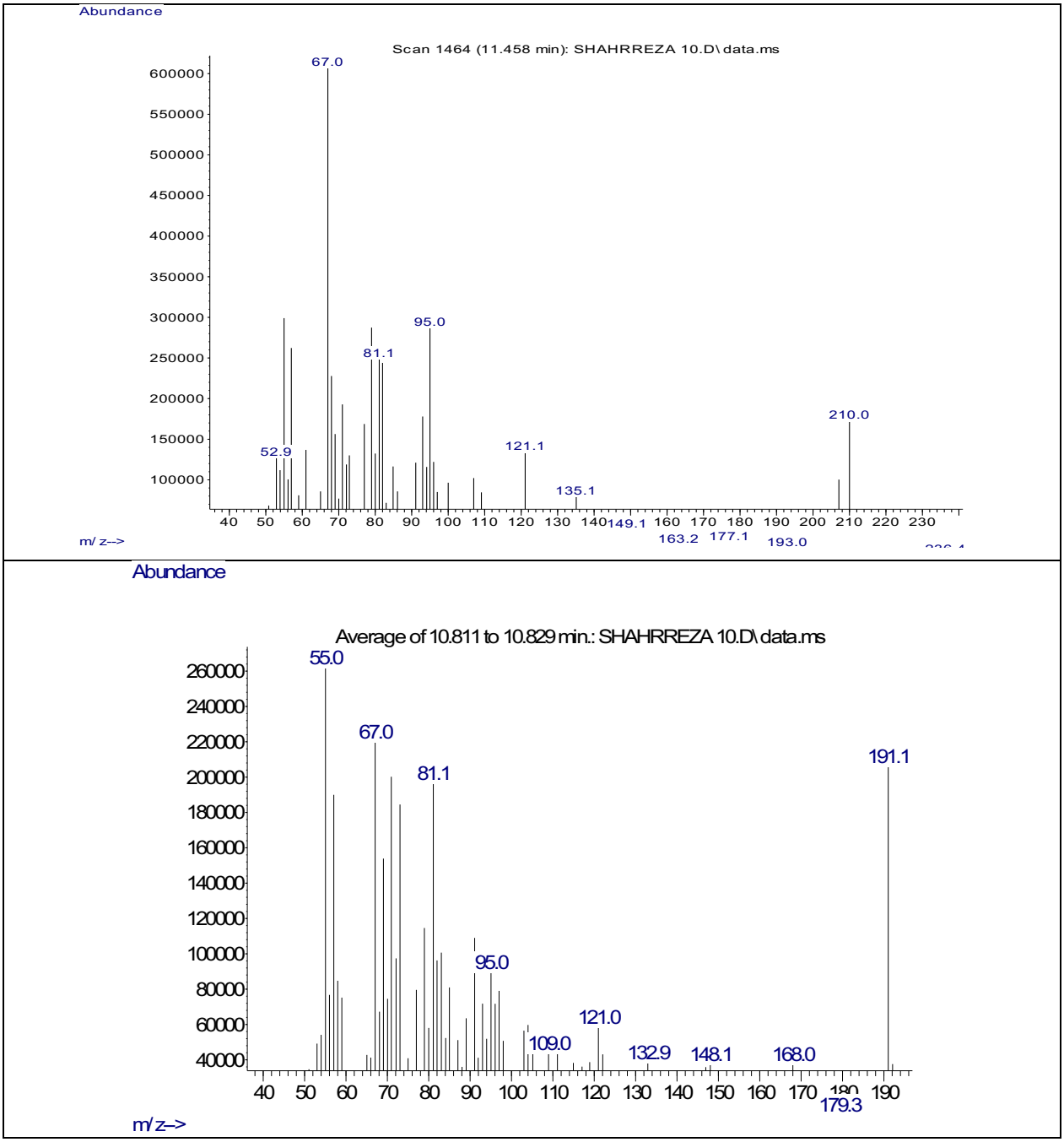

Figure 6. EI mass spectra of the compound from the SPME extract of female ovipositor 11.45 min (*Z,E*)- 9,11-tetradecadienal (top); 10.82 min (Z)-9-tetradecenal (bottom).

The ratios of the three components were 10:0.45:0.43 in Isfahan, 10:1.1:0.9 in South Khorasan, 10:0.9:0.9 in Sistan, and 10:2.5:2.1 in Lorestan, respectively. In addition, Frerot et al. (1997) used SPME for the isolation of Z11-16:Ac and Z11-16:OH from gland female *S. nonagrioides*, and identified their structures by GC/MS analyses of ions *m/z* 43, 55, 67, 82, 96, 222 and 41, 55,67, 82, 96, 222 and their ratio was found to be 9:0.5.

Mozaffarian et al. (2007) observed geometric and morphometric differences among carob moths from different populations in Iran due to the genetic changes in the population. These changes were considered to have resulted from the natural selection and adaptation to environmental conditions (Girling & Carde, 2006). Ziaaddini et al. (2010) indicated that there were differences in foraging behaviors of males and calling behaviors of females in different populations of carob moth in Saveh, Kerman and Arsanjan under the same conditions. However, the differences between populations did not prevent cross attraction and mating was not be prevented between the different populations (Phelan & Baker, 1986; Gemeno et al., 2000). Nevertheless, differences among the pheromones were evident for the populations used. In addition, the ratios of these components in from the different geographical regions differed. In response to the studies of Mozaffarian et al. (2007) and Ziaaddini et al. (2010), the authors of the present study decided to investigate these pheromone compounds from different parts of Iran.

In summary, SPME followed by GC/MS was an excellent technique for the analysis and study of volatiles of *A. ceratoniae* as an important pest of pomegranate. Three pheromone components (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal were identified. The ratios of these components were characterized and found to vary among the carob moth populations from different geographical regions of Iran. Further investigations are ongoing to prepare various ratios of three pheromone components of *A. ceratoniae* to be tested in the field in other regions of Iran.

Table 1. Variation of the retention time and the ratio of sex pheromone components of *Apomyelois ceratoniae* in four regions of Iran

	(*Z,E*)-9,11,13-tetradecatrienal Ratio (RT)	(*Z,E*)-9,11-tetradecadienal Ratio (RT)	(*Z*)-9-tetradecenal Ratio (RT)
Isfahan	10 (12.15)	0.45 (11.45)	0.43 (10.88)
South khorasan	10 (12.16)	1.1 (11.44)	0.9 (10.86)
Lorestan	10 (12.15)	2.5 (11.53)	2.1 (10.95)
Sistan	10 (12.16)	0.9 (11.57)	0.9 (10.86)

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