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Comparison of two different extraction techniques by SPME, in study of female specific volatile components of the Iranian populations of *Ectomyelois ceratoniae* Zeller (Lepidoptera.: Pyralidae)

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ABSTRACT

The carob moth, Ectomyelois ceratoniae Zeller, is a worldwide pest of nuts and fruits. This species is the most destructive pest of pomegranate in Iran. In this research, volatile components produced by carob moth were extracted by using Solid-phase microextraction (SPME) to compare the efficiency of different extraction techniques by SPME. SPME was conducted through two different methods: 1) extraction from headspace, 2) extraction through the contact of fiber with papillae anales of females or valva of males. Chemical analyses of extractions were conducted by Mass Spectrometry-Gas Chromatography (GC-MS). Results showed that more female specific components (3 components) were detected by extraction through the contact of fiber with papillae anales of females than the other metods of extraction by SPME. The results evidenced the role of extraction method on detection of female specific volatile components of carob moth and extraction through the contact of fiber with papillae anales of females could be optimized method for extraction of the female specific volatile components of carob moth.

Keywords: Carob moth, Specific volatile components, Extraction methods, SPME, Headspace, contact of fiber, GC-MS.

INTRODUCTION

Solid-phase microextraction is a solvent-free technique, it offers the possibility to analyse highly volatile and/or trace compounds that cannot be easily disclosed by gas chromatography when organic solvents are used (co-elution with the solvent or loss during concentration). Finnaly SPME allowes one to sample chemicals from living organisms with few disturbances in (semi-) natural environments necessary to the performance of peculiar behaviours associated with the chemical release [1]. By using SPME and GC-MS analyses, a sex pheromone blend for the stem borer, *Sesamia cretica* Lederer (Lepidoptera, Noctuidae), was identified. The pheromone gland of each female was extruded by gentle pressure on the abdomen and kept in

this position with forceps while the adsorbent part of a SPME fiber was gently rubbed on the gland surface [2]. Also headspace technique was used to identify the aggregation pheromones of the pest beetles *Scapanes australis* and *Strategus aloeus* by efficient and rapid isolation of their highly volatile components [3]. As yet chemical solvents including N-hexane and CS_2 were used for extraction of the sex pheromone of *E.ceratoniae* [4,5]. In this research tow different extraction techniques by SPME (headspace and contact of fiber) were used for extractin of female specific volatile components of the Iranian populations of *E.ceratoniae*. The present paper presents optimized extraction technique by SPME for extraction and detection of female specific volatile components of *E.ceratoniae.*

MATERIALS AND METHODS

Insect collection: Insects were collected from pomegranate orchards of Yazd province in Iran. Larvae were reared on semi-artificial diet including wheat flour 72%, honey 12%, glycerol 10%, wort 1% and distilled water 5% (Finney, 1967) in the laboratory, the pupae were segregated by sex and the moths allowed to emerge under the following conditions: 16:8 L:D photoperiod at 29±1°C in 70±10% R.H.

Isolation of volatile components: Volatile chemical components were extracted from 2- to -4-day-old male and female moths of first generation exhibiting the characteristic calling posture that were not mated. The SPME fiber was purchased from Supelco USA and consisted of 65 µm film of Polydimethylsiloxane/Divinylbenzene (PDMS/DVB).

1) extraction from headspace: This method was done in seven replications of male and female moths. Three SPME devices and three glassy vessels containing one female, one of them including one male and another one was empty were used in each replication. The SPME fiber was inserted into a hole that was created on the door of glassy vessel during the female calling period, at last three hours of scotophase (fifth to the eighth hour of scotophase; 16:8 L:D regime) at 29±1°C. Because the more percent of the calling females occurred at third and fourth ages and the time of calling was started after fifth hour of scotophase with the peak at eighth hour of scotophase.

2) extraction through the contact of fiber with papillae anales of females or valva of males: This technique was accomplished during the top time of female calling at the final hour of scotophase at 29±1°C in five replications of male and female moths. The SPME fiber was gently rubbed on the gland surface [6] on papillae anales of female while the female frequently evolved the papillae anales in calling period. Also the SPME fiber was rubbed on the valve of male at the same time.

GC-MS analyses: The fiber was then desorbed in the heated injector (250°C) of a Varian cp-3800 gas chromatograph (GC) coupled with a Varian Saturn 2200 mass spectrometry (MS). GC analyses were conducted on a nonpolar DB-5 column (30 m × 0.25 id). Conditions for the DB-5 column was: helium carrier gas flow of 1.5 ml/min, oven temperature program, 2 min at 100°C, then 6°/min to 230°C and held for 1 min at 230°C.

Detection of female specific volatile components: Chromatograms of SPME-sampled volatiles from males and females were compared with each other by GC-MS retention times and mass spectrum to detection of the female specific volatile components of carob moth.

RESULTS AND DISCUSSION

The results of comparison of chromatograms showed the different female specific volatile components in two extraction techniques by SPME. One female specific volatile component was detected in extractions by sampling of live female headspace by SPME and three female specific volatile components were detected in extractions by the contact of SPME fiber with papillae anales (table 1 and figure 1).

Table 1: Identification of female specific volatile components of *E.ceratoniae* by GC-MS, NIST (library of MS) in tow different extraction techniques by SPME

Extraction technique	Retention time (min)	Name of component	Replication
Headspace	13.36	Octadecanal	3
Contact of fiber	5.44	Z-8-Octadecen-1-ol acetate	4
	11.27	1-Hexadecanol, 2-methyl	4
	12.16	Tetradecanoic acid	4

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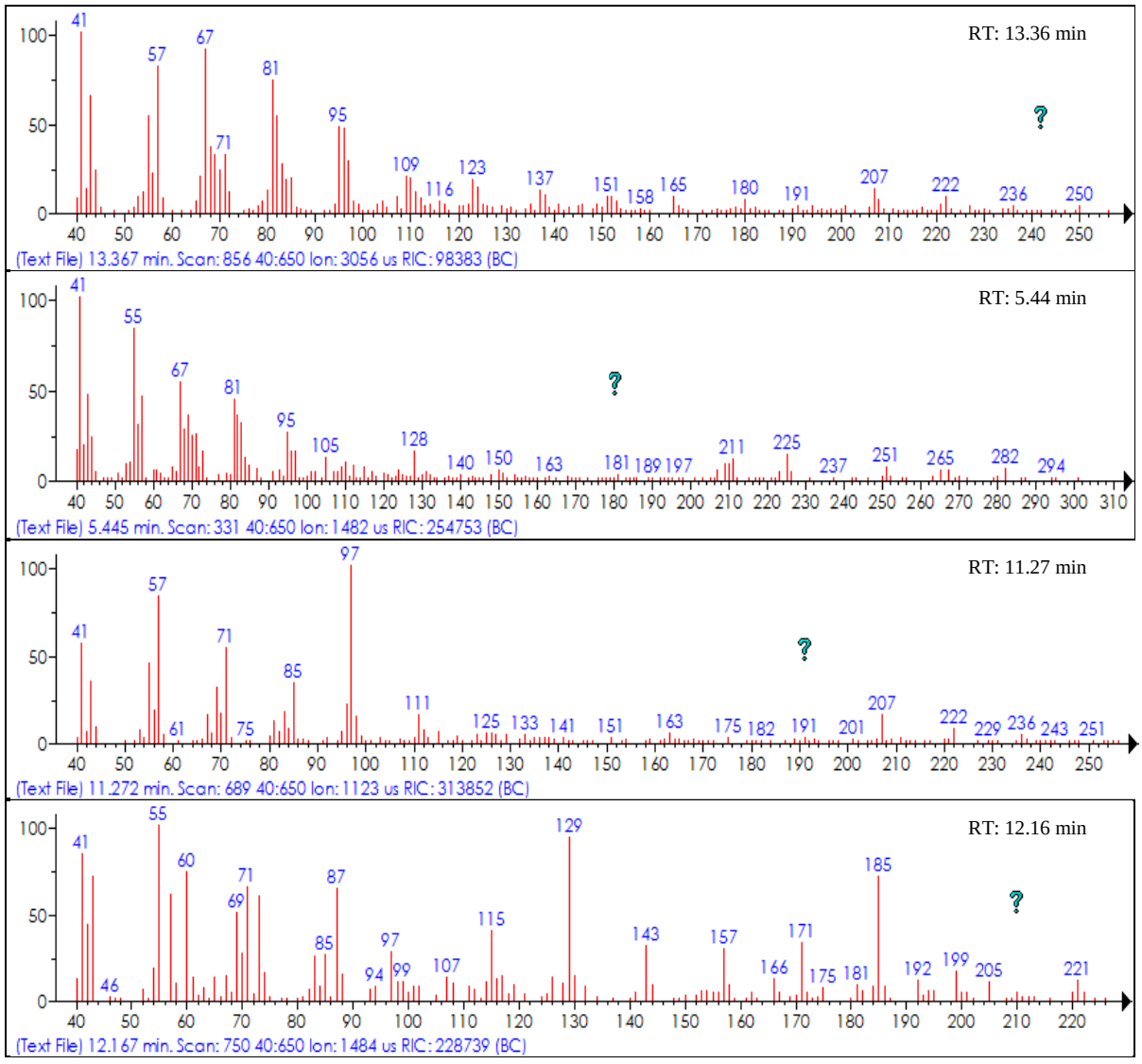

Fig. 1: Mass spectrum of female specific volatile components of *E.ceratoniae* identified by MS in tow different extraction techniques by SPME

Semiochemicals determine insect life situations such as feeding, mating, and egg-laying. They are thus potential agents for selective control of pest insects and methods for crop protection based on semiochemicals show advantages over methods based on conventional insecticides. Therefore, it is necessary to utilization of appropriate extraction technique for detection and identification of these components.

Based on the results of this research, extraction technique of the volatile chemical components was effective on the detection of female specific volatile components of *E.ceratoniae*. If, components were detected in our analyses were not similar to components were found by Baker *et al*, 1991 and Ziaaddini *et al*, 2009 [4,5]. Three sex pheromone components of carob moth on date were isolated and identified from the extract of female pheromone glands, using CS_2 solvent and variety of techniques including coupled gas chromatographic-electroantennographic recordings, coupled gas chromatographic-mass spectrometric analysis, microozonolysis, electroantennographic assays of monounsaturated standards, wind-tunnel bioassays and field trials. A sex pheromone blend for *E.ceratoniae*, was identified as consisting of (*Z*,*E*)-9,11,13-tetradecatrienal, (*Z*,*E*)-9,11-tetredecadienal and (*Z*)-9-tetradecenal [4]. Also, four components as carob moth (on pomegranate fruit and reared on pistachio) sex pheromone were identified from the extract of female pheromone glands, by injecting the Pheromone Biosynthesis Activating Neuropeptide, using N-hexan solvent and GC-MS coupled with electroantennographic detection system (three components were similar to the components were found by Baker *et al*, 1991 and one of them was identified as (*Z*,*E*)-9,11,13-tetradecatrienol) [5].

The aim of the utilization of tow different extraction techniques by SPME was the study of these techniques for access to appropriate technique for the detection of female specific volatile components by SPME. The results of comparison of chromatograms in different extraction techniques by SPME showed that extraction through the contact of fiber with papillae anales of females could be optimized method for extraction of the female specific volatile components from the female pheromone glands of carob moth because of the detection of three female specific volatile components by extraction through this technique. Moreover it needs to study the electrophysiologic role of extracted female specific components in this research by gas chromatography-electroantennodetectography. The results determined the efficiency of extraction method on the detection of female specific volatile components of carob moth. Determination of suitable method for extraction of female specific volatile components of *E.ceratoniae* are candidates of sex pheromone, plays a basic role on the improvement of sex pheromone efficiency for control of this pest and may be of practical importance for the development of integrated pest management systems in pomegranate orchards.

CONCLUSION

The results of this study showed the influence of extraction method on the detection of female specific volatile components and the contact of fiber with papillae anales of females can be appropriate and optimized technique for extraction of the female specific volatile components of carob moth by SPME.

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