Chemical Analysis of the Metathoracic Scent Gland of Eurygaster maura (L.) (Heteroptera: Scutelleridae)

E. Ogur^{1*}, and C. Tuncer²

ABSTRACT

Eurygaster maura (L.) (Heteroptera: Scutelleridae) is one of the most devastating pests of wheat in Turkey. The metathoracic scent gland secretions of male and female *E. maura* were analyzed separately by gas chromatography-mass spectrometry. Twelve chemical compounds, namely, Octane, n-Undecane, n-Dodecane, n-Tridecane, (E)-2-Hexenal, (E)-2-Hexen-1-ol, acetate, Cyclopropane, 1-ethyl-2-heptyl, Hexadecane, (E)-3-Octen-1-ol, acetate, (E)-5-Decen-1-ol, acetate, 2-Hexenoic acid, Butyric acid, and Tridecyl ester were detected in both males and females. These compounds, however, differed quantitatively between the sexes. In both females and males, n-Tridecane and (E)-2-Hexanal were the most abundant compounds and constituted approximately 90% of the total content. Minimal amounts of Octane were detected in males and Hexadecane in females.

Keywords: (E)-2-Hexenal, GC-MS, Metathoracic scent gland, n-Tridecane, Wheat.

INTRODUCTION

Eurygaster (Heteroptera: species Scutelleridae) are the most devastating pests of wheat in an extensive area of the Near and Middle East, Western and Central Asia, Eastern and South Central Europe, and Northern Africa (Critchley, 1998; Vaccino et al., 2006; Ravan et al., 2009). In Turkey, Eurygaster maura (L.),Eurygaster integriceps (Put.) and Eurygaster austriaca (Schrk.) are the most common species of the genus (Critchley, 1998). The species distributions vary by region. While E. maura is the dominant species in the Central Anatolia region, E. integriceps dominates in the Southeastern Anatolia, Aegean and Thrace regions (Koçak and Babaroğlu, 2005). Adults and nymphs of E. maura cause damage by feeding on the leaves, stems and grain kernels of the wheat plants (Lodos, 1986; Özkan and Babaroğlu, 2015), and can lead to economic losses as high as 100% in the absence of control measures (Lodos, 1986; Özbek and Hayat, 2003).

In the order Heteroptera, nearly all species have scent glands and many of these are colloquially referred to as "stink bugs" (Aldrich, 1988). Both nymphs and adults have scent glands in Heteroptera species (Abad and Atalay, 1994; Abad, 2000). The scent glands were first described in adult Heteroptera by Dufour in 1833, and in the nymphs by Künkel (1866) (Davidova-Vilimova, 2006). The name of the scent gland is based on the parts of the body where they are located (metathorax, abdomen) (Abad et al., 1994; Hassani et al., 2010; Kment and Vilimova, 2010; Kheyri et al., 2014). The "dorsoabdominal scent glands" and the "metathoracic scent glands" are of the greatest importance, with both often playing vital roles in defense. The dorsoabdominal scent glands, however, are developed in the nymphs, while the metathoracic scent glands are exclusively found in adults (Abad, 2000; Raska, 2009;

¹ Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya 42250, Turkey.

^{*} Corresponding author; Email: ekremogur@selcuk.edu.tr

² Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University, Samsun 55139, Turkey.



Kheyri *et al.*, 2014; Parveen *et al.*, 2014; Noge, 2015; Krajicek *et al.*, 2016; Rohanová *et al.*, 2016; Barao *et al.*, 2017).

Insects have developed a multitude of defense mechanisms to protect themselves from their enemies. In Heteroptera species, the MetaThoracic scent Gland (MTG) is one of the most effective defense mechanism against predation (Chapman, 1972; Aldrich, 1988). When the bugs are disturbed or molested, they produce large quantities of strong-smelling and irritating defensive chemicals, which are then released from the MTG (Aldrich, 1988; Durak and Kalender, 2007a; Raska, 2009; Abad et al., 2012; Parveen et al., 2014; Noge, 2015). Several researchers have reported that diverse substances may be secreted from the MTGs to serve different purposes or roles such as defense against predation, alarm, mating and aggregation (Aldrich, 1988; Ho and Millar, 2001; Marques et al., 2007; Hassani et al., 2010; Abad et al., 2012; Gonzaga-Segura et al., 2012; Krajicek et al., 2016; Weiler et al., 2017).

The main objective of this study was to determine the compounds produced in the MTGs of adult male and female *E. maura*. The MTG secretion of *E. maura* has previously been analyzed by Durak and Kalender (Durak and Kalender, 2007a), and the closely related species, *E. integriceps*, was analyzed by Durak (2006) and Hassani *et al.* (2010). In this study, our results are compared with the previous studies and the probable causes of the differences are discussed.

MATERIALS AND METHODS

Insect Material

Adult male and female *E. maura* were collected from wheat fields in Selçuklu, Konya, Turkey, from June through July 2012. Insects were kept in transparent plastic jars and reared and maintained in a climate chamber set at 26±1°C, 65±5%RH and a 16:8 light-dark photoperiodic regime

(Gözüaçık *et al.*, 2011). Bugs were maintained on fresh wheat plants until dissection.

Extraction

Adult E. maura males and females were killed individually by freezing to prevent premature discharge of the gland contents (Marquez et al., 2007; Hassani et al., 2010). The insects were dissected in sodium phosphate buffer (pH 7.2) (Durak, 2006). An adult E. maura was pinned in a petri dish with the dorsal side up. The dissection process consisted of cutting the dorsal abdominal edges of the insect cuticle up to the metathoracic region and then under the scutellum. The dorsal abdominal cuticle was pulled back and the viscera removed. The scent gland complex, located in the ventral abdominal metathoracic region, could then be accessed and removed with the aid of a small surgical scissors (Figure 1) (Zarbin et al., 2000). Twenty male and twenty female gland reservoirs were removed and immersed in 1 ml analytical grade hexane distilled from CaH2 (Zarbin et al., 2000; Durak and Kalender, 2007a; Hassani et al., 2010).

Chemical Analysis

The MTG secretions were analyzed separately for both sexes of E. maura. **Extracts** were analyzed Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent 7890A series fitted with an HP Innovax column (60 m×0.25 mm ID×0.25 µm film) and interfaced to an Agilent 5975C mass selective detector (electron impact ionization, 70 eV). Helium carrier gas was programmed for constant flow (1.2 mL min⁻¹). The GC programmed from 60°C/10 minutes initially, then 4°C min⁻¹ to 220 °C. After remaining at this temperature for 10 minutes, the temperature was raised 1°C min⁻¹ to 240°C and kept at this temperature for 30 minutes.

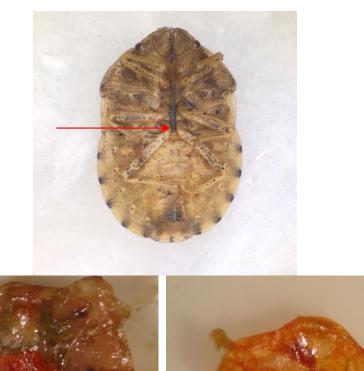




Figure 1. The dissection of Eurygaster maura metathoracic scent gland.

Thus, the total duration of the analysis was determined as 110 minutes. The temperature of the injector was 240°C. Compounds were tentatively identified by GC-MS, and identifications were confirmed by comparison of the retention times and mass spectra of reference samples. The relative amount of each compound was determined as the area under its respective GC peak. Wiley and Nist libraries were used to identify the compounds (Durak and Kalender, 2007a; Hassani *et al.*, 2010).

RESULTS

The chemical contents of the MTG of both sexes of *E. maura* were qualitatively the same with a total of 12 chemical compounds

being identified (Table 1), however, most of the compounds differed quantitatively between the sexes (Figures 2 and 3). Major components are shown in bold.

Analysis of the MTG of E. maura yielded 6 types of alkanes (Octane, n-Undecane, n-Dodecane, n-Tridecane, Hexadecane, Cyclopropane, 1-ethyl-2-heptyl), 1 type of aldehyde ((E)-2-Hexenal), 3 types of acetates ((E)-2-Hexen-1-ol, acetate, (E)-3-Octen-1-ol, acetate, (E)-5-Decen-1-ol, acetate) and 2 types of acids (2-Hexenoic acid, Butyric acid, tridecyl ester) in both males and females. The alkane, Tridecane", found he was to the predominant compound in the MTGs of both sexes of E. maura (60.22 and 56.78% in females and males, respectively). Another alkane, "Octane" (0.07%), was the least



Table 1. Compounds in the metathoracic scent gland secretions of male and female *E. maura.* ^a

Groups	Chemical compounds	Female (%)	Male (%)
Alkanes	Octane	0.07	0.13
	n-Undecane	0.48	0.55
	n-Dodecane	1.32	1.20
	n-Tridecane	60.22	56.78
	Hexadecane	0.16	0.08
	Cyclopropane, 1-ethyl-2-heptyl	0.33	0.27
Aldehyde	(E)-2-Hexenal	33.42	30.78
Acetates	(E)-2-Hexen-1-ol, acetate	1.78	8.62
	(E)-3-Octen-1-ol, acetate	0.20	0.21
	(E)-5-Decen-1-ol, acetate	1.25	0.99
Acids	2-Hexenoic acid	0.35	0.15
	Butyric acid, tridecyl ester	0.42	0.24

^a Major components are shown in bold.

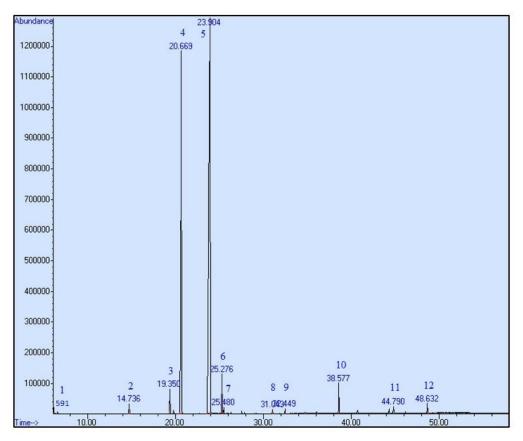


Figure 2. Gas chromatogram of an extract of the MTG secretion of *E. maura* females: (1) Octane, (2) n-Undecane, (3) n-Dodecane, (4) (E)-2-Hexenal, (5) n-Tridecane, (6) (E)-2-Hexen-1-ol, acetate, (7) Cyclopropane, 1-ethyl-2-heptyl, (8) Hexadecane, (9) (E)-3-Octen-1-ol, acetate, (10) (E)-5-Decen-1-ol, acetate, (11) 2-Hexenoic acid, (12) Butyric acid, tridecyl ester.

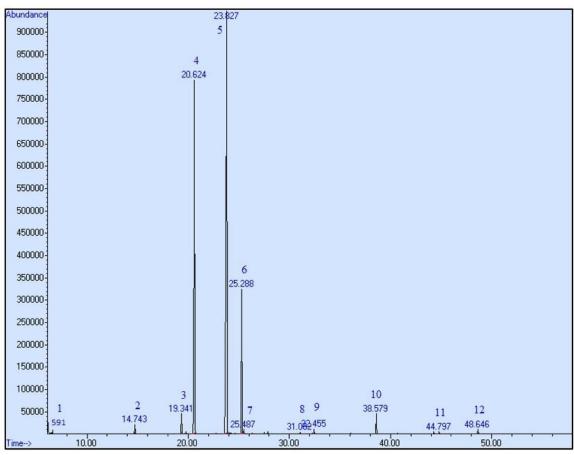


Figure 3. Gas chromatogram of an extract of the MTG secretion of *E. maura* males, 1, 2...12 as defined under Figure 2.

common of the 12 components present in the female glands, while in males, another alkane, "Hexadecane" (0.08%), was the least common of the glandular compounds.

The second most abundant compound found in both sexes was "(E)-2-Hexenal" (33.42% and 30.78% for female and male, respectively). The two compounds, n-Tridecane and (E)-2-Hexenal, comprised 93.64% of the total chemical compound in females and 87.56% in males.

DISCUSSION

The scent substances of Heteroptera are, for the most part, short to medium unbranched carbon-chain aliphatic substances, acids, aldehydes, ketoaldehydes, ketones, alcohols, and esters (Staddon, 1979; Aldrich, 1988; Krall *et al.*, 1999; Noge, 2015). In Heteroptera, the secretion of MTGs is generally characterized as a defensive function. However, the secretion is often involved in various other, mostly pheromonal, functions as well. Alkanes and aldehydes, the compounds that are most commonly found in the MTGs of Heteroptera, are effective in defense against predators as well as functioning as alarm pheromones (Raska, 2009; Zhang *et al.*, 2014).

The most common chemicals found in the MTG secretion are alkanes, aldehydes, esters, alcohols and terpenoids. Lactones, ketones, alcenes and miscellaneous other compounds are less commonly encountered chemicals (Farine *et al.*, 1993). Aldehydes and other hydrocarbons found in the MTG in several *Heteroptera* species have a dual



function. In this study, one aldehyde and 6 hydrocarbons were detected in the MTG of both males and females of *E. maura*. The function varies according to their concentration or the manner in which they are emitted (Farine *et al.*, 1992).

(E)-2-Hexenal, n-Undecane, n-Dodecane and n-Tridecane are known as toxins, irritants or repellents (Krall *et al.*, 1999; Zarbin *et al.*, 2000; Šanda *et al.*, 2012). These compounds are secreted by the bugs when they are disturbed or molested, indicating that these compounds are involved in defensive behavior. In our study, the aldehydes and alkanes, (E)-2-Hexenal, n-Undecane, n-Dodecane and n-Tridecane, were identified in the analyses of the MTG of both sexes of *E. maura* and thought to be the primary materials responsible for chemical defense.

In the present study, n-Tridecane was determined to be the most abundant compound in the MTG in both sexes of E. maura. Previously, it has been identified by other researchers as the most abundant compound in the MTG of many Pentatomoidea species, including Chlorochroa sayi Stål, C. uhleri (Stål) and C. ligata (Say), Graphosoma semipunctatum (Fabricius), Dichelops melacanthus (Dallas), Dolycoris baccarum (L.), Carpocoris fuscispinus (Boheman) (Pentatomidae), and Tessaratoma papillosa (Drury) (Tessaratomidae) (Ho and Millar, 2001; Durak and Kalender, 2007b; Marques et al., 2007; Durak, 2008; Laumann et al., 2009; Durak and Kalender, 2012; Zhao et al., 2012). This compound has also been detected in many other species Heteroptera (Aldrich, 1988; Borges et al., 2001; Zarbin et al., 2000). The ratio of this particular alkane in the MTG of female and male *E. maura* was 60.22% and 56.78%, respectively, of the total compounds found. In other insect species, such as D. baccarum *C*. fuscispinus (Heteroptera: and Pentatomidae), n-Tridecane was found to be approximately 50% of the total in the MTG of both adult females and males (Durak, 2008; Durak and Kalender, 2012).

According to previous studies, two special functions have been claimed for n-Tridecane; 1) to promote the penetration of the toxic scent carbonyls through the cuticle of arthropod predators and 2) to act as a fixative, delaying evaporation of the scent carbonyls from the surface of the body of the scent emitter. It also has to be considered that n-Tridecane may possibly have a defensive function in its own right (Staddon, 1979; Šanda *et al.*, 2012; Gregorovicova and Cernicova, 2016).

(E)-2-Hexenal, the second most abundant compound in both sexes of E. maura in this study, has been identified in many species of Lygaeidae (Staddon and Olagbemiro, 1984), Pentatomidae (Aldrich, 1988; Ho and Millar, 2001; Zarbin et al., 2000; Durak, 2006; Durak and Kalender, 2007b; Marques et al., 2007; Durak and Kalender, 2012; Noge et al., 2012), Rhopalidae (Aldrich, 1988), Coreidae (Steinbauer and Davier, 1995; Noge et al., 2012), Miridae (Drijfhout et al., 2002), Alydidae (Yasuda et al., 2007; Noge et al., 2012) and Scutelleridae (Durak, 2006; Durak and Kalender, 2007a; Abad et al., 2012). As in our findings with E. maura, Eurydema rugosa Motschulsky and E. pulchra (Westwood) contain considerable amounts of (E)-2-Hexenal and n-Tridecane together (Ishiwatari 1974).

It appears that E-2-Hexenal may play a primary role in all three functions, including pheromonal, allomonal, and kairomonal (Lockwood and Story, 1987). When sprayed suddenly, (E)-2-Hexenal is an alarm pheromone, but acts as an aggregation pheromone when exuded gradually and in small amounts. A similar situation, i.e. dispersal at high concentrations aggregation at low concentrations, was observed for n-Tridecane in the first instar of Nezara viridula (L.) (Lockwood and Story, 1985; 1987; Farine et al., 1992; 1993). It was reported that (E)-2-Hexenal worked synergistically with n-Tridecane and was more effective as repellents to insects when combined than when tested individually (Krall et al., 1999). Accordingly, n-Undecane, n-Dodecane and n-Tridecane show synergistic effects on toxicity and repellency when combined with aldehydes and acetates (Gunawardena and Herath, 1991).

In addition to the defensive pheromonal effects of compounds found in the MTG of many heteropterans, they may also play a kairomonal role in some of these insects. Lauman et al. (2009) tested the effect of crude extracts of the MTGs from five different host species on the hostsearching behavior of the scelionid egg parasitoids, Telenomus podisi Ashmead and Trissolcus basalis (Wollaston). The females of both species were attracted to crude MTG extracts of the preferred hosts Euschistus (Fabricius) and Ν. viridula. Additionally, the parasitoids responded to synthetic standards of individual compounds identified in these stinkbug glands. Trissolcus basalis responded positively to 4oxo-(E)-2-Hexenal and (E)-2-Decenal, while T. podisi responded positively to 4-oxo-(E)-2-hexenal. This parasitoid was also shown to respond positively to (E)-2-Hexenal and n-Tridecane, both of which are defensive compounds released from the MTGs by several stinkbugs.

The behavioral responses of the scelionid egg parasitoid, Trissolcus semistriatus (Nees von Esenbeck), to hexane extracts of the secretion of E. MTG maura were investigated using a Y tube olfactometer. Low concentrations of MTG secretions from both sexes attracted the parasitoids, but attraction was reduced with increased concentrations, where parasitoids tended to prefer clean air. Low concentrations of MTG secretion in both sexes increased the parasitisation rates by T. semistriatus. Conversely, increased concentrations resulted in decreased parasitisation rates. Use of the Y tube olfactometer demonstrated that the MTG secretion of E. maura, especially in males, plays active roles in intraspecific aggregation and dispersal depending on the concentration (Ögür, 2016).

(E)-2-Hexen-1-ol, acetate found in the MTG of *E. maura* during this study was also

detected in the MTG secretion of D. baccarum (Durak, 2008) and T. papillosa (Zhang et al., 2009). They stated that (E)-2-Hexen-1-ol, acetate released by irritated T. papillosa individuals may serve as a warning compound to their congeners, resulting in an alarming effect. Another alkane found in the E. maura MTG, Hexadecane, was also identified by Durak and Kalender (2007a) in E. maura and Zhang et al. (2014) in the MTG of Adelphocoris suturalis (Hemiptera: Miridae). The alkanes are reported to function as short-range attractants and defense pheromones (Lockwood and Story, 1985, Krall et al., 1999; Zhang et al., 2014).

Previous studies on the chemical analysis of the MTG of E. maura and E. integriceps have shown differences in their chemical compounds (Table 2). Durak and Kalender (2007a) analyzed the MTG secretion from females and males of E. maura using GC-MS and found 16 and 20 different chemical substances, respectively. In studies on E. integriceps, Durak, (2006) found 16 and 23 different chemical substances in the female and male, respectively, while Hassani et al. (2010) found a total of 7 and 7. In the first two studies above, quantitative as well as qualitative differences were found in the compositions of these substances when comparing males and females, but only quantitative differences were found in the third study.

In the Durak and Kalender (2007a) and (2006) studies, the chemical compounds from the MTGs of E. maura and E. integriceps were found to be very similar. Three major compounds found for both species were Tetracosane, n-Tridecane, and Octadecanoic acid. Hassani et al. (2010) found a total of 7 compounds in the MTG of adult E. integriceps when using in vitro and in vivo methods. Similar to our study on E. maura, they determined that (E)-2-Hexenal and n-Tridecane comprised nearly 95% of the total secretion in both females and males of E. integriceps. In the present study, we found that n-Tridecane and (E)-2-Hexenal comprised approximately 90% of the total



Table 2. Compounds in the metathoracic scent gland secretions of male and female *Eurygaster* spp. ^a

		Thi	This study	Durak and F	Durak and Kalender (2007a)	Durak	Durak (2006)	Hassani e	Hassanı et al. (2010)
	Chemical compounds	(E.	(E. maura)	(E.	(E. maura)	(E. inte	(E. integriceps)	(E. inte	(E. integriceps)
		Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)
Alkanes	Octane	0.07	0.13	pu	pu	pu	pu	pu	pu
	n-Undecane	0.48	0.55	pu	0.11	pu	pu	pu	pu
	n-Dodecane	1.32	1.20	0.3	0.34	0.30	0.17	pu	pu
	Pentadecane	pu	pu	pu	0.46	pu	2.19	pu	pu
	n-Tridecane	60.22	56.78	22.94	34	23.2	11.95	18.11	20.98
	Hexadecane	0.16	80.0	0.33	0.28	0.30	06.0	pu	pu
	Nonadecane	pu	pu	1.12	3.82	3.02	0.41	0.5	0.36
	Octadecane	pu	pu	1.68	0.43	1.54	2.12	pu	pu
	Tricosane	pu	pu	1.72	0.36	1.70	5.16	pu	pu
	Heptadecane	pu	pu	pu	pu	pu	2.02	pu	pu
	Tetracosane	pu	pu	41.51	3.82	42.53	4.24	pu	pu
	Hexacosane	pu	pu	2.25	2.91	6.75	1.26	pu	pu
	Octacosane	pu	pu	5.52	26.58	2.24	5.52	pu	pu
	Cyclopropane, 1-ethyl-2-heptyl	0.33	0.27	pu	pu	pu	pu	pu	pu
Alcenes	1-Nonadecene	pu	pu	0.45	1.43	0.42	pu	pu	pu
	Cyclodecene	pu	pu	1.29	pu	0.82	pu	pu	pu
	5-Decyne	pu	pu	pu	pu	pu	pu	0.32	0.44
Aldehyde	(E)-2-Hexenal	33.42	30.78	0.27	2.63	0.25	3.90	78.1	74.7
Acetates	(E)-2-Hexen-1-ol, acetate	1.78	8.62	pu	pu	pu	pu	2.59	3.14
	(E)-3-Octen-1-ol, acetate	0.20	0.21	pu	pu	pu	pu	pu	pu
	(E)-5-Decen-1-ol, acetate	1.25	0.99	pu	pu	pu	pu	pu	pu
	(E)-2-Hexenyl acetate	pu	pu	0.39	0.19	0.40	0.24	pu	pu
	Diisooctyl acetate	pu	pu	pu	1.00	pu	pu	pu	pu
	L-Histidinemethylesterdihydrochloride	pu	pu	pu	pu	pu	2.27	pu	pu
	Dodecylester	pu	pu	pu	pu	pu	2.63	pu	pu
	Methyl acetate	pu	pu	pu	pu	pu	0.40	pu	pu
Acids	2-Hexenoic acid	0.35	0.15	pu	pu	pu	pu	pu	pu
	Butyric acid, tridecyl ester	0.42	0.24	pu	pu	pu	pu	pu	pu
	n-Hexanoic acid	pu	pu	4.92	1.29	5.12	pu	pu	pu
	n-Hexadecanoic acid	pu	pu	1.89	1.51	1.50	pu	pu	pu
	Octadecanoic acid	pu	pu	8.96	4.21	7.33	35.65	pu	pu
	5-methyl-trimethylsilyl oxy-benzoic acid	pu	pu	pu	pu	pu	0.31	pu	pu
	1-Phenanthrene carboxylic acid	pu	pu	pu	pu	pu	11.19	pu	pu
Alcohol	3,7,11-(3-metyl-trideuterio)-trimetyl-(4,4,5,5-	pu	pu	pu	3.76	pu	pu	pu	pu
	tetradeuterio)dodeca-1,6,10-trien-3-ol	8	8			9	,		1
	1-Octadecanethiol	pu	pu	pu	pu	pu	06.0	pu	pu
Ether	Dimethyl ether	pu	pu	pu	pu	pu	1.38	pu	pu
Steroid	14-BETA-H-Pregna	pu	pu	pu	1.32	pu	2.19	pu	pu
Lactone	2(5H)-Furanone	pu	pu	pu	pu	pu	0.27	pu	pu
	2(5H)-Furanone, 5-Ethyle	pu	pu	pu	pu	pu	pu	0.1	0.11
Caraller Land									100

^a nd: Not detected; Major components are shown in bold.

secretion both in females (93.64%) and males (87.56%) of *E. maura*.

We speculate that the differences noted in the chemical compounds may be due to several factors, including the GC-MS device used, the libraries used for identifying the compounds, as well as possible differences in the collection time of the adult insects, and the diversity of the habitats and populations. Noge *et al.* (2015) discovered that secretory components differed during adult aging in the coreid, *Hygia lativentris* (Motschulsky).

Another possibility is that *E. maura* and *E.* integriceps may have been misidentified in one or more of studies. Eurygaster maura is extremely similar to E. integriceps in external morphology and notoriously difficult to separate even by taxonomists. The most reliable distinguishing character is the structure of the genitalia of Eurygaster species: the penis of E. maura has 2 hooks and 4 in E. integriceps (Kerzhner and Yachevskii, 1967). Another character is the internal spines of E. maura that are convoluted and are almost straight in E. integriceps (Kerzhner and Yachevskii, 1967; Lodos, 1986). Due to our concerns regarding the differences in chemical composition of the MTGs in these two closely related species, we usually reared E. maura male and females and then examined the male aedeagus and female genitalic structures in copulated couples according to the key provided by Kerzhner and Yachevskii (1967) to verify our identifications.

In conclusion, this study was an analysis of the chemical composition of MTGs in *E. maura*. Results showed that n-Tridecane and (E)-2-Hexenal were the major compounds and constituted approximately 90% of the total MTG content in both female and male *E. maura*. In most studies in Heteroptera, these two compounds have been identified as being primarily defensive in nature. Further studies are necessary to investigate the precise biological function of this alkane—aldehyde combination and chemical communication in *E. maura*. This will provide advantage to us in the management

of the pest. In summary, this study has provided useful information on the MTG compounds of *E. maura*, and will contribute to further studies on the MTG compounds in this and other species of Pentatomidae.

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Eurygaster maura (L.) تجزیه شیمیایی غده بو دهنده پس سینه ای (Heteroptera: Scutelleridae)

ا. اوگور، و ز. تونکر

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