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Fatty Acid Composition of Sexes and Body Parts in a Solitary Wasp, *Sphex flavipennis* (Insecta: Hymenoptera)

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ABSTRACT: Fatty acid composition in head, thorax, and abdomen of male and female specimens of the solitary wasp species *Sphex flavipennis* was investigated. Samples were collected from their natural habitats in Tokat province (Turkey). It is determined that, unsaturated fatty acids are high in females whereas saturated fatty acids are high in males. Total unsaturated fatty acids of body parts and sexes were found as: 80.19 % in female head, 75.73 % in male head; 81.57 % in female thorax, 81.43 % in male thorax; 79.03 % in female abdomen, 74.68 % in male abdomen. Total saturated fatty acids of body parts and sexes were found as: 19.57 % in female head but 24.67 % in male head; 14.83 % in female thorax, 16.91 % in male thorax; 19.77 % in female abdomen, 22.78 % in male abdomen. The highest amounts of fatty acids are oleic acid and linoleic acid ranging between 27.68 % - 52.65 % and 19.00 % - 37.36 %, respectively. Differences between body parts as well as male and female insects are due to diverse physiological and metabolic functions.

Keywords: Fatty acids, Hymenoptera, *Sphex flavipennis*

Soliter Yaban Arısı, *Sphex flavipennis* (Insecta: Hymenoptera)'in Eşey ve Vücut Kısımlarında Yağ Asit Kompozisyonu

ÖZET: Soliter yaban arısı *Sphex flavipennis* türüne ait erkek ve dişi örneklerin baş, toraks ve abdomen kısımlarında yağ asidi kompozisyonu araştırıldı. Örnekler Tokat ilinde doğal habitatlarından toplandı. Çalışmamızda doymamış yağ asitleri dişilerde, doymuş yağ asitleri ise erkeklerde yüksek bulunmuştur. Eşeyler ve vücut kısımlarına göre toplam doymamış yağ asitleri şu şekilde bulunmuştur: dişinin başında % 80.19, erkeğin başında % 75.73; dişinin toraksında % 81.57, erkeğin toraksında % 81.43; dişinin abdomeninde %79.03, erkeğin abdomeninde % 74.68. Toplam doymuş yağ asitleri şu şekilde bulunmuştur: dişinin başında % 19.57, erkeğin başında % 24.67; dişinin toraksında % 14.83, erkeğin toraksında % 16.91 in; dişinin abdomeninde % 19.77, erkeğin abdomeninde % 22.78. Yağ asitleri arasında en yüksekleri oleik asit ve linoleik asittir, miktarları sırasıyla 27.68% - 52.65% ve 19.00% - 37.36% arasında değişmektedir. Vücut kısımları ile erkek-dişi arasındaki farklılıklar çeşitli fizyolojik ve metabolik fonksiyonlardan kaynaklanmaktadır.

Anahtar kelimeler: Hymenoptera, *Sphex flavipennis*, yağ asitleri

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INTRODUCTION

Fatty acids perform many vital functions in insects such as reproduction, embryogenesis, metamorphosis, and flight (Canavoso et al., 2003). They also play regulatory role as being pheromones and hormones (Gilbert and Chino, 1974; Sushchik et al., 2013). They are crucial mediators of insect immunity and are responsible for membrane signaling (Uscian and Stanley-Samuelson, 1994; Tunaz et al., 2003; Stanley et al., 2012). Moreover they are the primary energy source during periods of nonfeeding, such as diapause and long migratory flights, and during nonfeeding stages of development (Beenakkers et al., 1985; Downer, 1985). Fatty acids serve as precursors in the biosynthesis of pheromones, waxes and eicosanoids and as structural components of membranes and defensive secretions, and they are essential components in the function of the cuticle (Blomquist and Dillwith, 1985; Stanley-Samuelson et al., 1988).

Sphex flavipennis is a solitary wasp species belonging to the Sphecidae family (Insecta: Hymenoptera) known as "thread-waisted wasp". Its members live in temperate regions all over the world and mostly dig nests in sandy areas. Adult wasps feed on nectar of various flowers, but their larvae feed on insects, such as grasshoppers, paralyzed by adult female wasp (Bohart and Menke, 1976). They are very active during daytime; especially females dig nests, catch and paralyze preys, and carry them to the nest etc. Being active predators they control and limit insect populations in the ecosystems while provisioning for their larvae.

Insect flight muscle, the most active tissue known, increases its oxygen consumption to 50-100 folds during flight (Beenakkers et al., 1984). This huge amount of energy is obtained from trehalose, proline, and ketone bodies which are produced from beta oxidation of fatty acids (Gilbert and Chino, 1974; Canavoso et al., 2001; Dooremalen and Ellers, 2010; Arrese and Soulages, 2010). Consequently, lipids play an important role in insect life in many aspects.

Many insect species belonging to different orders have been investigated in terms of fatty acid contents so far (Barlow, 1964; Fast, 1964; Thompson, 1973; Thompson and Barlow, 1974; Nurullahoğlu et al., 2004; Stewart-Jones et al., 2009; Yocum et al., 2011; Sushchik et al., 2013; Gołebiowski et al., 2013). In some of these studies it was stated that fatty acid composition in insects may differ between sexes and even different developmental stages of the same species (Sushchik et al., 2013; Gołebiowski et al., 2013). However, there is still limited number of investigations about fatty acid contents of different body parts of insects. Most of the studies on fatty acids have been carried out on several species that were reared in laboratory conditions (Fast, 1964; Thompson and Barlow, 1974; Canavoso et al., 2001; Murata and Tojo, 2002; Nurullahoğlu et al., 2004) and the situation in insects that feed naturally in their habitats is a matter of curiosity. The purpose of this study was to determine fatty acid composition of solitary wasp samples, belonging to *Sphex flavipennis*, collected from the field and compare it between sexes and body parts.

MATERIALS AND METHODS

Specimens Collection and Identification

Adult insects were collected using insect net from their natural habitats around Tokat province between 2009-2011. Specimens were identified by one of the authors (Gülmez) and confirmed by comparing with the entomological museum materials found in the Entomological Research Laboratory in Gaziosmanpaşa University, Tokat.

Extraction Procedure

Male and female specimens were separated and their bodies splitted into three parts (head, thorax, and abdomen) each of which then placed in distinct tubes. Before analysis, each body part was disintegrated under liquid nitrogen. After removing nitrogen, crude oil was obtained from disintegrating parts with light petroleum ether (b.p. 40–60°C) in a Soxhlet. The solvent was removed using rotary evaporator. The extracted oil was used for fatty acid analysis. The oils were saponified by the usual procedure according to the Standard IUPAC methods (IUPAC, 1988). Fatty acids were estherified using official methods (AOAC, 1990).

Gas Chromatographic Analyses

Gas chromatographic (GC) analyses were performed using a Perkin Elmer Clarus 500 Series

GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) equipped TR-FAME apolar capillary column (30 m x 0.25 mm and 0.25 m ID).

Helium (0.5 mL min^{-1}) was used as carrier gas. The injector temperature was set at 250 °C and the FID was operated at 260 °C. An initial column oven temperature of 100 °C was elevated to 220 °C at a rate of 2 °C min^{-1} and held for 0 min.

Identification of fatty acid components was accomplished based on comparison of their retention times with those of authentic standards (Supelco 37 Comp. Fatty acid Mix, 18919).

The relative peak area percentages of compounds were calculated based on the FID data.

Statistical Analysis

The means of three groups were compared through Duncan's Multiple-Range Test after all data were subjected to analysis of variance (Anova). Two group means were compared Paired-Samples T Test using SPSS statistical software programme (Norusis, 2002).

RESULTS AND DISCUSSION

In the present study, fatty acid composition of head, thorax, and abdomen of female and male *Sphex flavipennis* species were determined. Most of the fatty acids were significantly differed between sexes and body parts (Table 1). Our results are generally consistent with the findings of the previous studies on other Hymenoptera (Barlow, 1964; Thompson, 1973; Thompson and Barlow, 1974). The results concerning to unsaturated and saturated fatty acids are discussed separately below.

Unsaturated Fatty Acids

Total unsaturated fatty acids range from 74.68 % to 81.57 % (Figure 1) and their distribution in body parts of sexes were as follows: **head**: 80.19 % in female, 75.73 % in male; **thorax**: 81.57 % in female, 81.43 % in male; **abdomen**: 79.03 % in female, 74.68 % in male. The values between head and abdomen of sexes differ statistically significant ($p<0.01$) (Figure 1). Unsaturated fatty acids are used for diverse physiological functions, such as ovarian development and egg production in female insects (Murata and Tojo, 2002), therefore fatty acid levels may differ between sexes.

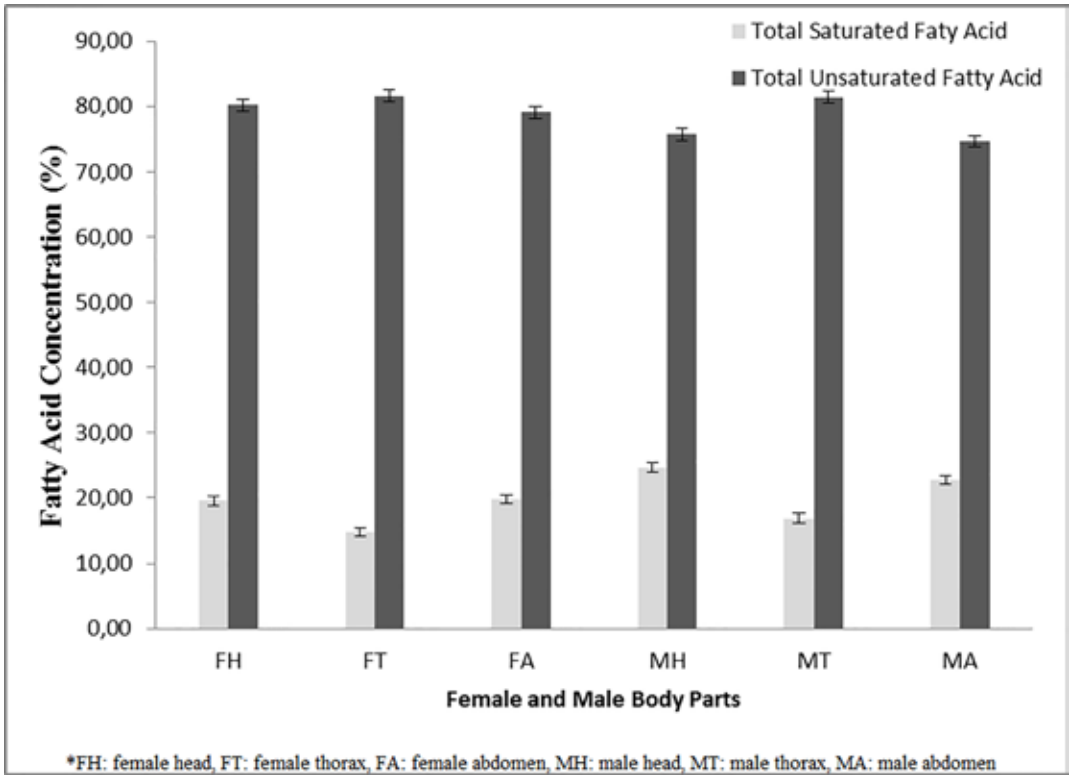

Figure 1. Total saturated fatty acid and total unsaturated fatty acid composition of body parts and sexes

Oleic acid is the highest of the unsaturated fatty acids, which was found as 31.88 % in female head, 27.68 % in male head; 36.91 % in female thorax 33.42 % in male thorax; 52.65 % in female abdomen and 47.43% in male abdomen (Table 1). All the differences between body parts and sexes are statistically significant ($p < 0.01$). Thompson and Barlow (1974) reported oleic acid ranging between 10 % and 54 % in 30 hymenopteran species and their hosts. Barlow (1964) has found oleic acid in two hymenopteran species (*Neodiprion sertifer* (Geoffroy) *Acanthomyops claviger* (Roger)) 33 % and 54 % respectively. Changing of oleic acid between body parts is thought to be resulted either from the food material, i.e., nectar of flowers, or unique functions of the body parts.

Table 1. Fatty acid composition of body parts and sexes of *Sphex flavipennis*

		Female			**Male**				
		Head	Thorax	Abdomen	Head	Thorax	Abdomen	SEM	P-value
C10:0	Capric Acid	0.09b	0.00e	0.04d	0.11a	0.06c	0.06c	0.01	0.01
C12:0	Lauric Acid	0.56bc	0.67b	0.42de	0.43cde	1.36a	0.41de	0.06	0.01
C14:0	Myristic Acid	0.52ef	0.55ef	1.18b	0.61e	0.47f	0.75d	0.06	0.01
C15:0	Pentadecylic Acid	0.31c	0.31c	0.18d	0.38bc	0.44a	0.44a	0.03	0.01
C16:0	Palmitic Acid	9.72e	8.29f	11.98d	12.77c	8.76f	13.52c	0.89	0.01
C16:1	Palmitoleic Acid	1.23bc	0.75d	1.15c	1.34b	0.56e	1.14c	0.08	0.01
C17:0	Heptadecylic Acid	0.17e	0.19d	0.11f	0.29b	0.33a	0.17e	0.01	0.01
C17:1		0.08e	0.02g	0.12d	0.08e	0.22c	0.08e	0.03	0.01
C18:0	Stearic Acid	8.07b	4.20e	5.70d	9.94a	5.36d	7.27c	0.36	0.01
C18:1n9c	Oleic Acid	31.88h	36.91f	52.65b	27.68i	33.42g	47.43c	1.75	0.01
C18:2n6c	Linoleic Acid	36.24b	34.41c	19.00f	37.36a	33.40d	21.29e	2.02	0.01
C18:3n3	Linolenic Acid	5.84d	3.61e	3.13fg	3.42ef	10.17a	2.82g	0.52	0.01
C20:0	Arachidic Acid	0.00c	0.47a	0.12b	0.00c	0.00c	0.00c	0.03	0.01
C20:3n6	Arachidonic Acid	0.00g	3.97a	0.48f	1.01d	1.53c	0.68e	0.25	0.01
C22:1n9	Erucic Acid	3.83b	1.28d	1.72c	4.52e	1.45d	0.00f	0.29	0.01
C24:0	Lignoceric Acid	0.13d	0.14cd	0.05e	0.14cd	0.14cd	0.18b	0.04	0.01
C24:1	Nervonic Acid	1.09b	0.62d	0.90c	0.32e	0.70d	1.24a	0.08	0.01
∑Saturated Fatty Aid		19.57f	14.83h	19.77f	24.67c	16.91g	22.78e	0.90	0.01
∑Unsaturated Fatty Acid		80.19b	81.57a	79.03c	75.73d	81.43a	74.68ef	0.76	0.01
UFA/SFA		4.10c	5.50a	4.00c	3.07e	4.82b	3.28d	0.84	0.01

SEM: Standard error of the mean; P: Probability. Different superscript letters within a line differ significantly (Duncan, $p < 0.01$).

Linoleic acid was found as: 36.24 % in female head, 37.36 in male head; 34.41% in female thorax, 33.40 in male thorax; 19.00 % in female abdomen, 21.99 % in male abdomen. It is statistically significant ($p < 0.01$) between all body parts of male and female. Thompson (1973) has found linoleic acid in a wide range, between 3 % and 43 %, in other Hymenoptera species. Barlow (1964) has found linoleic acid in two hymenopteran species (*Neodiprion sertifer* (Geoffroy) *Acanthomyops claviger* (Roger)) 45 % and 1 % respectively. Thompson and Barlow (1974) reported linoleic acid ranging between 7 % and 49 % in parasitic hymenopteran species and their hosts.

Linolenic acid was 5.84 % in female head, 3.42 in male head; 3.61 % in female thorax, 10.17% in male thorax. These differences between head and thorax of male and female individuals are statistically significant

($p < 0.01$). It was found as 3.13 % in female abdomen, 2.82 % in male abdomen, which is not statistically significant ($p > 0.05$). Although this fatty acid is higher in female head and abdomen than in male, it was found in male thorax almost three times higher than that of female.

Palmitoleik acid was 1.23 % in female head, 1.34 % in male head similarly it was 1.15 % in female abdomen, 1.14 % in male abdomen. Both of the values are not statistically significant ($p > 0.05$). It was found to be 0.75 % in female thorax, 0.56 % in male thorax, which is statistically significant ($p < 0.01$).

Arachidonic acid was not detected in female head, but was 1.01 % in male head; 3.97 % in female thorax, 1.53 % in male thorax; 0.48 % in female abdomen, 0.68 % in male abdomen. All of these differences are statistically significant ($p<0.01$).

Erucic acid was 3.83 % in female head, 4.52 % in male head; 1.28 % in female thorax, 1.45 % in male thorax; 1.72 % in female abdomen, not detected in male abdomen. Differences between head and abdomen of female and male are statistically significant ($p<0.01$), however the difference between thorax of male and female is not ($p > 0.05$).

Nervonic acid has been determined as 1.09 % in female head, 0.32 % in male head; 0.62 % in female thorax, 0.70 % in male thorax; 0.90 % in female abdomen, 1.24 % in male abdomen. Differences between head and abdomen of female and male are statistically significant ($p<0.01$), however the difference between thorax of male and female is not ($p > 0.05$).

Saturated Fatty Acids

Total saturated fatty acids range from 14.83 % to 24.67 % in the study (Table 1). They were found in body parts as follows: 19.57 % in female head, 24.67 % in male head; 14.83 % in female thorax, 16.91 % in male thorax; 19.77 % in female abdomen, 22.78 % in male abdomen (Table 1). All of the values between male and female body parts differ statistically significantly ($p<0.01$) (Figure 1). In general, saturated fatty acids in all body parts of males were higher than that of females. Since female wasps are more active than males, probably female wasps used saturated fatty acids for energy consumption more than males.

Palmitic acid has been found as 9.72 % in female head, 12.77 % in male head; 8.29 % in female thorax, 8.76 % in male thorax; 11.98 % in female abdomen, 13.52 % in male abdomen. The differences between head and thorax of male and female insects are statisticially significant ($p<0.01$).

Stearic acid has been found 8.07 % in female head, 9.94 % in male head; 4.20 % in female thorax, 5.36 % in male thorax; 5.7 % in female abdomen, 7.27 % in male abdomen. All the values between male and female body parts differ statistically significant ($p<0.01$) (Table 1).

Lauric acid, Miristic acid, Penthadecilic acid, Heptadecilic acid, Lignoseric acid, and Arachidic acid are not discussed here since they were found only in trace amounts (less than 1 %) (Table 1).

CONCLUSIONS

In this study, unsaturated fatty acids are found higher than saturated fatty acids in both female and male insects. Essential fatty acids, linoleic acid and linolenic acid, are high in the head and thorax, but low in the abdomen in both sexes. Since essential fatty acids cannot be synthesized *de novo,* it is thought that these fatty acids are taken from food plants via feeding. Essential fatty acids are metabolized for diverse biological functions to some extent in the abdomen, which helps explain their lower levels.

Non-essential fatty acids, either saturated or unsaturated, could be synthesized *de novo* when they are required. Therefore many fatty acids are regulated by living organisms according to their needs. For example, defensive alkaloids are stated to be derived from fatty acids, especially from oleic acid (Attygalle et al., 1994). The reason that oleic acid level is high in the abdomen is its necessity in that body part.

Future studies may be carried out to determine if fatty acid composition could be a useful tool to distinguish between insect taxa, especially morphologically identical ones.

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