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Identification and Analysis of the Labial Gland Secretions of the Male *Bombus* (*Psithyrus*) *vestalis* (Hymenoptera: Apidae) native Iran

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A b s t r a c t : The labial gland secretions from the male of the bumblebee *Bombus* (*Psityrus*) *vestalis* (GEOFFROY) collected from Vikan, Alamut (Qazvin Province, Iran) were analysed by gas chromatography/ mass spectrometry (GC/MS) and components of their extracts were identified. The major compound was a complex mixture of alkenols, alkenals, fatty acids, hydrocarbons, wax type esters and steroids. We found geranylcitronellyl acetate to be the main component (30.49 %). geranylcitronellol (24.2 %) and eicosadienal (13.5 %) were present in the secretion in lower amounts. We also identified 17 minor compounds. Morphological identification of male species by the general form of the genitalia and its components present here.

K e y w o r d s : *Bombus* (*Ps.*) *vestalis*, Labial Glands, Pheromone, Iran.

Introduction

Bumblebees are common in the mountains of Iran and are known to be important pollinators of both native and greenhouse plants. Over the last seventy years, 32 bumblebee species (include 28 social-bumblebees and 4 cuckoo-bumblebees) have been reported from Iran (MONFARED et al. 2007). Little known about these bees in Iran because they were been at most subjected of faunestic studies during past decades and no more study on their biology or chemotaxonomy have been aroused by now. Although there are some studies on labial glands of *B.* (*Ps.*). *vestalis* on other parts of the worlds (e.g. URBANOVA et al. 2004), but due to differences of geographical among species, it's seems that we need information about all worlds regions ecotypes. The labial glands of male bumble bees are the source of a complex mixture of compounds used as marking pheromones (KULLENBERG et al. 1973). Each bumblebee species produces a specific blend of compounds (see review VALTEROVA & URBANOVA 1997) and consists of a mixture of acyclic monosesquiand diterpenes (alcohols and aldehydes) and various straight-chain fatty acid derivatives (alcohols, esters, aldehydes, and hydrocarbons) (BERGSTROM et al. 1981). During premating behaviour, male bumblebees do three main kinds of behaviour including patrolling behaviour, perching behaviour and nest entrance waiting behaviour (SCHREMMER 1972; LLOYD 1981; BERGMAN 1997; HOVORKA et al. 1998). The patrolling behaviour is the most common type of pre-mating behaviour among the bumblebee and cockoo-bumblebee species that males attract females by marking places with a chemical

signal. This behaviour has been described by Darwin (1886, in FREEMAN 1968). Calam (1969) was the first to demonstrate that the secretions of the labial cephalic glands of male bumblebees are highly specific. Since then the cephalic gland secretions of many species have been studied (BERGSTROM & SVENSSON, 1973, SVENSSON & BERGSTROM 1979; BERGSTROM 1981; CEDERBERG et al. 1984; DESCOINS et al. 1984; BERGSTROM et al., 1985; BERTSCH 1997; PAMILO et al. 1997; BERGMAN (1997); TERZO et al. (2003) and BERTSCH et al. (2004). The analysis of male marking cephalic secretions has already led to the recognition of two groups of sibling species in the West-Palaearctic region. *Bombus* (*Pyrobombus*) *lapponicus* (FABRICIUS) has been separated from *B. monticola* SMITH because of the differences in their marking secretions (BERGSTROM & SVENSSON 1973; SVENSSON & BERGSTROM 1979; SVENSSON 1979, 1980). In the same way, it has been determined that *Bombus* (*Bombus*) *lucorum* (L.) includes other taxa, identified by most authors as *B. cryptarum* (FABRICIUS) and *B. magnus* VOGT (RASMONT 1981, 1983; RASMONT et al. 1986; BERTSCH 1997; PAMILO et al. 1997; URBANOVA et al. 2001; VALTEROVA et al. 2002; TERZO et al. 2003). On both occasions, a distinction drawn between the compositions of the cephalic secretions led to the discovery of ecological, ethological, geographical and even morphological evidence supporting distinctions that had been overlooked before (RASMONT et al. 2005). The difficulty of defining bumblebee species based on morphological characters led to the using of the marking secretions in their recognition. Individuals of the same species are able to search, find and recognize their sexual partners. Some bumblebee groups do not odour mark. The composition of the secretion is species-specifical (RASMONT et al. 2005). BERGSTROM et al. (1981) studied the temporal and spatial segregation between species and subspecies. Except for the geographical isolation, species patrolling in the same area segregate to some extent in time and space. Species occurring in the same time and habitat differ substantially in the composition of their marking pheromone to avoid inter-species mating (VALTEROVA et al. 1996). Chemical composition of the males' signal of *Psithyrus vestalis* has been described first time by VALTEROVA et al. (1996) and they found geranycitronellyl acetate to be the main component (48%). Modern instruments are considerably more sensitive, allowing analysis of the secretions from the glands of a single individual (TERZO et al. 2003). This is the first chemotaxonomy study on Apoidea of Iran a country with rich fauna and little known many aspects of biology and ecology of their bees.

Material and Methods

S p e c i m e n s c o l l e c t i n g : Males of individual *B.* (*Ps*.) *vestalis* species were collected during the years 2010-2012 from localities of Verk, Vikan, Alamoot and Moalem Kolaye (Qazvin Province), Also from Kamarbon, Pol zangule, Jadeh Chaloos (Alborz Province). Collected living insects were transported to the laboratory and then kept in a freezer until the labial glands were dissected. This species is a rare one in Iran. Although in the present study, we focused on detections of the secretions of male labial glands, but we studied coloration and other morphological characteristic, especially, genitalia which drawn out from end of body and dissected to show its elements including volsella, gonostylus, penis valve, sterna VII and VIII which are important in species identification.

C h e m i c a l S t u d y M e t h o d : The cephalic part of the labial glands was

dissected from the head of 5 males and placed in vials (glands from 1 males per vial) containing 20 µl hexane per gland. After 15 min of shaking and 2h standing in the refrigerator, the hexane extract was filtered and stored in a freezer (-18 °C) before analysis. The samples were analyzed using a gas chromatograph mass spectrometer (GC-MS) Fisons MD 800 with electron-impact ionization (70 eV). For the separation of components, a DB-5 column (5 % fenylmethyl silicone), 30 m × 0.25 mm film thickness 0.25 lm was used. A splitless injector mode (220 °C) and helium carrier gas (flow 0.94 ml/min) were used. The temperature program of analyses was 70 °C; 2 min; 10 °C/min to 320 °C; 15 min. Compounds were identified by comparing their mass spectra with those of the NIST Library (National Institute of Standards and Technology, USA) and by retention times and chemical ionization measured on an ion-trap instrument (Varian Saturn 2000) with MeCN as a reagent gas was used for the determination of the C=C bond positions.

Results and discussion

S p e c i e s d i a g n o s i s : Body length 15 to 17 mm, wingspan from 34 to 38 mm, under stereomicroscope (40×) hair of the clypeus and around of antenna, black, hair of the vertex black and yellow, hair of anterior thoracic collar dark yellow and Interalar band black, the scutellum yellow and black with dominance of black, hair of T1 in middle brown with low density, two marginal sides with a mass of yellow hair, hair of T2 and T3 black, T4 and T5 yellow, marginal sides of T6 with yellow hair and in median part with black hair, T7 black. The general form of the genitalia similar to Fig. 1-a, inner corner located near mid-point of its length without any inwardly-directed hooks, the volsella is nearly triangular in the distal section (Fig. 1-b), gonostylus with the distinct interio-basal process that associated with many long branched hair (Fig. 1-c), head of penis valve nearly straight from dorsal aspect (Fig. 1-d), Sternum 7 and 8 similar to Fig. 1-e and 1- f, respectively. Cuckoo bumblebees usually have tinted wings in both sexes. Cuckoo bumbles tend to have less dense hair (shiny thorax & abdomen) and their cuticles are thicker than other subgenera. Takes over the nest of *Bombus terrestris*, the female enters the nest and kills the queen, then laying her own eggs to be reared by the *Bombus terrestris* workers. Mated female cuckoo bees hibernate through the winter and emerge in the spring later than their hosts.

C h e m i c a l C o m p o u n d s : Twenty compounds were found in the labial gland secretions of the *B.* (*Ps.*) *vestalis* males (Table 1). The labial glands contain a mixture of acyclic diterpenes and various straight-chain fatty acid derivates (alcohols, esters, and both saturated and unsaturated hydrocarbons). geranylcitronellyl acetate is the main component (Fig. 1, peak 13 at 20:349 min, 30.49 %) of the labial gland and considerable amounts of geranylcitronellol (peak 8 at 17:36 min, 24.2%) and eicosadienal (peak 11 at 19:746 min) were detected. The mass spectera of main component (geranylcitronellyl acetate) show in Fig, 4. In this species Isoprenoids had higher amounts in comparison of other components. A typical chromatogram for the labial gland secretions of *B.* (*Psityrus*) *vestalis* is given in Fig. 2 and the compounds are summarized in Table 1.

Table 1: Compounds of the labial glands of *Bombus* (*Psityrus*) *vestalis* males and structural evidence of unsaturated compound.

NO	RT	COMPONENT	MASS SPECTERUM	PERCENT (MEAN)
1	17.733	1-7-Hexadecadiene	43-55-67-82-96-110-124-222	3.33
2	17.869	7- Hexadecanal	57-121-183-248	2.63
3	18.693	farnesol	41-55-69-81-95-109-123-136	0.31
4	18.796	farnesyl acetate	41-55-69-93-107-121-204	1.7
5	18.925	11-hexadecanol	55-83-111	0.43
6	18.989	octadecen-1-ol	41-55-82-96-110-124-138-250	0.11
7	19.124	Henicosane	41-57-85-113-141	0.21
8	19.415	Geranyl Citronellol	41-55-69-95-121-136-223-292	24.2
9	19.551	Docosene	41-55-81-95-109-135-149-308	2.74
10	19.746	Eicosadienal	41-55-81-95-109-123-137-292	13.5
11	19.857	15-Eicosenal	41-55-69-83-97-111-135-294	1.76
12	20.349	Geranyl Citronellyl acetate	41-69-95-121-136-163-265-334	28.3
13	20.417	cis-9-Eicosen-1-ol	41-55-69-82-96-109-123-278	6.86
14	20.545	Tricosane	41-57-71-99-127-155-324	3.47
15	20.979	1-Nonadecene	43-57-83-97-111-125-206-281	0.25
16	21.15	Eicosadienyl acetate	43-55-67-81-276-336	0.15
17	21.258	Tetracosane	55-81-95-123-138-336	1.41
18	22.159	Pentacosane	57-85-113-141-169-352	2.19
19	23.671	Heptacosane	57-85-113-141-169-380	3.73
20	25.073	Nonacosane	57-85-113-141-169-408	1.16

Analysis of the labial gland secretion of the Cuckoo-Bumblebees *B.* (*Ps*.) *vestalis* male carried by VALTEROVA et al. (1996) for the first time in the Czech Republic shown that geranycitronellyl acetate was the main component. We followed their method in analysis and found this substance in individuals of which identified as *B.* (*Ps*.) *vestalis*. The cuckoo-bumblebee species living in the Iran had not been thoroughly investigated from the chemical point of view. BERGMAN et al. (1996) identified compounds from male labial glands of *B. vestalis*, in their case from Scandinavia. VALTEROVA et al. (1996) also had been found nineteen compounds in the labial gland secretions of the *Psithyrus vestalis* males, native Czech Republic. We didn't considered substances which not known as pheromone in components like dodecane, tridecane etc. Cuckoo bumblebees recorded by now from Iran are six species including *B.* (*Ps.*) *sylvestris* (LEPELETIER), *B.* (*Ps*.) *vestalis* (GEOFFROY), *B.* (*Ps*.) *bohemicus* SEIDL, *B*. (PS.) *campestris* (PANZER), *B*. (*Ps*.)

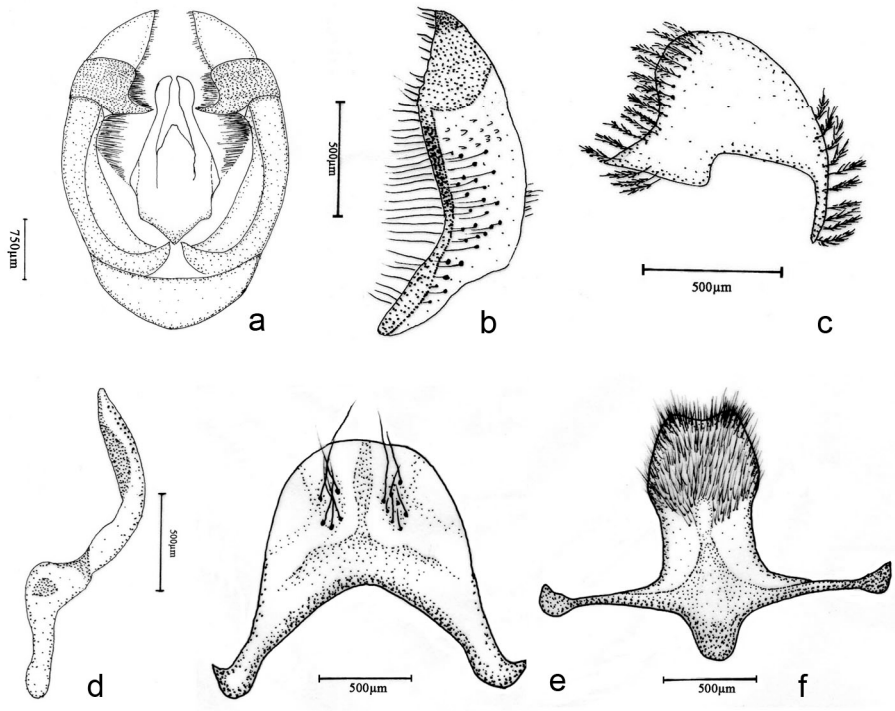

Fig. 1a-f: Genitalia pattern of *B. vestalis* male: (**a**) Genitalia, (**b**) Volsella, (**c**) Gonostylus, (**d**) Penis valve, (**e**) sternit 7, (**f**) Sternit 8. (original, drawn by first author, PA).

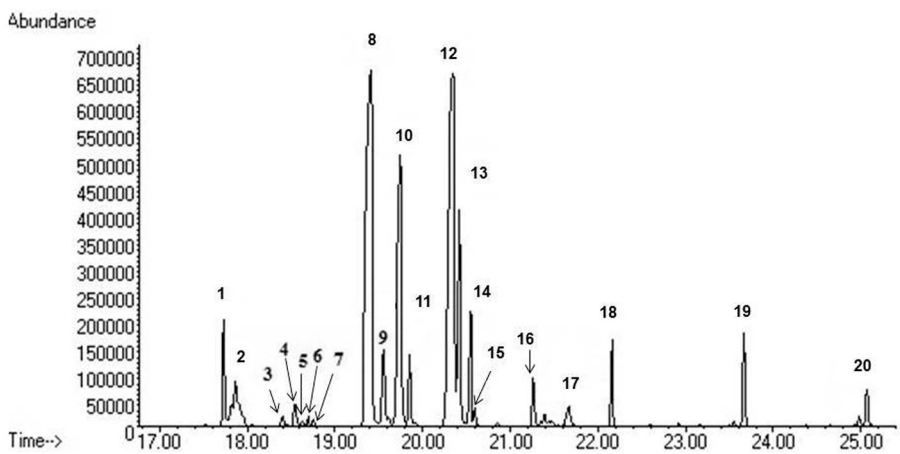

Fig. 2: Gas chromatogram of the labial gland secretion of *Bombus* (*Psityrus*) *vestalis* males 17 up to 25 min retention time.

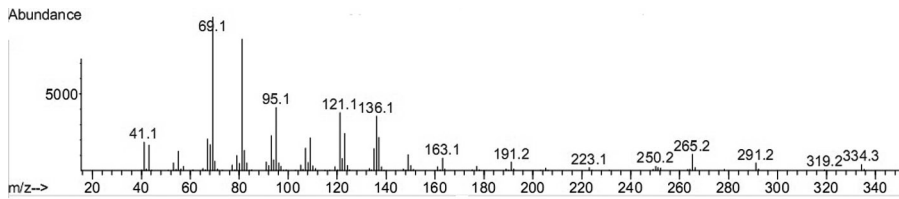

Fig. 3: Mass spectra of geranyl citronellol acetate.

maxillosus KLUG, *B.* (*Ps.*) *quadricolor* (LEPELETIER) (MONFARED et al. 2007). Females of these species are parasites of other subgenera of *Bombus* which are rare and may trapped by happened, so male of them would be very few in field. The secretions of the labial cephalic glands of male bumblebees are highly specific (CALAM 1969). These findings have been used in purpose of chemotaxonomic studies like what RASMONT et al. 2005 carried out on proving of unity of two species by different colorations were the same species. RASMONT et al. 2005 shown that Cephalic secretions of the bumblebee subgenus *Sibiricobombus* VOGT suggest *Bombus niveatus* KRIECHBAUMER and *Bombus vorticosus* GERSTAECKER are conspecific. They found a total of 40 compounds were identified in *B. niveatus* and *B. vorticosus* and they were the same in both taxa. Another example of using this method was study by HOVORKA et al. 2006 which studied male cephalic labial gland secretions of two bumblebee species of the subgenus *Cullumanobombus* including *B. semenoviellus* and *B. cullumanus*. The main components in both species were the same and was geranylgeranyl acetate. This compound also has been observed in the labial gland secretion of males of several bumblebee and cuckoo bumblebee species (HOVORKA et al. 2006). Comparing components with results of VALTEROVA et al. 1996 shown that main components in our experiment (30 %) was lower than what they found (48 %). Some components like tricosane, docosene, heptacosane are found in almost all bumblebees species. Components like eicosodienyl acetate and some aldehides were the same in two experiments.

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