Towards Precise Identification of The Medically Important Flesh Fly, *Sarcophaga* (*Liopygia*) *argyrostoma* (Robineau-Desvoidy, 1830) (Diptera: Sarcophagidae)

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ABSTRACT

Although of its medical importance and its role in the forensic investigation field, flesh fly, *Sarcophaga* (*Liopygia*) *argyrostoma* is not well taxonomically studied. This work aimed to use different tools (morphological and biochemical) toward precise identification of this species. By using SEM, different types of antennal sensilla (used for host location among other functions) had been classified to be applied as potential accurate taxonomic character. Trichoid sensilla are the most common and numerous types on *S. argyrostoma* antennae, in addition chaetica, basiconica and campaniform types are found to be represented by a few numbers. Gas chromatography-mass spectrometry (GC/MS) analysis characterized thirty-eight cuticular hydrocarbons components (CHCs) found in the flesh fly integument. The hydrocarbons identified were belonged to seven categories (i.e. Alkane, Alkene, Cycloalkane, monocyclic hydrocarbons, Alkyne, Polycyclic and Cycloalkane hydrocarbons), with chain length ranged from C_5 to C_{35}. Cuticular hydrocarbon profile now used precisely in insect taxonomy. Most studies on *Sarcophaga* were carried out on larvae, it's very important to study adult flies to suggest precaution and control measures against adults to reduce their harmful role in diseases transmission.

Keywords: Sarcophaga argyrostoma, Taxonomy, Medical importance, Myiasis, SEM, CHCs.

INTRODUCTION

Sarcophagidae Hagen, 1881 is a medium-sized family of Diptera which include about 2600 species. Sarcophagidae has worldwide distribution, especially in tropical and warm temperate climate regions[1]. Practically, species of Sarcophaginae are difficult to identified based on external feature and can only be precisely identified using characters of male genitalia[2]. Sarcophagid spp. applied in forensic investigations as they have been found on carcasses throughout the decomposition process, being slightly less abundant only during the advanced stages of decomposition[3].

Several sarcophagid spp. recorded as facultative myiasis agents[4]. *Sarcophaga (L.) argyrostoma* is classified as included in the category of "nosocomial myiasis agents" because they have been found in wound myiasis affecting hospital patients who are immobilized[5].

Most studies on myiasis were carried out on larval stage of flies (maggots) as the larvae is the direct causative agent of myiasis[5].

The members of Sarcophagidae received little attention in Egypt. Complete descriptions of the immature stages of *S.* (*L.*) *argyrostoma* (R.-D.)[6], [7]. They applied classical description with SEM figures in some cases, but no modern documentation techniques were applied.

New approach has been introduced for species identification (e.g. DNA barcoding[8], Cuticular hydrocarbon profile[9]).

Scanning electron microscopy (SEM) is particularly useful for illustrating external characters. During recent years, several SEM based papers appeared, dealing with larval morphology of calyptrate flies of veterinary and medical importance[10]. In addition to the physiological function performed by sensilla, they have a useful tool in taxonomic and phylogenetic studies. Different species in the order Ephemeroptera were taxonomically compared using scanning electron microscopy (SEM)[11].

As regards the importance of the insect antennal sensilla as they play an important role in host location, food selection, oviposition site selection, chemical communications, mechanoreceptor and in various behaviors during adult life, numerous studies have characterized the antennal sensilla of different insect species[12].

On the other hand, few studies were carried on other regions of the body and their taxonomic value e.g. the sensilla of tarsi and ovipositors of six fruit flies[13]; labial sensilla and their distribution in Reduviidae (Triatominae and Peiratinae) and suggested that interspecific diversity and intraspecific similarity in the shape and numbers of labial sensilla could be used as taxonomic characters[14].

Cuticular hydrocarbons (CHCs) are essential to the survival of the insects, because their primary function is to prevent dehydration[15] and they protect insects against invasion by microorganisms[16]. The CHCs are synthesized by secreting cells derived from epidermal cells and are transported to the cuticle via hemolymph by lipophorin proteins[17]. Cuticular hydrocarbons are

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heritable and stable end products of genetically controlled metabolic pathways[18]. There is no significant differences between male and female concerning cuticular hydrocarbons in different instars[19] and the cuticular and internal hydrocarbons are similar[20]. Applying CHCs as chemotaxonomic tool, was investigated by different researchers. CHCs studied as chemotaxonomic tool for different categories e.g. Termites[21] and *Triatoma dimidiate*[22]. The ratio of epicuticular hydrocarbons to internal hydrocarbons was 28:1[23].

The aim of this study is to provide a morphological documentation of the adult *S.* (*L.*) *argyrostoma* (R.-D.) in addition to estimate the cuticular hydrocarbon components, as taxonomic identification tools. Also suggest prevention strategy by depend on precise taxonomic identification of adult flesh fly for effective control of the source of myiasis.

MATERIALS AND METHODS

Insects:

The insect specimens collected by bait trap from different localities in Egypt (Wadi El-Natroun, Kafr Sakr, Cairo, Ismailia). The male genitalia were dissected , dry mount the flies, and identified by using taxonomic key constructed by **Alfred**[24].

Scanning:

The specimens were coated by gold sputter coater (PSI-Module, USA). A fully computer-controlled scanning electron microscope (Model: Trace 1310; Thermo scientific). Using high vacuum, it was scanned with the electron beam at 30 kV from different angles. The SEM photograph was conducted at the National Research Center, Cairo, Egypt. Sensilla on the antennae of adult *S. argyrostoma* were identified and measured. Measurements (μm) obtained from photomicrographs of 3-5 individual sensilla of the same type to calculate the means.

Hydrocarbon extraction and analysis

Cuticular hydrocarbons were extracted using hexane as a solvent from adult specimens, separated from other lipid components and analyzed by gas chromatography-mass spectrometry (GC/MS) as described by **Page *et al*.**[25].

Gas Chromatography – Mass Spectrometry (GC/MS).

GC/MS analysis was conducted in "The Regional Center for Mycology and Biotechnology", Al-Azhar University. Samples were run on Thermo Scientific TRACE 1310 Gas Chromatograph, fitted with a silica capillary column DB-5, (Length 30 m. x Internal diameter 0.25 mm. x film thickness 0.25 μm), carrier gas of helium (flow rate 1 ml/min.). One microliter of sample was injected into the injector in pulsed splitless mode. The injector temperature was at 300 °C. The GC temperature program was started at 40 °C (5 min.) then raised to 275 °C (5 min.) at 5 °C/min. Mass spectrometric was operated in electron impact ionization mode with an ionizing energy of 70 ev. The ion source temperature was 300 °C. The electron multiplier voltage (EM voltage) was maintained 1650 v. above auto run. The instrument was manually turned using perfluorotributyle amine (PFTBA).

Compounds were identified by comparison of the spectra to the Wiley & NIST MASS SPECTRAL DATABASE and by comparison to literature relative retention indexes.

RESULTS

1- Taxonomic notes:

Family: Sarcophagidae

Diagnosis: Common oviduct with bilobed incubatory pouch. Bacilliform sclerites (divided male sternite 10) shortened and more or less perpendicular to the median plane. Abdominal sternites without sensilla trichodea. Posterior larval spiracles placed in a recession or cavity. Peritreme of posterior spiracles of second and third instar larva incomplete and without a distinct ecdysial scar[1].

Genus *Sarcophaga* Meigen, 1826: 14

Type species. *Musca carnaria* Linnaeus, 1758, designated by Partington (1837:697).

Subgenus: *Liopygia* Enderlein

Liopygia Enderlein, 1928a:41.

Type species: *Musca ruficornis* Fabricius, 1794, by original designation.

Sarcophaga* (*Liopygia*) *argyrostoma

Myophora argyrostoma Robineau-Desvoidy, 1830:340. South Africa, Cape Province, Cape of Good Hope.

Diagnosis[1]: Body length 7–17 mm. Male. Frontal vitta, antenna and palps black. Thorax grey with black longitudinal stripes. Legs black. Abdomen with checkerboard-like pattern. Frontal vitta moderately wider frontoventrally. Funiculus about 2.2 as long as pedicel. 1–2 rows of postocular setae. Parafacial bristles short and weak, arranged in two vertical rows. Scutellum with short setae. Wings with medio-cubital crossvein (m-cu) more or less sigmoid; cell R5 open. Abdominal tergite III without medial marginal setae. Sternite V with numerous short marginal hair-like bristles.

Female. Lighter coloured. Frons equal to or slightly narrower than eye width. Orbital plates wide. Frontal stripe only 1.25–1.5 times wider than orbital plate. Palps long and clearly broaden apically. Legs. Fore femoral organ distinct and with a few cross-striations. Mid femur with row of setae and an apical row of short stout bristles. Mid-femur

organ in the form of an elongate ovate patch, blackish grey coloured with diametrical lines, situated on posterior surface of mid femur. Mid femoral organ large with many cross-striations. Abdominal tergite VI brown with long and short marginal bristles and hairs. Sternite VII with marginal tubercle near anterior margin and 1–2 pairs of short lateromarginals.

2- Antennal sensilla:

The antennae of flesh fly, *S. argyrostoma* (Figure 1), are inserted between compound eyes in a frontal depression called antennal fossa. The antenna is composed of three regions, the scape, pedicel, and flagellum which carries arista at its dorso-proximal end.

Scape (Figure 2):

Scape, is the most proximal and shortest antennal segment. Sensilla on the scape are:

- Chaetica II (Ch II): Length (~ 80.64 – 140 µm., $\overline{X}$ 113.50 µm) locate at the dorsal part of the rim ofscape. Chaetica sensilla of the scape with grooved surface, pointed at tip, a number of Ch II are slightly curved and other are straight.
- Trichoid I (Tr I): Majority of scape surface furnished with fine trichoid sensilla of type I. sensilla of. Length nearly uniform (20µm.), recumbent, slightly dense.

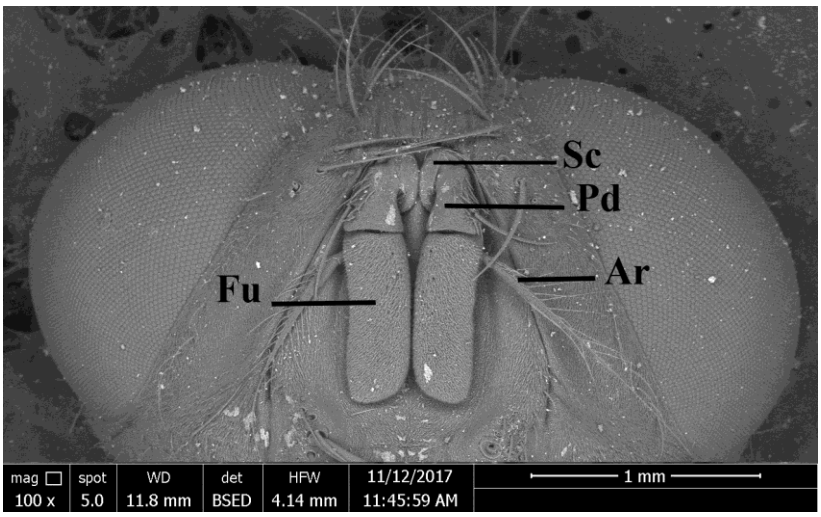

Figure 1: Photomicrograph of *Sarcophaga argyrostoma*, Antennae. Ar, Arista; Fu, Funiculus; Pd, Pedicel; Sc, Scape.

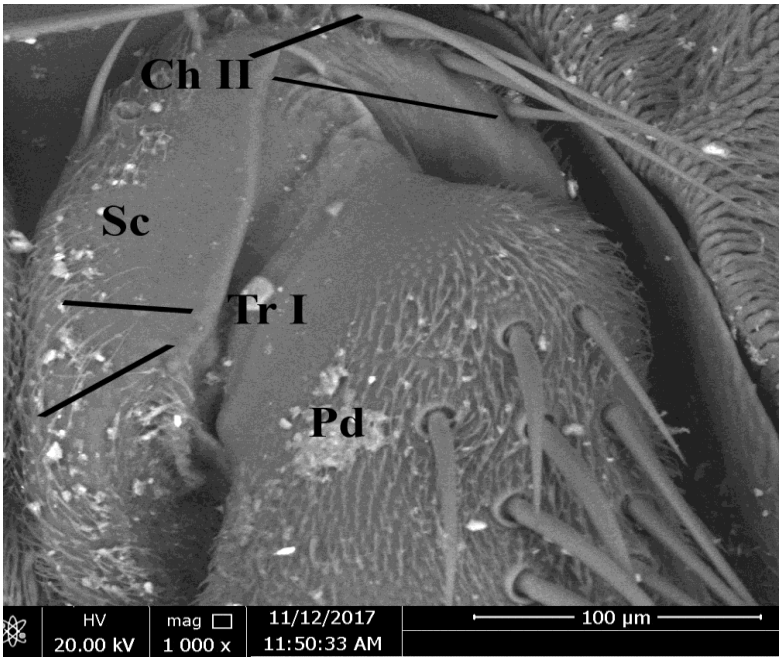

Figure 2: Photomicrograph of *Sarcophaga argyrostoma*, antennal scape. Ch II, Chaetica II sensilla; Pd, Pedicel; Sc, Scape; Tr I, Trichoid I sensilla.

The Pedicel (Figure 3):

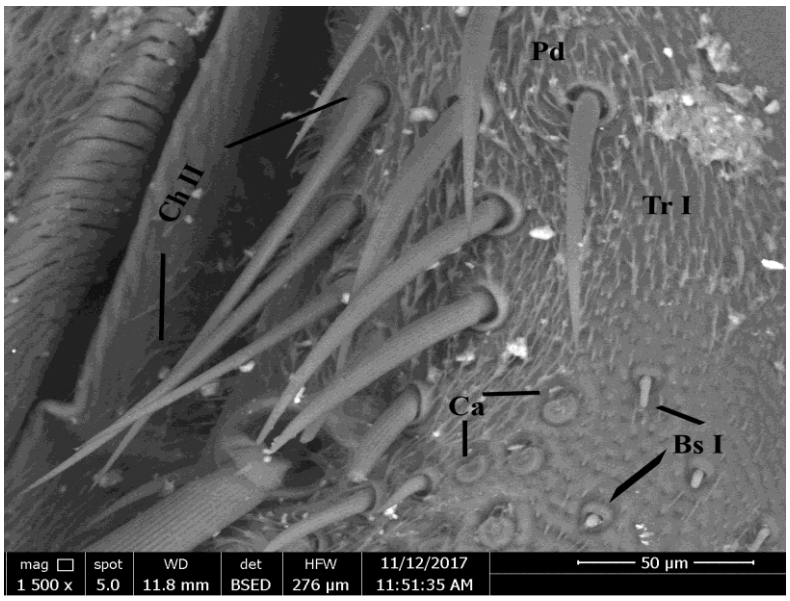

Figure 3: Photomicrograph of *Sarcophaga argyrostoma*, antennal pedicel sensilla. Ba I, Basiconica I; Ca, Capaniform sensilla; Ch II, Chaetica sensilla II; Pd, Pedicel; Tr I, Trichoid I sensilla.

- Chaetica I sensilla: Length (~ 28.61 – 39 µm., $\overline{X}$ 33.81 µm). About five sensilla mixed with trichoid II.
- Trichoid I sensilla: Length (~ 7 – 16 µm., $\overline{X}$ 12 µm). Recumbent sensilla, slightly dense at base, widely sparse at rest of pedicel.
- Chaetica II sensilla: Length (~ 80.11 – 151 µm., $\overline{X}$ 102.64 µm). A bundle of straight and slightly curved sensilla at basal outer part of pedicel. Pointed, with grooved surface (about ten sesillum). Each sensillum with socketat base surrounded by elevated edge.
- Basiconica I sensilla: Length (~ 4 – 16 µm., $\overline{X}$ 10.2 µm). Straight, scattered, blunt, with smooth surface. At inner side of pedicel
- Campaniform sensilla: Dome like, at inner side of pedicel, feebly elevated.
- Bristle (Figure 5): Single bristle arise from each antenna externally at midlength of pedicel, surrounded by chaetica II. Length 320 µm. 0.41 as long as funiculus and about three times as long as chaetica II (3.12 times). Its surface grooved, pointed at tip, slightly curved.

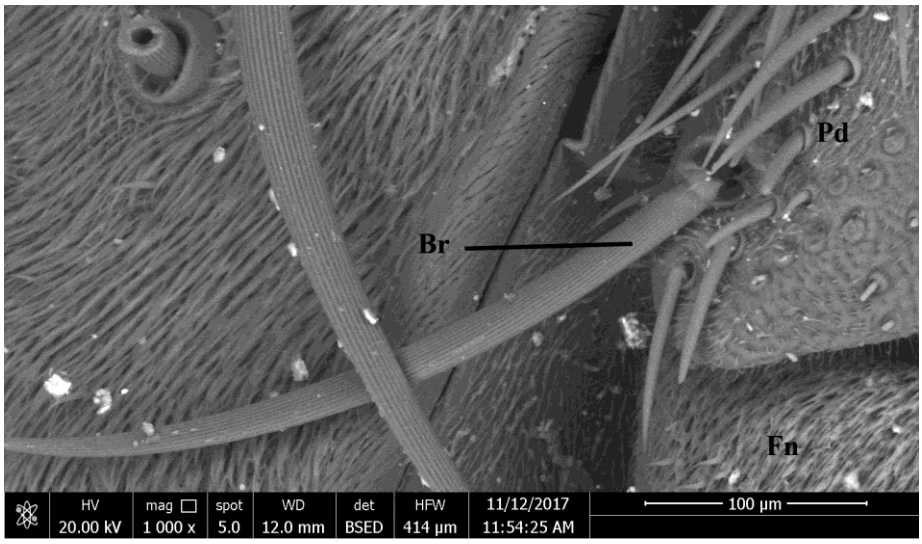

Figure 4: Photomicrograph of *Sarcophaga argyrostoma*, antennal pedicel bristle. Br, Bristle; Fn, Funiculus; Pd, Pedicel.

Funiculus (Figure 5 & 6):
Subrectangular segment, arista two-segmented, located at external side, arise near base, about 1.2 times length of funiculus.
- Funiculus densely covered with trichoid sensilla type I (Tr I), in between sparse chaetica sensilla type I.
- Arista bristle like, plumose (with long setae) except basal internal part which is bare.

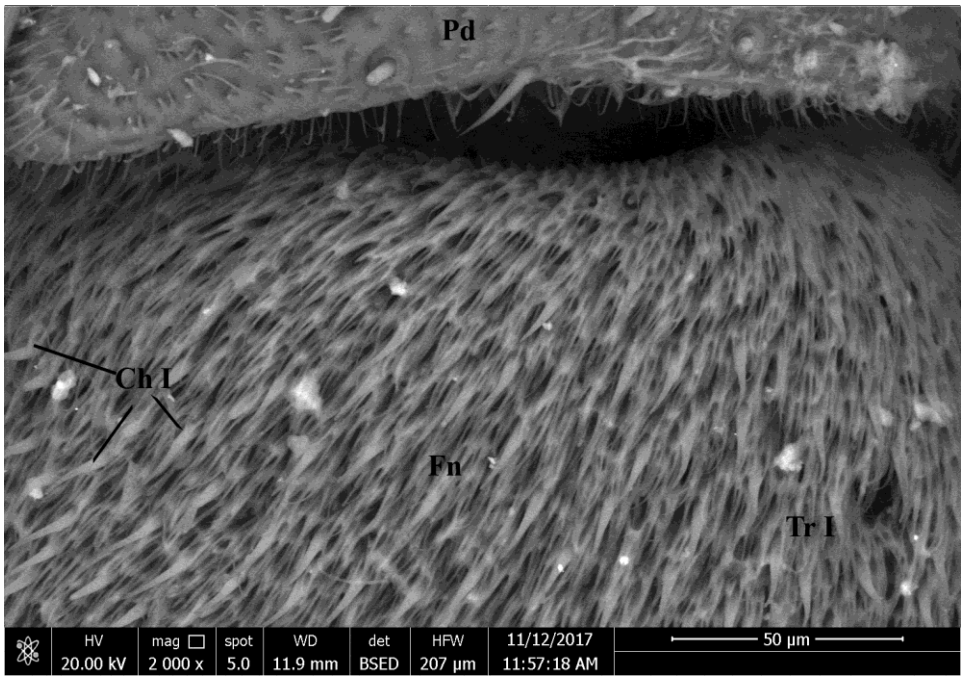

Figure 5: Photomicrograph of *Sarcophaga argyrostoma*, Antennal Funiculus. Ch I, Chaetica sensilla type I; Fn, Funiculus; Pd, Pedicel; Tr, Trichoid sensilla type I.

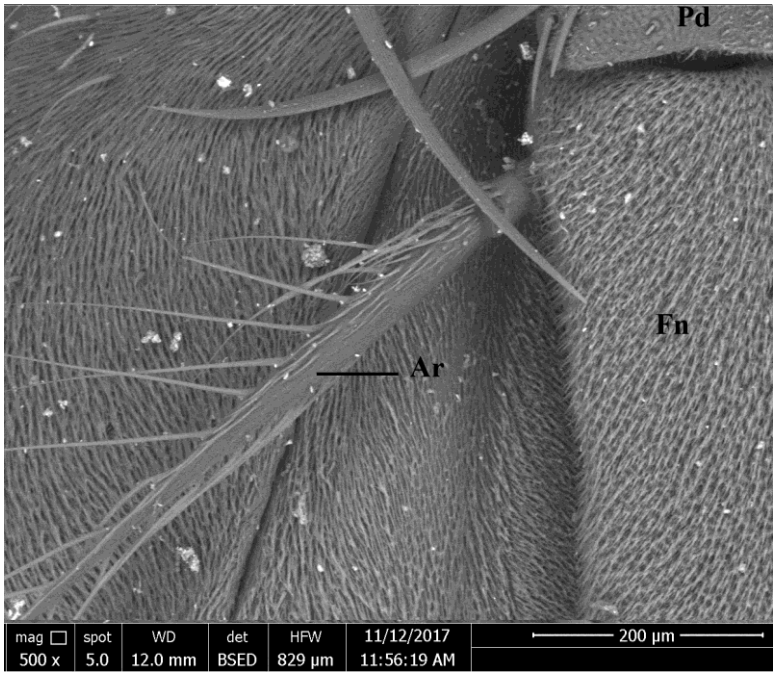

Figure 6: Photomicrograph of *Sarcophaga argyrostoma*, Arista. Fn, Funiculus; Pd, Pedicel.

3- Cuticular hydrocarbons

Chromatographic separations of hydrocarbons (Fig. 7) indicated the presence of more than 38 hydrocarbons. As shown in table (1), *S. argyrostoma* had a mixture of hydrocarbons with chain lengths varying from C_5 to C_{35}. The hydrocarbon of *S. argyrostoma* was classified within eight categories namely, alkane (13components (34.2%)), alkene (8 components (21.1%)), cycloalkane (6 components (15.8%)), monocyclic hydrocarbons (5 components (13.8%)), Alkyne (3 components (7.9%)) and one compound was classified within each of Polycyclic and Cycloalkyne hydrocarbons; one component is unknown. The most abundant hydrocarbon in *S. argyrostoma* was Benzene, (2-methyloctyl) (4.41%) followed by Nonane (3.15%), Cyclohexane, 1,3-dimethyl (3.12%), Dodecane, 2,6,10-trimethyl (2.94%), Dodecane (2.72%), Cyclohexane, eicosyl (2.03%), Benzene, methyl (1.87%), Xylene (1.42%), Cyclopentane, 1,3-dimethyl (1.08%) and Octane (1.09%). Twenty eight hydrocarbons represented as traces (i.e. less than 1%).

The mass results (Fig. 7) revealed hydrocarbons with carbon atom numbers ranging from C5 to C35. The number of carbon atoms of hydrocarbon components did not have clearly dominant group (carbon numbers C_8, C_9, C_{11}, C_{12} and C_{18} were represented in three components for each; eight carbon numbers C_7, C_{10}, C_{13}, C_{15}, C_{16}, C_{17}, C_{19} and C_{35} were represented in two components for each and seven components each represented in one component, C_5, C_{14}, C_{20}, C_{21}, C_{22}, C_{26} and C_{32}).

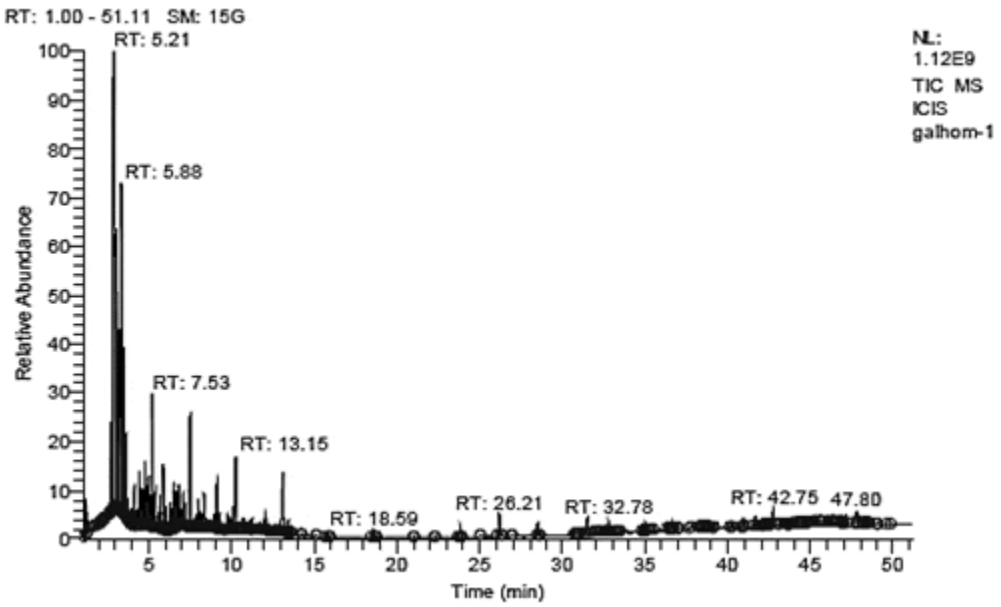

Figure (1): Chromatogram obtainedby GC/MS: Cuticular hydrocarbons of *Sarcophaga. argyrostoma*

Table (1): Cuticular hydrocarbon components of *Sarcophaga argyrostoma*.

RT	Compound Name	Area %	Molecular Formula	Molecular Weight	Classification
1.231	Butane, 2-methy	0.66	C5H12	72	Alkane
2.70	Cyclopentane, 1,3-dimethyl-,	1.08	C7H14	98	Cycloalkane
2.78	5-Undecene, 3-methyl	0.27	C12H24	168	Alkene
3.26	Benzene, (2-methyloctyl)	4.41	C15H24	204	Monocyclic
3.44	Benzene, methyl	1.87	C7H8	92	Monocyclic
3.49	Cyclohexane, 1,3-dimethyl	3.12	C8H16	112	Cycloalkane
3.63	Octane	1.09	C8H18	114	Alkane
3.96	Octane, 2,6-dimethyl	0.18	C10H22	142	Alkane
4.10	Cyclohexane, 1,3,5-trimethyl	0.57	C9H18	126	Cycloalkane
4.31	Cyclodecane, methyl	0.03	C11H22	154	Cycloalkane
4.76	Xylene	1.42	C8H10	106	Alkane
5.21	Nonane	3.15	C9H20	128	Alkane
5.88	Cyclohexane, eicosyl	2.03	C26H52	364	Cycloalkane
5.98	Undecane, 2,6-dimethyl	0.37	C13H28	184	Alkene
6.05	Cyclohexane, 1,2-diethyl	0.57	C10H20	140	Cycloalkane
6.16	1,19-Eicosadiene	0.10	C20H38	278	Alkyne
6.29	pentadecane, 8-methylene	0.59	C16H32	224	Alkene
6.38	4-Nonene, 5-butyl-	0.35	C13H26	182	Alkene
6.62	Benzene, 1,3,5-trimethyl	0.72	C9H12	120	Monocyclic
8.00	Decane, 4-methyl-	0.56	C11H24	156	Alkane
8.64	Octadecyne	0.03	C18H34	250	Alkyne
9.32	2,3-Dimethyldecane	0.56	C12H26	160	Alkane
9.54	10-Heneicosene	0.14	C21H42	294	Alkene
9.94	2,4,4,6-Tetramethyl-6-phenyl-2-heptene	0.46	C17H26	230	Monocyclic
10.25	Dodecane, 2,6,10-trimethyl	2.94	C15H32	212	Alkane
10.91	Docosane	0.12	C22H46	310	Alkane
11.16	naphthalene, decahydro-2-methyl	0.19	C11H20	152	Cycloalkyne
12.04	Nonadecane	0.46	C19H40	268	Alkane
12.22	9-methylheptadecane	0.23	C18H38	254	Alkane
13.15	Dodecane	2.72	C12H26	170	Alkane
15.14	Dotriacontane	0.01	C32H66	450	Alkane
15.99	Tetradecane, 2,6,10-trimethyl	0.04	C17H36	240	Monocyclic
18.59	1-Tetradecene	0.18	C14H28	196	Alkene
22.33	15-methyltricyclo[6.5.2(13,14).0 (7,15)]pentadeca-1,3,5,7,9,11,13-heptene	0.01	C16H14	206	Polycyclic
26.21	1H-Indene,2-butyl-4-hexyloctahydro	0.56	C19H36	264	Alkyne
26.99	17-Pentatriacontene	0.00	C35H70	490	Alkene
28.52	Octadecene	0.28	C18H36	252	Alkene
43.83	Cholest-1-eno[2,1-a]naphthalene, 3',4'-dihydro-	0.09	C35H52	472	Unknown

DISCUSSION

Three subfamilies have been included in family Sargophagidae they are Miltogramminae, Paramacronychiinae and Sarcophaginae. The latter is the most diverse, and includes species which are forensically important[1]. The species of Sarcophagidae are accommodated in 108 valid genera[1], but the generic and subgeneric concepts are far from stable in the family.

Sarcophaginae adults is extremely similar morphologically (the species with three black stripes in the mesonotum, meron with bristles, undeveloped subscutellum, and abdomen checkered or spotted)[2]. Because of this morphological similarity this species are difficult for identification[26]. Identification of this species was accomplished through morphological characters of male genitalia[27].

Although the previous studies, needed more information about the ultrastructure of adult sarcophagid antennal sensilla. Their external morphology and distribution on antennae have been studied during present study. The sensilla on the antenna of *S. argyrostoma* are divided into five types, based on their shape: trichoid (type I), Chaetica (I and II), basiconica (type I), campaniform and bristle (macrotrichia). Chaetica sensilla were found at the different parts of antennae, this result disagree with that of Bisotto-de-Oliveira *et al* [28] who found that other dipteran species (e.g. Tephritid species *Anastrepha fraterculus*, not locate on flagellum ,microtrichia were distributed over the whole antenna. Giannakakis and Fletcher[29], recorded six types of sensilla on the antennae of *Dacus tryoni*. Dickens *et al*[30], recognized six types, but used different classification (i.e. non-porous sensilla (NPS) for chaetica, and multipore sensilla (MPS) for trichoid and basiconic.

Chaetica sensilla, observed on the scape, pedicel and funiculus of *S. argyrostoma* are common throughout other dipteran flies[12]. This type of sensilla is originated from a socket and regarded as mechanoreceptor sensilla[31] (Wang *et al.*, 2012).

Many advantages have been achieved when using cuticular hydrocarbons in taxonomy. The CHC profile of each species may reach to more than hundred components showing differences in number of carbon atoms, structure and size[32], this variation used as taxonomic characters. Identification of Cryptic species form a big obstacle as they very similar externally for taxonomists[33]. CHC components can be used to separate cryptic species[34] . Another advantage is in case of identified rare specimens, where the CHC analysis technique keep intact specimens as they soaked for few minutes in Hexane and washed[32].

Thomas and Dennis[19] found no significant differences in percentage composition of hydrocarbon components between sexes or with the ages of the pupae of *Manduca sexta* (L.) as well as in different instars. Kather and Martin[32] found interspecific variation in cuticular hydrocarbon profile due to age, sex, sexual maturity and cast (in social insects).

The most probable related group of the Sarcophagidae is the family Tachinidae[1] as they both possess a single dorsal phallic sclerotization, i.e. fused dorsolateral processes.

It is very important to correctly identify the species involved in myiasis. Correct identification of the myiasis agent provides more detailed information on the responsibility that health units must have towards patients[5].

In conclusion, adult need intensive studies to adopt prevention strategy by eliminate adult and as a result no eggs laid or larvae grow.

CONCLUSION

The present study suggests studying the myiasis causing insects towards planning prevention strategy to avoid the diseases. This work recommended morphological and biochemical approaches to identify adult *S. argyrostoma.* The results indicated that this approach is qualified to be accurate identification tool and enabling subsequent effective control strategy.

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