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Use Cuticular Hydrocarbons as Chemotaxonomic of The Pamphagidae Pamphagus elephas (Insecta, Orthoptera) of Algeria

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***Abstract*—**The cuticular hydrocarbons of *Pamphagus elephas* (Orthoptera: Pamphagidae) has been analysed by gas chromatography and by combined gas chromatograph-mass spectrometry. The following hydrocarbon classes have been identified in insect cuticular hydrocarbons are: n-alkanes and methylalkanes comprising Monomethyl-, dimethyl-and trimethylalkanes. Sexual dimorphism is observed in long chain alkanes (C24-C36) present on male and female. The cuticulars hydrocarbons of *P.elephas* ranged from 24 to 36 carbons and incluted n-alkanes, Dimethylalkanes and Trimethylalkanes. n-alkanes represented by (C24-C36,72,7% on male and 79,2% on female), internally branched Monomethylalkanes identified were (C25, C30-C32,C35-C37;11% on male and 9,4% on female), Dimethylalkanes detected are (C31-C32, C36; 2,2% on male and 2,06% on female) and Trimethylalkanes detected are (C32, C36; 3,1% on male and 4, 97 on female). Larvae male and female (stage 7) showed the same quality of n-alkanes observed in adults. However a difference quantity is noted.

***Keywords*—**Cuticular hydrocarbons, Gas chromatography, Mass spectrometry, *Pamphagus elephas*,, Sexual dimorphism

I. INTRODUCTION

Most of the Pamphagidae species (Insecta: Orthoptera) can readily be identified by morphological characters.

The grasshopper studied in this work is *Pamphagus elephas*. That is difficult to identify by taxonomic techniques. Most of investigation effectuated on insects showed that hydrocarbon composition not only separates species but also between male and female at different stage of development. A hydrocarbon chemotaxonomy could provide information on the evolution of hydrocarbon biosynthesis in the insect at different stages. The focus of this work is the identification of hydrocarbons components present on males and females of adults and larvae of *P. elephas*, specifically examines patterns in the compounds that demonstrate the sexual dimorphism. Demonstration of sexual dimorphism in the cuticular hydrocarbons of female and male can provide clues for the determination of a sex pheromone [1]. Additionally cuticular hydrocarbons have been used to differentiate species of Orthoptera [2], Coleoptera [3], Diptera [4], Hymenoptera [5], [6] and others. The identification of cuticular hydrocarbons by gas chromatography/mass spectrometry (GC/MS) allows the differentiation of sex dimorphism by examining not only the gas chromatogram but also the mass spectra.

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II. MATERIALS AND METHODS

A. Insects

The Pamphagidae, *Pamphagus elephas* L. is endemic species of North Africa and although living in different biotopes in Algeria [7]. Adult males and females were collected by hand near plateau of El Anasser, North Algiers. second instar larvae were reared in the laboratory from eggs obtained from a colony of field collected adults.

B. Chemical Analysis

Insect's adults were extracted individually in glass vials rinsing them repeatedly with 2 ml using a Pasteur pipette at room temperature for 27°C. This brief extraction period was used to ensure that the components recovered were primarily surface lipids [8]. The volume used is 10 ml of hexane. The hexane extract was evaporated to dryness under a stream of nitrogen, and suspended in 50 µl of hexane, and an aliquot 10% was analyzed by combined gas chromatography/mass spectrometry (Hewlett-Packard 5730A /5971A). Samples were injected into a capillary column (25 m Hewlett-Packard HP5-1 cross-linked phenyl (5%) and methyl-silicone (95%); 0,25mm, internal diameter, 0.33 µm film thickness) with helium as the carrier gas. The oven was held at 320°C for 3 min after injection (splitless) and the temperature was raised at a rate of 15°C/min to a final temperature of 320°C which was maintained for a total run time of 50 min. The column was connected to a mass spectrometer, and mass spectra were recorded every 0.8 s at 70 eV. Components were identified by their mass spectra, which were compared to those of standards, and were matched by computer Vectra Qs/165. Methylbranched hydrocarbons were identified by their mass spectra [9]. Equivalent chain lengths were determined by using standard n-alkanes (Data Bank), and quantitation was based on peak areas of total ion chromatograms.

In presenting the data we based our analyses on the important proportions of different components in each of the classes' hydrocarbon (n-alkanes, monomethylalkanes, dimethylalkanes and trimethylakanes).

III. RESULTS

A. *Total Hydrocarbons*

The total ion chromatogram for the female and male are presented in Figure 1 and 2 respectively. The displayed chromatograms contain some overloading of major components and represent the analyses used to provide quality mass spectra of level hydrocarbons. Comparison the sets of chromatograms reveals that the females and males contain the same components. However a difference quantity is noted both in sex. The n-alkanes ranged nC_{24} from nC_{36} comprised over 72% and the discontinuous chain of methylalkanes represented in average 16 % of the hydrocarbons extracted from the surface for this species

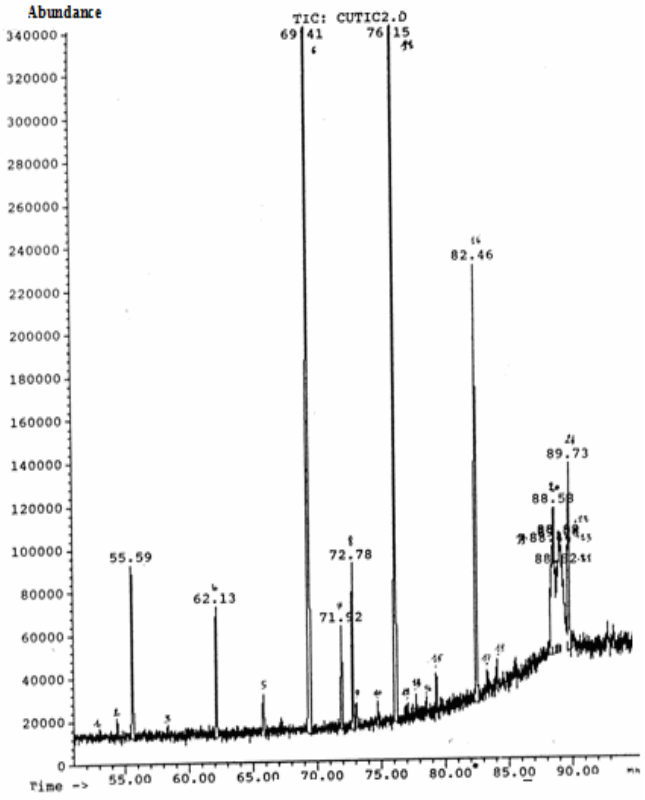

Fig. 1 Chromatogram obtained by GC/MS: cuticular hydrocarbons on male adult stage

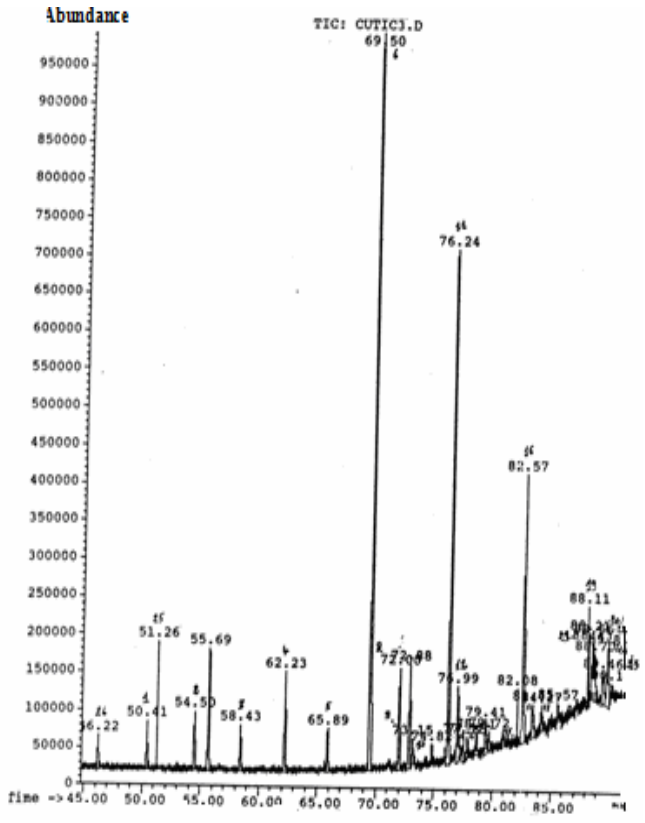

Fig. 2 Chromatogram obtained by GC/MS: cuticular hydrocarbons on female adult stage

TABLE I
CUTICULAR HYDROCARBONS IDENTIFIED ON FEMALE OF *P. ELEPHAS*

Peak n° GC-MS	Time retention (minute)	Peak identification	Carbon number	Relative content %
26	46.22	?	?	?
1	50.41	n-tetracosane	24	1.5
25	51.26	monomethyltetracosane	25	+
2	54.50	n-pentacosabne	25	1.8
3	58.43	n-hexacosane	26	1.4
4	62.23	n-heptacosane	27	3.1
5	65.89	n-octacosane	28	1.3
6	69.50	n-nanocosane	29	26.1
7	71.99	monomethylnanocosane	30	3.5
8	72.00	n-triacontane	30	3.4
9	73.15	dimethylnanocosane	31	0.8
10	74.82	monomethyltriacontane	31	0.5
11	76.24	n-hentriacontane	31	18.4
12	76.99	trimethylnanocosane	32	2.7
13	77.50	dimethyltriacontane	32	0.8
14	77.81	monomethylhentriacontane	32	0.6
15	79.72	n-dotriacontane	32	0.7
16	82.57	n-tritriacontane	33	10.8
17	83.44	n-tetratriacontane	34	1.6
18	84.17	monomethyltetratriacontane	35	1
19	88.11	n-pentatritriacontane	35	2.7
20	88.25	trimethyl tritriacontane	36	0.4
21	88.43	dimethyltetratriacontane	36	0.5
22	88.55	monomethylpentariacontane	36	1.9
23	89.46	n-hexatriacontane	36	1.5
24	89.84	monomethylhexatriacontane	37	3

TABLE II
CUTICULAR HYDROCARBONS IDENTIFIED IN MALE OF *P. ELEPHAS*

Peak n° GC-MS	Time retention (minute)	Peak identification	Carbon number	Relative content %
1	50.42	n-tetracosane	24	+
2	54.50	n-pentacosane	25	+
3	58.42	n-hexacosane	26	+
4	62.13	n-heptacosane	27	2,4
5	65.90	n-octacosane	28	0.07
6	69.41	n-nanocosane	29	34.9
7	71.92	monomethylnonacosane	30	1.9
8	72.78	n-triacontane	30	3.3
9	73.15	dimethylnonacosane	31	+
10	74.82	monomethyltriacontane	31	+
11	76.15	n-hentriacontane	31	23.7
12	77.50	trimethylnonacosane	32	+
13	77.85	dimethyltriacontane	32	+
14	78.85	monomethylhentriacontane	32	+
15	79.72	n-dotriacontane	32	0.07
16	82.46	n-tritriacontane	33	9.3
17	83.43	n-tetratriacontane	34	0.08
18	84.16	monomethyltetratriacontane	35	+
19	88.43	n-pentatritriacontane	35	3.5
20	88.58	trimethyl tritriacontane	36	4.9
21	88.42	dimethyltetratriacontane	36	1.9
22	88.98	monomethylpentariacontane	36	2.9
23	89.06	n-hexatriacontane	36	1.6
24	89.72	monomethylhexatriacontane	37	4.5

B. N-Alkanes

Adults

The compounds n-alkanes identified on male and female of *P. elephas* ranged from C24 to C36. The approximate quantitative amounts and structures can be inferred from Fig. 3 which show that the cuticular hydrocabons quality of female are similar to male, but with some differences within the quantity (Table 1and 2).

The distinguishing characteristics for females and males are presented respectively by peaks 6, 11 and 16 (Fig.1; 2).

The results showed that n-nanocosane (Peak 6) is the major compound (34,9 %).

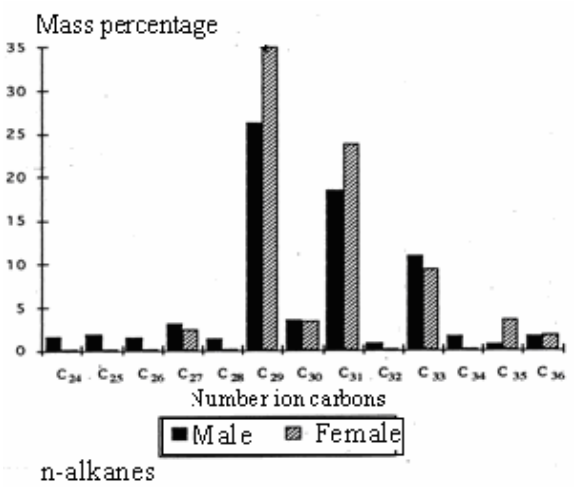

Fig. 3 Cuticular hydrocarbon: continuous chain of n-alkanes from adults of *P. elephas*

Metylalkanes

Females and males showed a discontinuous series.. Generally methylalkanes with odd carbon numbered dominate in both sex. Metylalkanes represented an average of 11% of total of cuticular hydrocarbons.

Monomethylalkanes

Females and males possess the same quality of chain ion carbon (C_{30} to C_{32} and C_{35} to C_{37}) (Fig 4). However, C_{25} was identified only on male cuticular.

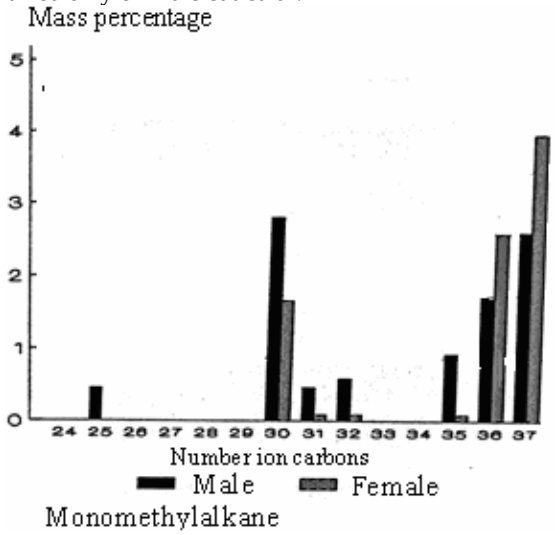

Fig. 4 Cuticular hydrocarbon: discontinuous chain of monomethylakanes from adults of *P. elephas*

Dimethylalkanes

The total quantity of dimethylalkanes was very low and represented respectively on male and female 2,1 % and 2,00% of total of cuticular hydrocarbons. Females and males showed the same quality of chain ion carbon (C_{30} to C_{32} and C_{36}) but the quantity was different (Fig.5).

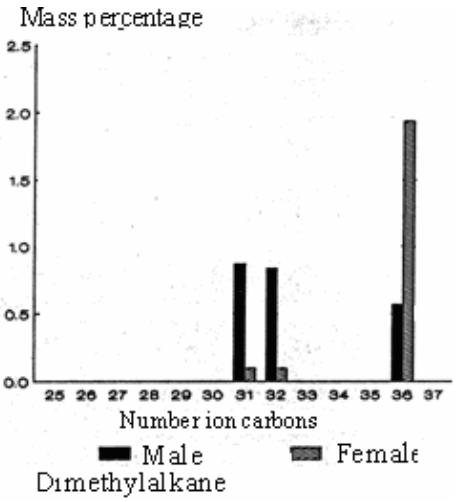

Fig. 5 Cuticular hydrocarbon: discontinuous chain of dimethylalkanes from adults of *P. elephas*

Trimethylalkanes

The total quantity of trimethylalkanes was also very low and represented respectively on male and female 3,1 and 4, % of total of cuticular hydrocarbons. The same quality of chain ion carbon (C_{32} and C_{36}) was identified but the quantity was different in both sex. (Fig.6)

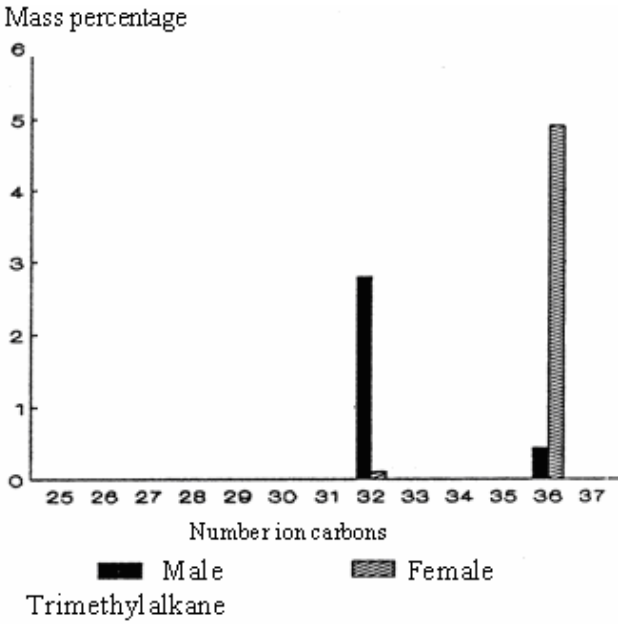

Fig. 6 Cuticular hydrocarbon: discontinuous chain of trimethylalkanes from adults of *P. elephas*

Larval

Chromatograms obtained by CG identified the major cuticular hydrocarbons (n-alkanes) on both sex are represented in figure 7a to l. Larval (2 to7) and adult stages have the same qualitative n-alkane ranged from C_{24} to $C_{36,}$ but larval stage from 1 to 5 showed some differences within the quantity compared to adult stages on both sex. Larval (6 and 7) have the same quality and quantity of adults in both sex. The n-nanocosane (C_{29}) is in very lower abundance and very difficult detectable on the chromatogram.

n-tetracosane was the major components in the larval cuticular hydrocarbons, but the amount of cuticular hydrocarbon present in the larva increased to become the predominant hydrocarbon component in the adults.

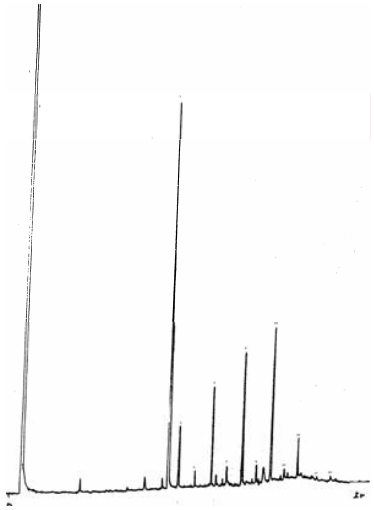

Fig. 7 a Chromatogram obtained by GC: n-alkane on male larvae stage 2

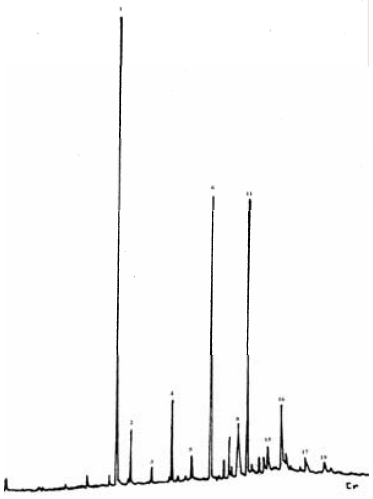

Fig. 7 b Chromatogram obtained by GC: n-alkane on male larvae stage 3

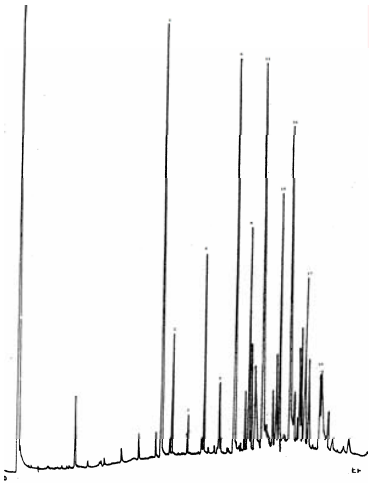

Fig. 7 c Chromatogram obtained by GC: n-alkane on male larvae stage 4

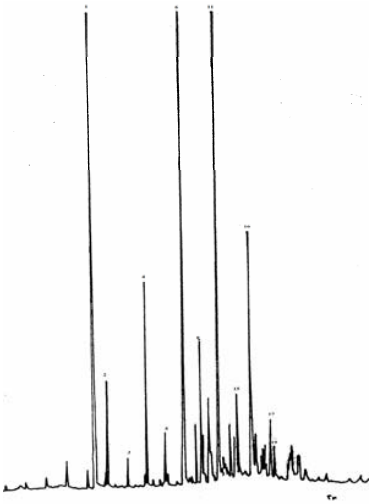

Fig. 7 d Chromatogram obtained by GC: n-alkane on male larvae stage 5

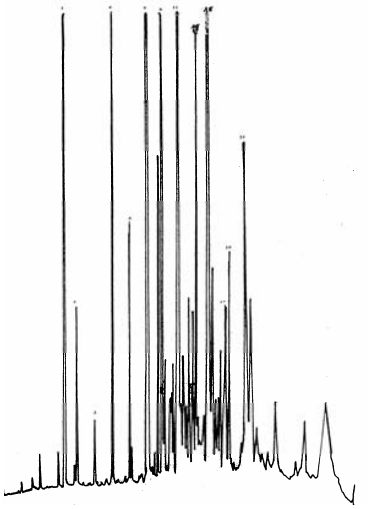

Fig. 7 e Chromatogram obtained by GC: n-alkane on male larvae stage 6

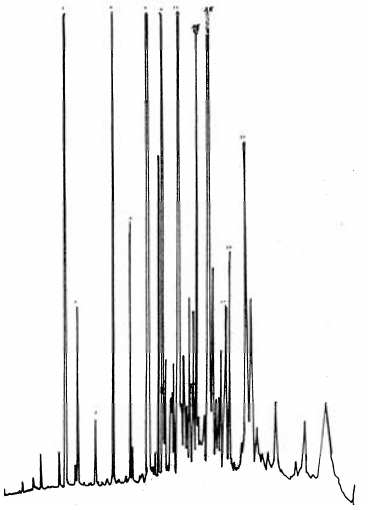

Fig. 7 f Chromatogram obtained by GC: n-alkane on male larvae stage 7

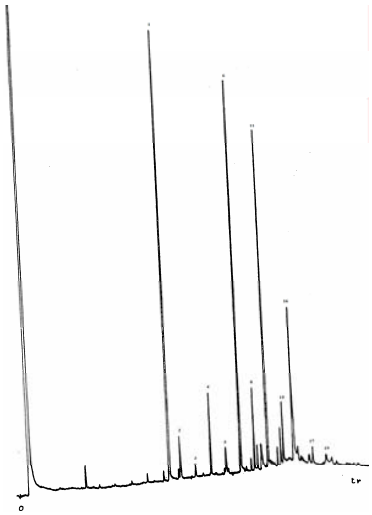

Fig. 7 i Chromatogram obtained by GC: n-alkane on female larvae stage 4

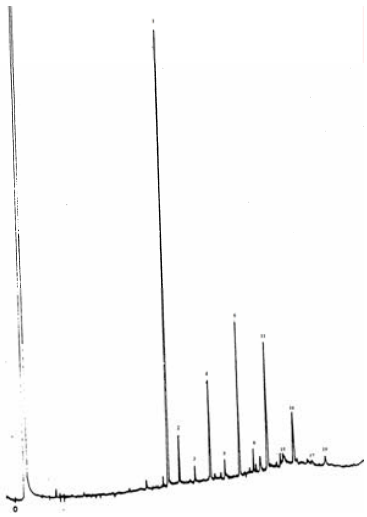

Fig. 7 g Chromatogram obtained by GC: n-alkane on female larvae stage 2

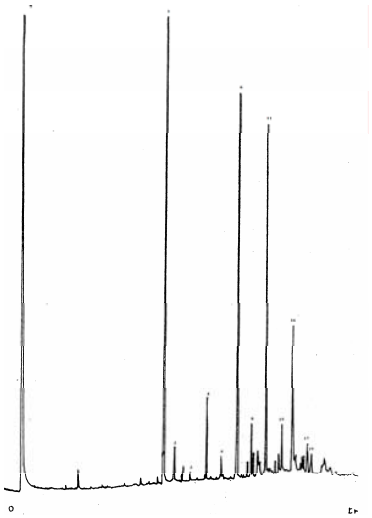

Fig. 7 j Chromatogram obtained by GC: n-alkane on female larvae stage 5

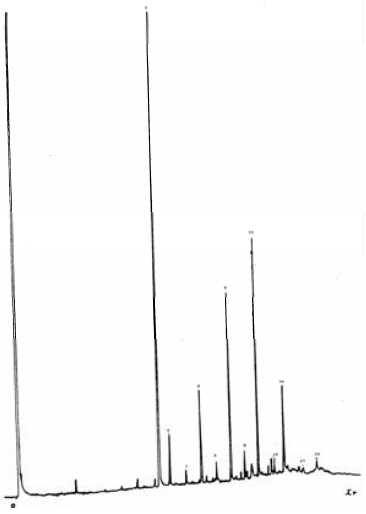

Fig. 7 h Chromatogram obtained by GC: n-alkane on female larvae stage 3

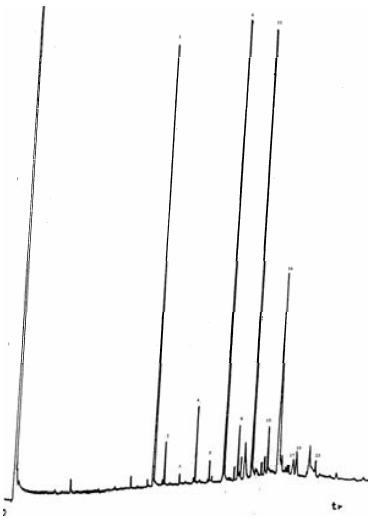

Fig. 7 k Chromatogram obtained by GC: n-alkane on female larvae stage 6

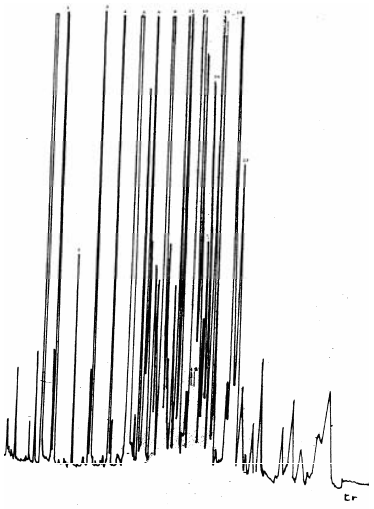

Fig. 7 l Chromatogram obtained by GC: n-alkane on female larvae stage 7

III. DISCUSSION

Using the same extraction technique as that used here, [9] found a few specimens of *Schistocerca americana* (Drury) with more than 60% n-alkanes in the cuticular In *Schistocerca* species, n-nonacosane has nearly always been recorded as the most abundant component with n-henriacontane usually next in abundance [10]. Generally, n-heptacosane was the most abundant compound in many insecta species [11]. In all the grasshopper species examined to date, the predominant n-alkane is nC_{27}, nC_{29} or nC_{31} [10]. However, quantity different existed between males and females. In *P.elephas* individuals on both sex and stages the n-alkane represented in average respectively 75% of the total cuticular hydrocarbons. In our data the most abundant compounds were nC_{29} and $nC_{31.}$ The same results were obtained for *Locusta migratoria cinerascens* and *Locusta migratoria* [12], but for *Locusta migratoria migratoria* and *Schistocerca gregaria* there was the nC_{26} and nC_{27} as the most abundant compounds [13]. However dimethylalkanes are usually the most abundant branched compounds in other species of Acrididae, like *Locusta migratoria migratoriodes* [12], the male individuals of *Schistocerca gregaria* and some species of *Melanoplus* genus [11]. The total quantity of methylalkanes compounds was very low in *Halmenus robustus* and in the *Schistocerca* species [10].

IV. CONCLUSION

Both sexes of *Pamphagus elephas* contain prominent cuticular hydrocarbons with rare methylalkanes. Sexual dimorphism is clearly seen by GC/MS. However the quality of cuticular hydrocarbons is the same on female and male. In contrast of cuticular hydrocarbons from larvae stages to adult stages.

This work may prove most useful in the examination of hydrocarbons from larvae to adult in both sex. Chemotaxonomic characters derived from GC peak areas can also be used to produce a key to examine the other species of Orthoptera.

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