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LARVAL SALIVARY GLAND PROTEINS OF THE NASAL BOTFLY *CEPHALOPINA TITILLATOR* CLARK (DIPTERA - OESTRIDAE) AND THE FLESH- FEEDING FLY *SARCOPHAGA AEGYPTIACA* SALEM (DIPTERA - SARCOPHAGIDAE)

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SUMMARY

The determination of salivary gland total protein content and electrophoretic pattern of the different 2nd . 3rd larval instars and prepupae of *C. titillator* and *S. aegyptiaca* were studied.

Protein content in salivary gland of *C. titillator* decreased sharply from 2nd instar larvae to 3rd one and increased in the prepupae. While in *S. aegyptiaca* protein content in salivary gland increased in 3rd instar than in 2nd instar and prepupae. The total number of protein bands detected in the salivary glands of the different larval instars in both *S. aegyptiaca* and *C. titillator* were 3, 10, 9, 15, 12 and 13 in 2nd , 3rd larval instars and prepupae respectively.

INTRODUCTION

Importance of the nasal botfly *C. titillator* had been reviewed by Mustafa (2002)

Also the flesh fly *S. aegyptiaca* has a considerable medical and veterinary importance due to its role in causing human and animal myiasis (Zumpt, 1965; Baumgartner & Greenberg, 1984 and Colwell&O`connor ,2000).

There is now abundant evidence that in insects as in other animals the salivary glands play a predomenantly digestive role. The saliva secreted by them is expelled from the mouth to moisten the food in which the insect is feeding and the enzymes it contains partially digest the food which is then taken into the mouth in a sime-fliud state . numerous enzymes have been detected in saliva (Wigglesworth, 1965); however, there is also evi-

dence that the saliva of some insects does not contian any enzymes , digestion in these insects being carried out by enzymes originating in the gut. In some insects little is known about the role played by the salivary glands of dipterous insects but surprisingly those of *Drosophila* have been well investigated, (Ashburner, 1970 and Lane et al., 1972).

The aim of this study is to estimate the protein content and electrophoretic variations in the general pattern of salivary gland protein in the 2nd , 3rd and prepupal larval instars in both insects.

MATERIALS AND METHODS

1. Collecting larvae from slaughtered camels:

C. titillator larvae 2nd , 3rd instars and prepupae were collected from camel heads of both sexes, slaughtered at Cairo abattoir. Each head was cut sagittally using a hand saw. The cut started at the nose tip, then through the nasal maxillary and frontal sinuses till the base of the skull including the brain meninges.

The larvae were collected from the pharyngeal region in plastic vials washed in distilled water then examined by stereoscopic binocular (X100) for separating 2nd , 3rd instar larvae and prepupae according to El-Khateeb (1992).

S. aegyptiaca were reared under laboratory conditions of 25 - 30°C and 60 - 70 R.H. Adults were offered water, sugar and fresh meat as a source of protein for ovarian maturation and as an ovipositional site.

The larvae were fed on meat. The flies were reared according to Omar (1974).

2. Sample preparation:

The 2nd , 3rd instar larvae and prepupae of *C. titillator* and *S. aegyptiaca* were dissected individually in Ringer's solution by using fine needles, forceps and a pair of scissors, the integument was pinned on both sides, the fat body, the tracheal system, muscles and the alimentary tract were removed and then the salivary gland were taken off Mustafa (2002) (in separating the alimentary canal). Fifteen salivary glands from 15 larval instars were used in each investigation.

3. Protein estimation:

Quantitative protein analysis were determined using Folin phenol reagent technique according to Lowry et al. (1951). Alkaline protein solution was measured spectrophotometrically at 545 nm using double beam UV 160 Schimadzu spectrophotometer. Bovine albumine was used as standard. The experiment of each analysis was repeated 3 times.

4.Sodium Dodecyl Sulphate Polyacryl-Amide Gel Electrophoresis (SDS-PAGE):

Salivary glands of 2nd , 3rd instar larvae and prepupae of *C. titillator* and *S. aegyptiaca* were analyzed by SDS-PAGE based on the method of

Laemmli (1970). Samples were added to an equal volume of 0.5 mM tris-HCl, pH 6.8, containing 10% (W/V) SDS, 10% glycerol and 1%(W/V) bromophenol blue. All samples were homogenated and centrifuged at 3000 r.p.m. for 5 min. before applying onto the gel. 12% polyacryamide mini-gels (protein II electrophoresis cell, Bio-Rad) were used. Running conditions were 200 v for 45 min. at room temperatur e (27- 30°C).

The gels were calibrated with a broad range molecular weight (mw) marker protein (Bio-Rad), myosin 209, β-galactosidase 120, soybean-trypsin inhibitor 30.2, lysozyme 21.9 and aprotinin 7.4 Kda. Following electrophoresis, polypeptides were visualized using 0.1% Comassie brilliant blue R 250 in fixative (40% methanol and 10% acetic acid) overnight at room temperature, and then destained the following day using several changes of 40% methanol/10% acetic acid.

Densitometer scanner was used in estimating the relative concentration of the identified bands. T-test and ANOVA were used to test the significant difference and to compare the total protein concentration estimated for the different stages of *C. titillator* and *S. aegyptiaca* larvae.

RESULTS AND DISCUSSION

I. Total protein content:

Table (1) shows that total protein content in the salivary gland were markedly higher in 2nd instar larvae and prepupae of *C. titillator* than that in *S. aegyptiaca*. But protein content in the salivary gland of 3rd instar larvae of *C. titillator* was less than that of *S. aegyptiaca*.

Table (1): Total protein content in 2nd, 3rd larval instars and prepupae of *C. titillator* and *S. aegyptiaca*

Larval instars	Protein concentration (gm/L) ± SE and analysis of variance (ANOVA) a, b, c	
	C. titillator	*S.aegyptiaca*
2nd	36.20 ± 0.01 (a)	29.30 ± 0.01 (a)
3rd	17.23 ± 0.006 (b)	30.14 ± 0.06 (a)
Prepupae	24.14 ± 0.006 (c)	21.55 ± 0.006 (b)

Figures followed by unlike letters for each species were significantly different using ANOVA.

In *C. titillator* there was a significant difference between the protein content of salivary gland in the 2nd instar larvae, 3rd instar larvae and prepupae, also there was a significant difference between 2nd instar larvae and 3rd instar larvae (a, b, c). It might be attributed to long life span and environment, physiology and behaviour of this in-

sect which spends almost 10-11 monthes infesting camels. (Tzaprun,1935). There was a significant difference between 2nd instar larvae salivary gland protein content and prepupae of *S. aegyptiaca*. also significant difference was reported between 3rd instar larvae protein content of salivary gland and prepupae (a, a, b). However there was a non - significant difference between 2nd and 3rd instar larvae , it might be due to short life span .

The statistical analysis using T-test showed that protein concentration in the salivary gland of 2nd, 3rd instar larvae and prepupae of *C. titillator* and *S. aegyptiaca* was extremely significant at $p<0.001$ (T-value = 363.16, 186.56, 212.30 and d.f. = 4) respectively.

Results obtained are in agreement with the data obtained by Stephen and Steinhauer (1957); El-Domiaty (1968) and Abdel-Samie (2000) for locusts, where marked quantitative and qualitative differences in protein also exist between the developmental stages of the same species. Also Firling (1977) stated that changes of the protein content in the developmental stages of insects suggest its involvement in metabolism and probably reflect the balance between synthesis, storage transport and degradation of structural and functional protein as well as a response to a particular physiological conditions.

II. Electrophoretic Separation of Proteins:

Figure 1 shows the electrophoretic pattern of *S. aegyptiaca* and *C. titillator* larval salivary gland proteins of 2nd , 3rd instars and prepupae. Lane M is the marker protein used and its molecular weight around 209 and 17.5 kda , lane A, a 2nd , B, b 3rd instar larvae and C, c were the prepupae of *S. aegyptiaca and C. titillator* respectively.

Nine common bands 1, 2, 3, 5, 7, 8, 9, 12 and 13 were detected in *C. itillator* salivary glands of 2rd , 3nd instar larvae and prepupae. This might indicate that these proteins are characteristic to *C. titillator* salivary glands especially band No. 1, 2 and 3 as they possess the highest relative percentages as represented in tables (2, 3 and4). Possibly are enzymes such as amylase,invertase and anti-

Table (1A) : ANOVA of *C. titillator* and *S. aegyptiaca* 2nd , 3rd instar larvae and prepupae

Sample	Source of variance	Degree of freedom	Sum of squares	Meaqn squares	Variance ration (F-test)	Significance
C. titillator	Between	2	553.01	276.51	23042.50	P= 0.01
	Within	6	0.07	0.012		
S. aegyptiaca	Between	2	134.65	67.325	14961.11	P= 0.01
	Within	6	0.27	0.0045		

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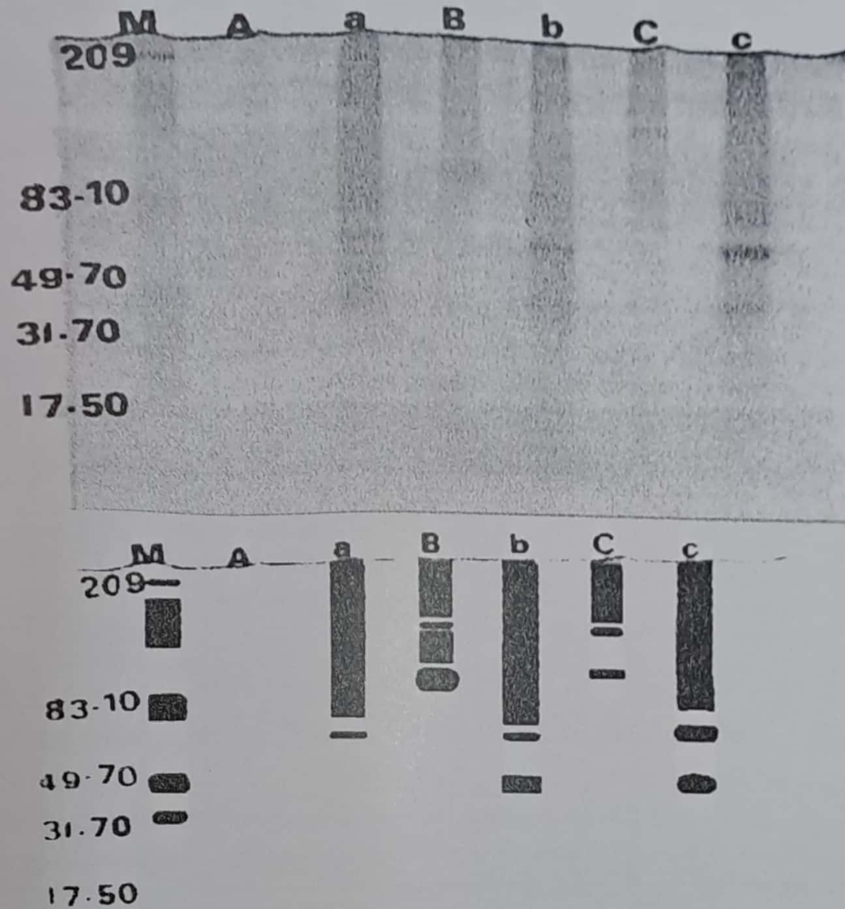

Fig. (1): Electrophoretic separation of salivary gland proteins of 2nd, 3rd instar larvae and prepupae of *S. aegyptiaca* and *C. titillator* lanes A, a, B, b, C, c respectively Lane M. is the marker protein used.

coagulant.

Three major bands 3, 6 and 7 were observed in *S. aegyptiaca* in 2nd, 3rd instar larvae and prepupal stages.

C. titillator have 3 major protein bands 1, 2 and 3 with molecular weights 271, 217 and 172 Kda. respectively. On the other hand, *S. aegyptiaca* have 3 major bands with molecular weights 170.9, 101.23 and 90.186 Kda.

Table (2) indicated that salivary gland of 2nd instar larvae of S. aegyptiaca have 1 band of molecular weight below 209 Kda., 2 bands above

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83.1 Kda. ,also the relative mobilities for the detected bands are shown .

While in the 2nd instar larvae of *C. titillator* 2 bands were separated above 209 Kda., 4 bands above 83.1 Kda., 4 bands below 83.1 Kda. and 5 bands below 49.7 Kda.

Band 6 with molecular weight 101.23 Kda. and relative mobility 0.13 disappeared in 2nd instar larvae of *C. titillator* .

Table (3) indicated that salivary gland of 3rd instar larvae of *S. aegyptiaca* were separated into 2 bands above 209 Kda.. 3 bands above 83.1 Kda., 3 bands below it and 2 bands below 49.7 Kda.

Bands 5 and 10 with relative mobility (Rm) and molecular weight 0.12, 0.27, 103.7 and 57.479 respectively disappeared in 3rd larval instar of *S. aegyptiaca* , and shows that 3rd instar larvae of *C. titillator* have 2 bands above 209 Kda.., 4 bands above 83.1 Kda., 4 bands below 83.1 Kda. and 2 bands below 49.4 Kda.

Table (4) indicated that salivary gland of prepupae of *S. aegyptiaca* was separated into one band above 209 Kda. 4 bands below it, 1 band at 83.1 Kda., 2 bands below 83.1 Kda. and 1 band above 49.7 Kda.

While prepupae of *C. titillator*, indicated that it was separated into 1 band above 209 Kda., 2

Table (2): The relative percentage(in lane), relative mobilities and molecular weights (Kda) of the electrophoretic bands of salivary glands of 2nd larval instar of *Sarcophaga aegyptiaca* and *Cephalopina titillator*

Number of detected bands	%		Rm		Mol.W.	
	S. aegyptiaca	*C. titillator*	*S. aegyptiaca*	*C. titiallator*	*S. aegyptiaca*	*C. titillator*
1		4.51		0.024		268.78
2		3.35		0.044		217.58
3	0.977	2.07	0.067	0.066	170.9	172.63
4		1.83		0.088		136.97
5		1.98		0.11		108.29
6	1.25		0.13		101.23	
7	1.35	1.68	0.15	0.16	90.186	86.363
8		1.86		0.19		75.848
9		1.6		0.21		70.648
10		1.49		0.26		60.266
11		2.59		0.30		51.758
12		3.19		0.36		43.404
13		4.28		0.38		41.339
14		0.903		0.41		37.906
15		1.74		0.44		34.198
16		1.77		0.49		29.863
Total	3	15				
Sum	3.58	34.8				
In lane	100	100				

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Table (3): The relative percentage (in lane), relative mobilities and molecular weights (Kda) of the electrophoretic bands of salivary glands of 3rd larval instar of *Sarcophaga aegyptiaca* and *Cephalopina titillator*

Number of detected bands	%		Rm		Mol.W.	
	S. aegyptiaca	*C. titillator*	*S. aegyptiaca*	*C. titiallator*	*S. aegyptiaca*	*C. titillator*
1	4.73	4.44	0.023	0.027	271.49	260.78
2	2.14	4.31	0.045	0.044	215.4	217.58
3	1.23	1.68	0.067	0.066	170.9	172.63
4	1.96	0.369	0.1	0.1	116.6	116.6
5		1.50		0.12		103.7
6	3.71	1.55	0.13	0.15	97.877	91.498
7	2.25	1.74	0.16	0.17	83.904	81.156
8	0.895	1.54	0.19	0.20	77.403	73.824
9	6.31	1.60	0.23	0.23	66.701	66.701
10				0.27		57.479
11		4.69				
12	0.305	8.56	0.36	0.36	43.404	43.404
13	1.04		0.37	0.38	41.902	41.339
14						
15						
16						
Total	10	12				
Sum	24.6	33.7				
In lane	100	100				

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Table (4): The relative percentage(in lane), relative mobilities and molecular weights (Kda) of the electrophoretic bands of salivary glands of prepupae of *Sarcophaga aegyptiaca and Cephalopina titillator*

Number of detected bands	%		Rm		Mol.W.	
	S. aegyptiaca	*C. titillator*	*S. aegyptiaca*	*C. titiallator*	*S. aegyptiaca*	*C. titillator*
1	7.32	7.33	0.027	0.023	260.78	271.49
2	3.69	5.98	0.058	0.057	188.99	190.91
3	2.25	4.8	0.078	0.074	153	159.28
4						
5	2.19	3.03	0.11	0.12	110.39	105.71
6	7.44		0.14		94.634	
7	1.72	2.31	0.17	0.15	83.1	89.322
8	1.54	1.11	0.18	0.20	78.457	72.833
9	2.68	1.34	0.23	0.23	66.701	66.701
10						
11	1.08	1.00	0.29	0.3	53.178	52.641
12		1.95		0.36		43.404
13		13.5		0.38		41.339
14		0.928		0.41		37.194
15		0.577		0.44		34.291
16		14.3		0.47		31.595
Total	9	13				
Sum	29.9	58.2				
In lane	100	100				

bands below it. 2 bands above 83.1 Kda., 2 bands below it. 1 band above 49.7 Kda. and 5 bands below 49.7 Kda.

Band 6 with Rm 0.14 and molecular weight 94.634 Kda. was disappeared in the prepupal stage of *C. titillators*.

Band 4 appeared in 3rd instar larvae of *C. titillator* and *S. aegyptiaca*, was disappeared in 2nd instar S. aegyptiaca and prepupae of *C. titillator* and *S. aegyptiaca*.

Bands 12, 13, 14, 15 and 16 disappeared from the salivary gland of the prepupae of *S. aegyptiaca*, possibly they are non digestive proteins.

Kinnear et al. (1971) working with *Calliphora stygia* found that electrophoresis of a salivary secretion from glands of 3rd instar larvae separated the protein into 18 bands, some of which were present at the feeding stage only to disappear at the wandering stage and vice versa. Price (1974) stated that in *C. erythrocephala* 3 major bands were found at the feeding stages, and some minor bands were just discernible, while at the wandering stage the fastest running of the three major bands decreased in intensity and the two slower running bands increased.

From this study it appeared that certain bands were characteristic to certain families in each of the two insects under investigation. These individual fractions appear and disappear at various stages in the life cycle independently of each other suggesting that each protein is under independent genetic and hormonal control (Warren and Brelan, 1969). Wigglesworth, (1972), reported that the three lobes of the salivary glands of *Acricotopus* (Chironomidae) produce proteins with different amino acid composition.

Innocenti et al (1995) mentioned that salivary glands of *O.ovis* contribute most ,if not all, of the protein content detectable in larval secretory product by native and denaturing gel electrophoresis. This conclusion is in line with earlier evidence by Skelly and Howells (1987) who showed that the salivary glands of *Lucilia cuprina* maintain a high immunogenic activity even when denatured by SDS.

Eid and Omar (2000) investigated salivary glands of *C.titillator* and *O.ovis* third stage larvae concluded that the salivary glands contain polypeptides which gives it the priority to be an effective vaccine.

Kuo and Patton (1975) succeeded in visualize 49 bands in the different developmental stages of *Schistocerca gregaria* and *Locusta migratoria*.Similar results were obtained by Muse and Balogun (1994) for *Zonocerus variegates*. The authors revealed that a variable number of protein bands was seen during the development of the adult insect with protein bands increasing in

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intensity during development, there was little difference in the protein profiles of young and old adults.Abdel-Samie (2000) reported similar results on the different phases of *S. gregaria* and *L. migratoria*.

It is believed that the structural design of an organism is regulated so as to satisfy the requirements of its functional systems. If this principle (principle of symmorphosis), first proposed by Taylor and Weibel (1981), is fundamentally correct, then any persistent change in the general behaviour of an organism should be accompanied by corresponding adjustments in the structural components associated with that behaviour.

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