Al-Azhar Bulletin of Science

Volume 24 | Issue 2 Article 21

12-1-2013
Section: Botany, Microbiology and Zoology

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How to Cite This Article

EL-SHEHABY, MOHAMED (2013) "BIOCHEMICAL AND GEOGRAPHICAL STUDIES ON POTATO TUBER MOTH, PHTHORIMAEA OPERCULELLA (ZELLER) IN SOME GOVERNORATES OF ARAB REPUBLIC OF EGYPT," *Al-Azhar Bulletin of Science*: Vol. 24: Iss. 2, Article 21.
DOI: https://doi.org/10.21608/absb.2013.6424

Al-Azhar Bull. Sci. Vol. 24, No. 2 (Dec.): pp. 121-132, 2013.

BIOCHEMICAL AND GEOGRAPHICAL STUDIES ON POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* (ZELLER) IN SOME GOVERNORATES OF ARAB REPUBLIC OF EGYPT

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Abstract

Simple proteins, lipids and carbohydrates were examined by acrylamide –gel electrophoresis in the whole body(larva or prepupa ,pupa and adult) of the potato tuber moth *Phthorimaea operculella* in three governorates in the Delta of Egypt . Polymorphic in the whole body of potato tuber moth, variations in simple proteins ,lipoproteins ,lipoglycoproteins according to their mobility on polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue ® ,Sudan black B and Periodic acid Schiff's were detected. Twenty bands were further characterized simple proteins, lipoproteins, glycoproteins, lipoglycoproteins. The different protein concentrations was stage and locations dependent it reached a peak just before the last molt .Sodium dodecyl - sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) resolved the protein into single polypeptide with molecular weight in the three governorates tested in Delta of Egypt ranged between209kDaltons to 19.37 k Daltons

Keywords: *Phthorimaea operculella,*

Introduction:

The potato tuber mot, *Phthorimaea operculella* is one of the primary insect pests of potato crops (Kirkham, 1995). The larvae damage leaves and tubers by their mining causing damage in storage (Raman,1988) and field(Fenemore,1988) *P.operculella is* commonly controlled by various synthetic pesticides and an integrated pest management (IPM) programme. The protein components of insect haemolymph comprise a functionally and structurally heterogeneous array of macromolecules that include vitellogennins, lipoproteins, hormone-binding proteins, storage proteins and enzymes. Detailed information concerning structure, developmental appearances, hormonal regulation of biosynthesis and function is available for certain classes of these proteins and has recently been reviewed (Wyatt and Pan , 1978).In the holometabolous insects the so-called storage proteins are quantitatively the most important class of proteins in larval haemolymph .Typically only two to four physicochemically distinct storage protein species occur (Thomson , 1975), however, they have been shown to comprise as much as 80-90% of total soluble protein on a weight basis .The concentration of storage proteins in a number of insect species reaches a maximum during the final larval instar then declines in the haemolymph during pharate pupal development concurrent with the accumulation of polypeptides with identical electrophoretic (Collins and Downe, 1970).

Herein, the objective of the present investigation was to study biochemical analysis of proteins, lipoprotein, glycoprotein as well as SDS PAGE technique. In

order to the overall aim of the work was to determine the geographical origin of the pest in three different governorates in the Delt of Egypt.

Materials and Methods

Insects collected from three governorates (Dakahlia, Gharbia and Behaira) The strains of the potato tuber moth *P.operculella* were reared in the laboratory as described by Fenemore (1977). maintained at 25 ±1ºC and 12L:12D Photoperiod.

1. Total protein

The total protein concentration (gm/gm body weight) was determined spectrophotometrically using the Biuret method by Josephson and Gyllensward (1975).

2. Polyacrylamide Gel Electrophoresis

The basic principle of protein electrophoresis is the movement of the charged protein molecules through a supporting medium towards an electrode with the opposite charge. After electrophoresis, the protein fractions were visualized by staining with COBB (Coomassie brilliant blue R-250 stain), which is specific for proteins and used 8% concentration of gel,according to the method was described by Laemmli (1970).

3- Protein bound lipids (lipoproteins):

Electrophoresis was done by the same procedure used for separating simple proteins .The gel patterns were stained to reveal lipoproteins with a filtrated solution of SBB (Sudan Black B) for 24 hrs. Excess stain was removed by 7.5% acetic acid until the background became colorless. Areas containing lipoprotein bands stained black.

4. Protein bound carbohydrates (glycoproteins):

Electrophoretic separation of glycoprotein's was accomplished using the same technique for separating simple proteins or lipoproteins according to (Chippendal and Beek 1996).The visualization of glycoprotein fractions was achieved by using Periodic Acid Schiff's (PAS)stain which is specific for carbohydrates.

5- Refractionation of protein bands by SDS-PAGE(LKB Application Note 306,1977):

In a solution of SDS and β-mercaptoethanol, proteins dissociate into subunits (polypeptide chains)in which the diameter of the rods is although to be constant, while the long axis varies in proportion with the molecular weight (MW). The latter value can be determined by comparing the relative electrophoretic mobility of unknown proteins with the mobility of known protein markers.

6-Determination of molecular weights of proteins:

Molecular weights (MW) is a property often used in the identification of organic compounds such as protein. SDS-PAGE had been carried out for the determination

of MW of proteins in the presence of a standard protein marker .We used gel pro documentation for analysis of data.

Results

1-Protein concentration

Illustrated figure (1) represent the total protein concentration of the whole body homogenate of larval, pupal and adults stages collected from Gharbia, Beheira and Dakahlia governorates . The mean total protein concentration of the larval stage of (the whole body tissue) were 0.177,0.165,and 0.153 gm/gm body weight in Gharbia, Beheira and Dakahlia governorates, respectively. There is difference is a significant between three larval stages of the three governorates studied, the total protein concentration of the pupal stages samples collected from the same governorates were 0. 141, 0.159 and 0.141 gm/gm body weight , respectively .The difference between them is non significant and the total protein concentration of the adult stage of potato tuber moth whole body tissue recorded 0.147, 0.147 and 0.144 gm/gm body weight. The differences in the total protein concentration among samples of this stage of potato tuber moth is non significant .

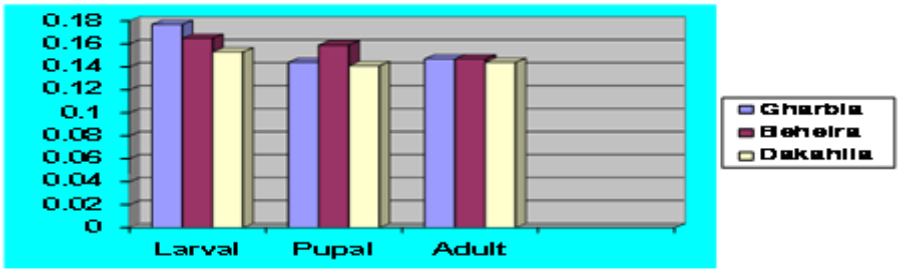

Fig (1) Total protein concentrations (gm/gm body weight of insects) in different stages of potato tuber moth *Phthorimae operculella*.

2-Simple protein patterns:

On using non -denaturing PAGE, the general protein patterns of the whole body tissue were separated into 20 bands according to their relative mobility (Rm) values in the Potato tuber moth samples which were collected from Gharbia, Beheira and Dakahlia. Generally there were no clear differences in different potato tuber moth samples. The total number of protein bands in each larval stage of the Gharbia ,Beheira and Dakahlia were 10 bands, but in both pupal stage in Beheira and Dakahlia were 16 bands, and 17 bands in Gharbia samples on the other hand,the adult stage of the same governorate had 17 bands as shown in Fig (2) and table (2).

3-Lipoprotein:

Protein staining with Sudan B indicated that it was a lipoprotein .It revealed 11bands of lipoprotein (No 1,2,3,4,5,7,9,10,13,18,19 and 20) were present in nine

potato tuber moth with Rm values (0.036,0.087, 0.088,0.11,0.22,0.26,0.34,0.37,0.46, 0.69,0.80 and 0.89) as shown in Fig (3) and table (2)

4- Glycoprotein:

Protein staining with PAS was an indication of glycoprotein .The number of bands of larval stage in three samples of potato tuber moth were 2 bands (No 1and 4) with Rm values (0.036 and 0.11). The common bands of larval stage in three samples of potato tuber moth were 2 bands (No 1and 4) with Rm values (0.036 and 0.11). These bands were characteristics of larval stage of potato tuber moth .as shown in Fig (4) and table (2).

Table (1) : Relative concentration (%) of different non denaturated bands in samples of potato tuber moth from three different governorates

Band no	Rm	Larval stage			Pupal stage			Adult stage		
		Gh	Be	Dak	Gh	Be	Dak	Gh	Be	Dak
1	0.036	10.73	12.98	6.99	5.72	2.81	1.97	4.17	7.49	4.16
2	0.087	25.26	-	-	11.47	11.50	9.4	8.16	10.51	11.72
3	0.088	-	-	-	12.22	7.40	9.89	13.96	14.75	0.19
4	0.11	0.17	11.19	10.60	21.97	16.03	1.14	18.8	4.15	18,13
5	0.22	40.36	12.26	14.82	6.71	5.36	12.0	25.65	25.16	25.62
6	0.23	-	-	-	-	-	-	27.99	28.46	27.15
7	0.26	53.61	53.06	2.67	2.23	44.52	41.50	2794	44.71	37.77
8	0.30	-	-	-	6.72	47.81	3.96	-	-	-
9	0.34	64,94	9.9	18.40	0.46	52.16	2.84	51.87	52.8	52.99
10	0.37	-	-	-	5.07	56.30	2.34	-	-	-
11	0.39	-	-	-	1.34	4.96	7.17	-	-	-
12	0.44	8.90	7.12	8.55	5.45	2.15	3.10	2.12	2.17	2.04
13	0.46	-	-	-	1.78	1.45	1.53	3.60	3.43	2.45
14	0.51	-	-	-	2.33	5.68	5.77	4.65	5.59	6.52
15	0.55	-	-	-	3.39	-	-	1.15	2.08	2.33
16	0.57	6.62	4.58	4.55	1.0	0.96	1.31	2.98	1.72	2.14
17	0.64	4.21	3.43	3.42	3.31	2.76	1.62	3.77	3.29	3.06
18	0.69	-	-	-	-	-	-	4.12	5.50	4.38
19	0.80	-	-	-	-	-	-	22.10	4.36	4.13
20	0.89	14.19	20.72	25.42	25.51	23.33	27.85	28.73	17.52	22.1

Gh: Gharbia Be: Beheira Dak: Dakahlia

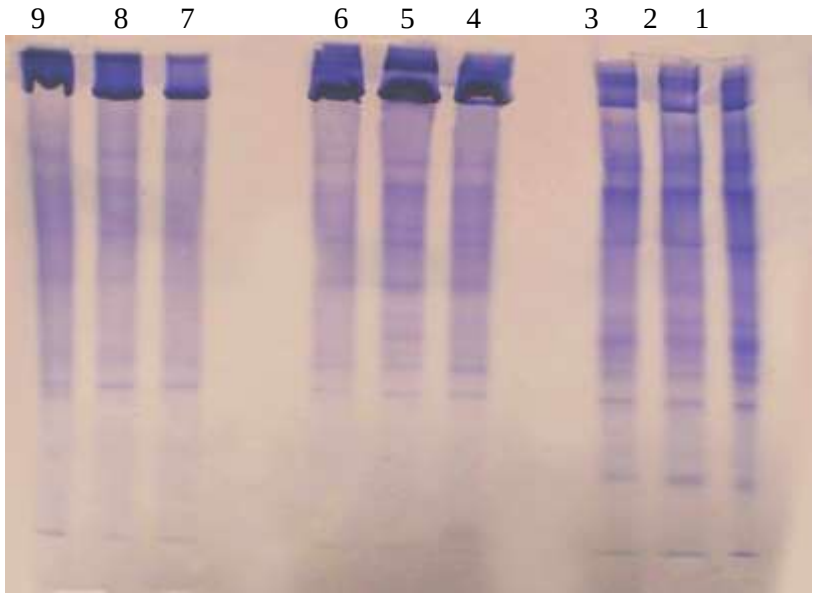

Fig (2) : Polyacrylamide gel of –native protein patterns in the nine samples of potato tuber moth. Lanes 1-3 represent samples of larval stage. Lanes 4-6 represent samples of pupal stage . Lanes 7-9 represent samples of adult stage. Samples collected from Gharbia, Beheira and Dakahlia governorate, respectively .Protein band numbers are indicated on the side of the gel.

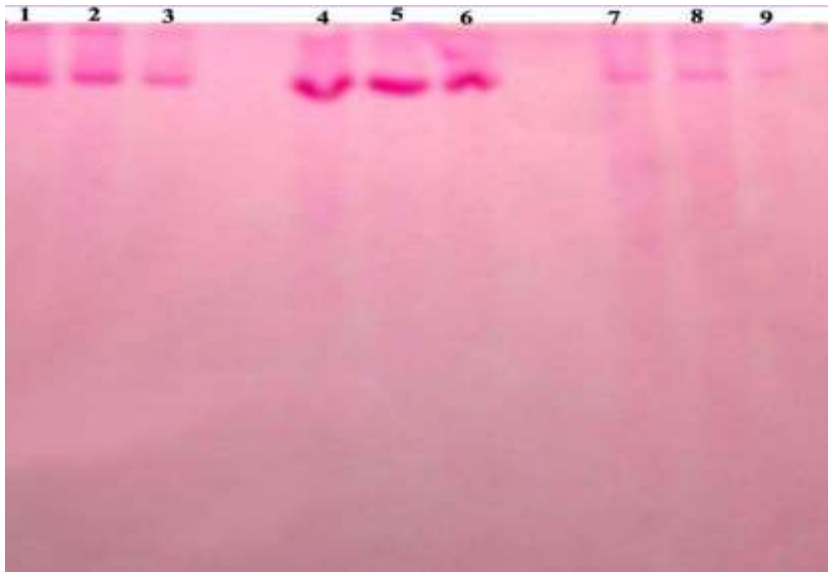

Fig (3) : Polyacrylamide gel of lipoprotein patterns in the nine samples of potato tuber moth. Lanes 1-3 represent samples of larval stage. Lanes 4-6 represent samples of pupal stage .Lanes 7-9 represent samples of adult stage .Samples collected from Gharbia, Beheira and Dakahlia governorate, respectively. Protein band numbers are indicated on the side of the gel.

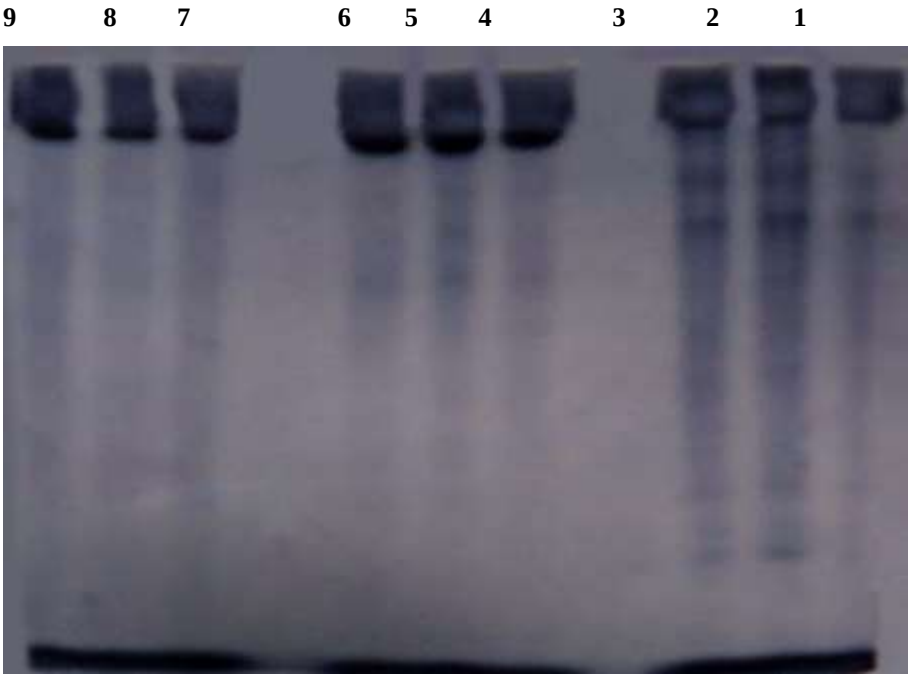

Fig (4) : Polyacrylamide gel of glycoprotein patterns in the nine samples of potato tuber moths. Lanes 1-3 represent samples different instars of larval stage .Lanes 4-6 represent samples of pupal stage .Lanes 7-9 represent samples of adult stage .Samples collected from Gharbia, Beheira and Dakahlia governorate respectively. Protein band numbers are indicated on the side of the gel.

5-Lipoglycoproteins:

Protein stains strongly with both Sudan Black B,PAS and CoBB indicating that it was a lipoglycoprotein. Such protein is conjugated with both carbohydrates and lipids. It revealed 2 bands of lipoglycoprotein (No 1 and 4 bands) were present in three larval stage of Potato tuber moth.

The number of lipoglycoprotein bands of pupal stage in three samples of Potato tuber moth were 2 bands (No 1 and 4) with Rm values (0.036 and 0.11).

The number of lipoglycoprotein bands of adult stage in three samples of potato tuber moth were 2 bands (No 1 and 3) with Rm values (0.036 and 0.088).as shown in table (2) .

6- Refractionation of protein patterns :

SDS –PAGE revealed that the proteins of the whole body tissues of larval ,pupal and adults stages were separated into 29 bands by using COBB stain in the three governorates tested we noticed the bands number 9 and 11 present in all stages but some bands found in larva stage only and some other found pupal stages and adults stages as shown in table in (3) Fig (5).

Table (2) : Different patterns of the protein bands detected in the nine potato tuber moth samples collected from three different governorates:

band no	RM	Larval stage			Pupal stage			Adult stage		
		Gh	Be	Dak	Gh	Be	Dak	Gh	Be	Dak
1	0.036	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein
2	0.087	Protein	"	"	Lipoprotein	Lipoprotein	Lipoprotein	Protein	Protein	Protein
3	0.088	"	"	"	Protein	Protein	Protein	Lipophorprotein	Lipophorprotein	Lipophorprotein
4	0.11	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Lipophorprotein	Protein	Protein	Protein
5	0.22	Protein	Protein	Protein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein
6	0.23	"	"	"	"	"	"	Lipoprotein	Lipoprotein	Lipoprotein
7	0.26	Protein	Protein	Protein	Lipoprotein	Lipoprotein	Lipoprotein	Protein	Protein	Protein
8	0.30	"	"	"	Protein	Protein	Protein	Lipoprotein	"	"
9	0.34	Protein	Protein	Protein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein
10	0.37	"	"	"	Protein	Protein	Protein	"	"	"
11	0.39	"	"	"	Protein	Protein	Protein	"	"	"
12	0.44	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein
13	0.46	"	"	"	Protein	Protein	Protein	Lipoprotein	Lipoprotein	Lipoprotein
14	0.51	"	"	"	Protein	Protein	Protein	Protein	Protein	Protein
15	0.55	"	"	"	Protein	"	"	Protein	Protein	Protein
16	0.57	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein
17	0.64	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein
18	0.69	"	"	"	"	"	"	Lipoprotein	Lipoprotein	Lipoprotein
19	0.80	"	"	"	"	"	"	Lipoprotein	Lipoprotein	Lipoprotein
20	0.89	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein	Lipoprotein

Gh: Gharbia Be: Beheira Dak: Dakahlia

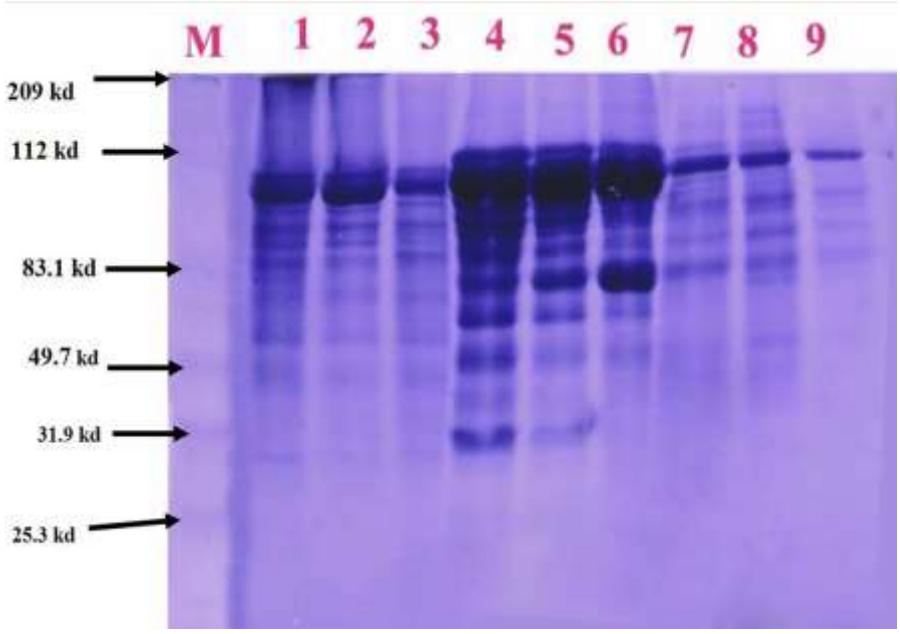

Fig. (5) SDS Polyacrylamide gel of denatured protein patterns in nine samples of potato tuber moth.Lanes 1-3 represent larval stage .Lanes 4-6 represent pupal stage.Lanes 7-9 represent adults stage. Samples collected from Gharbia , Beheira and Dakahlia governorates , respectively .

Discussion

Electrophoresis provides a wonderful biochemical means for identification of type species as careful controlled electrophoretic analysis separate proteins into fractions that have species –specific mobility (Hudson, 1976; Nilima *et al*., 1987; Costa *et al*.,1993 and Gunning *et al* .,1995) .

The protein contents of the tested Potato tuber moth ,*Phthorimaea operculella* populations were high in the larval stage than the pupal and adult stages and difference between concentration of protein in larval stage of three governorates were the small .this result is in agreement with Collins and Downe (1970). In the present work ,the general protein electrophoresis of the whole body tissue were separated into 20 bands .Among these bands some were to certain potato tuber moth *Phthorimaea operculella* samples .These phenomenon was studied extensively among other insects (Hubby,1963 and Mohamed 1996).The tested potato tuber moth *Phthorimaea operculella* samples had 9 common bands (No 1,4,5,7,9,12,16,17,and 20) most of protein profiles of the populations samples showed very similar profile. This suggests that within the limits of sampling ,there is a high degree of genetic similarity between populations throughout the tested patterns which can not be used solely for identification and discrimination between insect pecies, because they are affected greatly with diet and physiological status of individual insect (Silva de Moraes *et al* 1996).The presence of identical proteins in different tissue of insect

Table (3): Rm values and molecular weight of SDS of protein bands detected in nine samples of potato tuber moths different stages collected from Gharbia ,Beheira and Dakahlia governorates

Band No	RM	Mol. W	Larval stage			Pupal stage			Adult stage		
			Gh	Be	Dak	Gh	Be	Dak	Gh	Be	Dak
1	0.022	209	-	+	-	-	-	-	-	-	-
2	0.09	155.48	+	-	-	-	-	+	+	+	-
3	0.12	134.58	-	+	-	+	+	-	-	+	+
4	0.15	112	+	-	+	-	+	+	+	+	+
5	0.18	107.81	-	-	+	+	-	+	+	+	-
6	0.19	105.64	+	+	-	-	+	-	+	+	+
7	0.21	103.33	-	-	+	+	+	-	+	+	+
8	0.22	101.60	+	+	+	-	-	+	+	+	-
9	0.24	98.99	+	+	+	+	+	+	+	+	+
10	0.26	96.53	+	+	-	-	+	-	+	+	+
11	0.28	94.08	+	+	+	+	+	+	+	+	+
12	0.30	90.61	+	+	-	+	+	+	+	-	-
13	0.31	89.45	-	+	+	-	+	-	-	+	-
14	0.33	87.72	+	+	-	-	-	+	+	-	-
15	0.35	84.83	+	+	+	+	+	-	-	+	+
16	0.36	83.10	+	-	+	+	-	+	+	+	+
17	0.39	78.33	+	+	-	+	+	-	-	-	-
18	0.41	74.34	+	+	-	-	+	+	+	+	-
19	0.42	72.76	-	-	+	+	+	+	-	-	-
20	0.48	61.62	-	+	-	+	-	+	-	-	-
21	0.49	60.02	+	+	+	-	-	-	-	+	-
22	0.50	56.65	+	-	+	+	+	+	+	-	-
23	0.54	49.70	+	-	-	+	+	+	-	+	-
24	0.56	47.06	-	+	+	-	-	-	-	-	-
25	0.60	40.14	+	+	+	-	-	-	-	-	-
26	0.63	36.68	-	-	-	+	-	-	-	-	-
27	0.66	31.90	-	-	-	+	+	-	-	-	-
28	0.69	26.62	+	+	+	-	+	-	-	-	-
29	0.74	19.37	-	-	-	+	-	-	-	-	-

Gh: Gharbia Be: Beheira Dak: Dakahlia

Table (4): Relative concentration (%) of different denaturated bands in the nine samples of potato tuber moth from three different governorates

Band	Larval stage			Pupal stage			Adult stage		
No	Gh	Be	Dak	Gh	Be	Dak	Gh	Be	Dak
1	-	1.89	-	-	-	-	-	-	-
2	8.77	-	-	-	-	7.53	5.27	2.5	-
3	-	14.9	-	3.12	3.01	-	-	3.23	3.91
4	12.1	-	7.78	-	0.82	1.1	1.26	1.34	8.8E-04
5	-	-	0.60	0.72	-	7.4E-03	8.4E-03	3.4E-02	-
6	0.17	0.22	-	-	4.2E-02	-	0.87	2.54	5.48
7	-	-	2.4E-02	4.4E-03	1.8E-03	-	2.84	4.46	-
8	3.2E-03	2.1E-02	1.04	-	-	0.23	4.05	4.46	-
9	0.54	0.70	2.55	0.27	0.42	1.43	0.58	5	5.19
10	1.64	1.99	-	-	0.92	-	4.53	3.83	15.6
11	2.38	0.18	0.45	1.21	0.89	4.69	-	0.38	-
12	1.45	1.96	-	1.77	2.85	5.98	0.59	-	-
13	-	0.57	3.85	-	0.13	-	-	3.73	-
14	1.83	2.47	-	-	-	3.4	3.99	-	-
15	0.32	0.63	0.67	2.07	3.19	-	-	8.09	16
16	1.73	-	6.01	2.23	-	9.15	0.59	1.93	69.9
17	4.2	5.18	-	0.47	0.21	-	-	-	-
18	4.92	5.78	-	-	4.79	4.01	13.5	10.5	-
19	-	-	13.3	4.07	5.01	4.31	-	-	-
20	-	13.9	-	0.63	-	7.14	-	-	-
21	7.82	16.1	13.5	-	-	-	-	11	-
22	6.1	-	7.1	6.56	8.59	0.22	18.4	-	-
23	7.42	-	-	2.51	14.8	10.2	-	17.1	-
24	-	9.08	7.42	6.91	-	-	-	-	-
25	5.8	10.3	7.24	8.54	-	-	-	-	-
26	-	-	-	6.81	-	-	-	-	-
27	-	-	-	0.19	1.15	-	-	-	-
28	17.6	15.8	14.4	-	45.3	-	-	-	-
29	-	-	-	45.2	-	-	-	-	-

Gh: Gharbia Be: Beheira Dak: Dakahlia

was previously reported for *Piers rapae* (Kim and Seo,1981) . In the present work ,the general proteins of the whole body tissue of larval stage had more number of protein than the pupal and adult stage. During the metamorphosis of holometabolous insects the last larval stage must prepare itself for the non feeding pupal instar .Such behavior involves the synthesis of relevant amounts of haemolymph proteins during the feeding phase of the last larval instar, and their accumulation in haemolymph. At, or near ,the time of metamorphosis, Some haemolymph proteins are taken up into the fat body and other tissue (Levenbook and Bauer, 1984).In the present investigation ,there were 20 lipoprotein bands present in the three potato tuber moth ,*Phthorimaea operculella* samples.Three of these

lipoproteins (No 1,4,20) were common in all potato tuber moth ,*Phthorimaea operculella*

Haunerland *et al* (1986) analyzing the haemolymph of the silk worm ,*Bombyx mori* found polymorphic variations in three lipoproteins namely, LPs ,L Pm, and LPf according to their mobility on PAGE and staining with Sudan Black B. These three lipoproteins are stage specific because they appear during the development from the late larval stage to the middle stage of the adult .The same possibilities was studied by Hammam (2000) who stated that lipoproteins were useful to distinguish species within six whitefly *Bemsia tabaci* samples .One of these lipoproteins might be characteristic feature of *B tabaci* .

In the present investigation two glycoproteins (No 1 and 4) were detected in the body tissues of all stages of potato tuber moth, they are common bands and may represent characteristic glycoproteins of this insect.

The present study suggests that the change of identity of proteins usually results from the again or loss of both or either of carbohydrates and or lipid protein of the peptide molecule in different metabolic pathways these results are in agreement with Eid *et al*(1979) who reported that some differences in patterns reflected both quantitative changes in the protein of various tissue of *Spodoptera littoralis* (6th instar larva).

In SDS denaturing condition ,the present data showed that proteins dissociate into their subunits (wheeler and Kawooya ,1990 ; Mortinez and wheeler 1991) stated that closely migrating bands sometimes look similar while they are not . However, the present work showed that ,SDS –patterns have no clear differences between the stages of potato tuber moth which were collected from three governorates .They were 7 common bands in larva, 6 common bands in the pupal and 7 common bands in adult stages of potato tuber moth examined . Some bands were characteristics to certain stage of potato tuber moth, we showed some bands in larval stages but disappear in pupal stage and adult. This studied not enough to determine differences between populations in the three governorates but we shall make to fingerprint and sequences

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