Changes of protein patterns of the whitefly adults, *Bemisia tabaci* (Genn.), induced by lambda-cyhalothrin

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Abstract: Changes in protein patterns (Profiles) of parent strain (PS) of whitefly, *Bemisia tabaci* (Gennadius), resulting from susceptible strain (SS) selected with lambda-cyhalothrin under laboratory conditions were studied using SDS-PAGE method. The SDS – PAGE analysis revealed large numbers of peptides ranging between 9-173 KDa. They were arranged in 33 peptide groups in descending ranges of 5 KDa. The peptides of a slow mobility (89 - 173 KDa) were 17 peptides, the peptides of a moderate mobility (29 -88 KDa) were 12 peptide groups and the peptides of a fast mobility (9 -28 KDa) were 4 peptide groups.The protein profiles of generations of parent strain (GPS) of whitefly showed the specific separated peptides, compared with the separated peptides in protein profiles of SS and parent strain (PS) of insect. The dendogram placed the detected peptides of SS, PS and GPS of tested *B. tabaci* adults treated with lambda-cyhalothrin in different clusters that differed in their similarity. The dendogram showed that the detected peptides in protein profiles of GPS of G_2, G_4, G_5, G_{11}, G_{16} and G_{18} formed categories of one cluster with similarity between them. The high similarity level of 0.73 was recorded between the generations of G_{16} and G_{18}. The obtained results indicated that the differences of separated protein peptides of tested *B. tabaci* evidence the genotype variation of whitefly.

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1. Introduction

The whitefly *Bemisia tabaci* (Genn.) [Hemiptera: Aleyrodidae] is one of the most damaging pests of numerous crops worldwide as well as Egypt. The ability of *B. tabaci* to develop resistance to insecticides after only a few applications makes its control problematic in the long run **(Radwan, *et al.*, 2009)**. For the past thirty years *B. tabaci* control in Egypt has been based almost exclusively on conventional insecticides such as organophosphates, pyrethroids and carbamates. Little work quantifying resistance in *B. tabaci* in Egypt has been published. More recent data on insecticide efficacy from field trials on cotton shows that many traditional insecticides are no longer effective and 10 to 12 insecticide applications per season are now common in the cotton crop. Rotating insecticides with different modes of action is one of the most commonly recommended approaches to delay insecticide resistance **(Elbert and Nauen, 2003; El-Kady and Devine, 2003).** Resistance to insecticides can be considered as a major obstacle to effective whitefly control. Changes in protein patterns of treated larvae of whitefly may be play an important role in inducing the resistance **(Gao and Zheng, 1990).**

The sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) method is the most widely used for analysis protein mixture qualitatively or quantitative based on the separation of proteins according to their molecular masses **(Wilson and Walker, 1995)**. In addition, the protein electrophoresis is a powerful, relatively simple and inexpensive **(Millership, 1993)**. The SDS-PAGE of egg extract of the sweet potato whitefly (*B. tabaci*) showed one major band (approximately 190 KDa) and two minor bands (approximately 75 KDa and 67 KDa) (**Tu *et al.*, 1997)**. The PAGE revealed that the total proteins of the three tissues (haemolymph, fast body and gut) of *Earias insulana* last instar larvae were separated into 23 bands **(Zidan *et al.*, 2002)**. **Farghaly (2010)** used the field experiments to detect resistance to organophosphate, carbamate, and pyrethroids insecticides in the field population of whitefly *B. tabaci*. These tests indicated that resistance in four field strains increased gradually to pyrethroids, carbamates and OP insecticides, but resistance to pyrethroids were the strongest. The electrophoretic analysis of protein using SDS-PAGE revealed that there were many differences in protein patterns and the bands differed in intensity and molecular weights between laboratory and field strains. There were 3 protein bands present only in the lab-strain with the following molecular weights 96.5, 89.9 and 50.7 KDa, respectively. On the other hand,

there was one protein of 13.2 KDa detected only in the four field strains.

This work is aimed to study the changes in protein patterns based on the detected peptides using SDS-PAGE technique, when larvae of *B. tabaci* (susceptible strain) were sprayed by lambda-cyhalothrin at $LC_{50,}$ compared to parent and susceptible strains as control.

2. Materials and Methods

Insecticide.

Lambda-cyhalothrin as Karate 2.5% EC **[**A reaction product comprising equal quantities of(*S*)- α-cyano-3-phenoxybenzyl(*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop -1- enyl)-2, 2- dimethyl cyclopropane carboxylate and (*R*)- α-cyano-3-phenoxybenzyl(*Z*)-(1*S*,3*S*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethyl cyclopropane carboxylate *Roth*: (*S*)- α-cyano dimethyl cyclopropane carboxylate and (*R*)- α-cyano-3-phenoxybenzyl 3-phenoxybenzyl(*Z*)-(1*R*)-*cis*-3-(2-chloro-3,3,3 - trifluoropropenyl) -2,2- (*Z*) -(1*S*)-*cis*-3-(2- chloro -3,3,3- trifluoropropenyl)-,2-2- dimethyl cyclopropane carboxylate(1:1), Syngenta. Agro., Switzerland] was used in this study.

Bemisia tabaci

The susceptible strain (SS) of whitefly insect [*Bemisia tabaci* (Genn.)], maintains without exposure to insecticides in the laboratory, was obtained from Pest Rearing Department of the Central Agricultural Pesticides Laboratory. The whitefly insects were confined with potted cotton, placed in an environmental chamber under conditions of 27 ± 2º C, 55 ± 5 % R.H. and a photoperiod of 18: 6 (light: dark).

Development of resistance to the lambda-cyhalothrin.

The parent strain (from susceptible strain) of *B.tabaci* was reared on cotton plants under constant laboratory conditions for produce many generations of insect, till the built up the resistance against lambda-cyhalothrin, as described by **Coudriet *et al.* (1985).** The lambda-cyhalothrin was applied against adult stage of insect at level LC_{50} (producing 50 % mortality of the insect adults) under laboratory conditions. The susceptible strain of *B.tabaci* was bioassayed with lambda-cyhalothrin to measure the initial LC_{50} value (3.51 ppm) as described by **Abbott (1925) and Finney (1971).** Then the susceptible strain was sprayed with insecticide at 3.51 ppm that considered as parent strain for the following generations. Then the resulting parent strain was sprayed again with 3.51 ppm, the LC_{50} value of insecticide was measured to determine the new LC_{50}. After generation of G_4, the insects adult were sprayed with recommended dose of insecticide (12.5 ppm) till the end of experiment (G_{18}). Then, the development resistance in parent strain generations was detected at G_2, G_4, G_6, G_8, G_{10}, G_{12}, G_{14}, G_{16} and G_{18}. After each two generation, the new LC_{50} (ppm) was calculated after statistical analysis and applied against the following generations.

Spray program:

Both susceptible and parent generation were sprayed with lambda-cyhalothrin at LC_{50} of 3.51 ppm. The generations of $G_1$and G_2 were sprayed with lambda-cyhalothrin at LC_{50} of 30.73 ppm. The generations of G_3 and G_4 were sprayed with lambda-cyhalothrin at LC_{50} of 148.54 ppm. The generations for G_5 to G_{18} were sprayed with lambda-cyhalothrin at LC_{50} of 12.50 ppm (every generation).

Sampling:

Samples of *B. tabaci* adults were taken from each generation of parent strain (GPS) that sequence sprayed with insecticide lambda- cyhalothrin, parent strain (PS) and susceptible strain (SS) were obtained by an aspirator. About 50 adult insects as a sample for each generation in an Eppendorf were obtained and kept at - 20°C for protein electrophoretic studies.

Preparation of protein samples:

About 50 adult insects adult from each sample of *B. tabaci* were homogenized in an Eppendorf (with glass trunk) contained 50 µl distilled water. The homogenate was centrifuged at 3000 rpm for 15 minutes at 4° C and then was used in electrophoretic analysis. The total protein content in *B. tabaci* adults sample was determined according to the Scico test (Biuret Method) **[Domas, 1975]**.

SDS –PAGE method

Separation of total cellular proteins of *B. tabaci* adults, treated with Lambda cyhalothrin, on the basis of molecular weight by electrophoresis in denatured polyacrylamide gel was determined using SDS-PAGE method as adopted by **Laemmli (1970).** Vertical slab gel units were used. Protein from *B. tabaci* adults was denatured by heating in the presence of low molecular weight thiol (2-mercaptoethanol) and sodium dodecyl sulphate. Four volumes of the adult protein suspension (20µl) were mixed with one volume of sample buffer 5X (5µl). Then, boiled in a water bath for 2 min and quickly transferred to ice water and kept until loading of the gel.

The denatured gels prepared as separating gel 12% and stacking gel 4 %. The gels were prepared from monomer solution of 30 % acrylamide and 0.8 % bis-acrylamide. Ammonium persulphate and N, N, N, N - tetramethylene - ethylene - diamine (TEMED) were used as initiators for cross - linking and polymerization. By pipette, the separating gel solution into the assembled vertical slab gel unit in the casting mode to a level of 3cm. from the top. The gel was left to polymerize at room temperature. The stacking solution was added to fill the gel caster. Then, the comb was inserted into each gel caster and the gel was

allowed to polymerize at room temperature. This allows the formation of wells.

For loading of samples and running, the comb was removed slowly from the polymerized gel and rinsing each well with the tank buffer using a syringe. Wells were washed three times with the tank buffer. The lower and upper chambers were filled with the tank buffer. Using a loading tips equal amounts of adults proteins (about 20 µl of denatured adult's protein) in each well, Wide Molecular Weight Protein Marker provided by Sigma (U.S.A) in the same gel. The lid was placed on the unit and run in the anode direction for 6 hr at 2 mA per each well.

After electrophoresis was completed, the power supply was turned off, the cell lid was removes, and the inner cooling core was pulled out carefully of the lower chamber and poured of the upper buffer. The gel was removed from the other glass plate and was placed in the staining solution of Commassie blue R-250 with gentle shaking for an hour. It was distained for over night in distaining solution I (50% methanol and 10% acetic acid) and then in distaining solution II (5% methanol and 7% acetic acid) until the gel between the protein bands become colorless.

Protein fingerprint characterization:

The gel was photographed and scanned. The molecular weight and percentage of protein in each band were determined comparing with Sigma protein marker (13 Proteins from 6.5 to 205 KDa). The system of EPSON Scaner GT9600 and the software of PHORETIX 1D Advanced, from the company of Total Lab, England, were used.

Pairwise similarity matrix (Clustering):

The pairwise similarity matrix (Clustering) based on matching co-migrating band positions between pairs of protein profiles of different *B. tabaci* were evaluated using Computer analysis. The DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among *B.tabaci* adults.

3. Results

Results of SDS – PAGE for protein profiles of GPS (G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11}, G_{15}, G_{16} and G_{18}) resulting under treatment of insecticide Lambda-cyhalothrin, PS and SS of *B. tabaci* adults, are presented in Table (1) and Fig (1). The separated proteins of different molecular weights were categorized in 33 groups peptides with the descending ranges of 5 KDa starting with P_1 (Protein no.1) ranging from 169 -173 KDa to P_{33} (Protein no.33) ranging from 9 -13 KDa. The electrophoretic results revealed that the protein profiles of *B. tabaci* adults were distinguished into 9 -17 peptides for GPS, 16 peptides for PS and 18 peptides for SS. The number of protein bands was 14, 11, 15, 14, 16, 9, 14, 17, 9, 14 and 12 in protein profiles in the GPS of G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11}, G_{15}, G_{16} and G_{18}. The molecular weight of peptides in protein profiles of *B. tabaci* adults were in the range of 9 -173 KDa for GPS, 24 -158 KDa for PS and 9 -158 KDa for SS.

Peptides of high molecular weight:

The peptides are of a slow mobility (89 -173 KDa) comprising 17 groups showing differences among *B. tabaci* adults (Table, 1 and Fig., 1). The numbers of high molecular weight peptides were in the range of 2 to 6 peptides in protein profiles of GPS of *B. tabaci*, compared to 5 and 6 peptides in both PS and SS, respectively. The peptides numbers were 3, 4, 5, 4, 6, 3, 4, 6, 2, 4 and 3 bands in the generations of G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11}, G_{15}, G_{16} and G_{18}, respectively. The peptide P_1 (169 -173 KDa) was detected in protein profiles of GPS of G_5, G_6 and G_{11}. The peptide P_2 (164 -168 KDa) was detected in protein profile of GPS of G_{16} only. Results also showed that P_4 (154 -158 KDa) was detected in protein profiles of the generations of G_2, G_3, G_5, G_{11} and G_{16} as well as PS and SS. The peptide P_5 (149 -153 KDa) was detected in the generations of G_3, G_4, G_5, G_7, G_{11}, G_{15}, G_{16} and G_{18} as well as PS. The peptide P_6 (144 -148 KDa) was detected in the generations of G_1, G_2, G_4 and G_5 as well as SS. Results also indicated that the peptide P_7(139 -143 KDa) was detected in protein profiles of PS generations of G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11} and G_{18}, while the peptide P_8 (134 -138 KDa) was detected in protein profiles of the generations of G_4, G_7 and G_{11}.

The peptides P_9 (129 -133 KDa) and P_{10} (124 -128 KDa) were detected in protein profiles of SS only. The SDS-PAGE results revealed that the peptide P_{13} (109 -113 KDa) was detected in protein profiles of the generations of G_2, G_5 and G_{16}. The peptide P_{14} (104 -108 KDa) was detected in protein profiles the GPS of G_1, G_6, G_{11}, G_{15} and G_{18} as well as PS and SS. The peptide P_{15} (99 -103 KDa) was detected in protein profiles of the generations of G_3 and G_7 as well as PS. The peptide P_{16} (94 -98 KDa) was detected in protein profiles of the generation of G_3 as well as PS and SS. Results revealed that the peptides P_4 (159 -163 KDa), P_{11} (119 -123 KDa), P_{12} (114 -118 KDa) and P_{17} (89 – 93 KDa) no detected in protein profiles of the generations of *B. tabaci* adults treated with lambda – cyhalothrin as well as PS and SS (Table,1).

Peptides of moderate molecular weight:

The peptides are of a moderate mobility (29 -88 KDa) comprising of 12 groups showing the differences among the tested *B. tabaci* adults (Table, 1 and Fig., 1). The number of moderate molecular weight peptides were in the range of 6 to 9 peptides in protein profiles of GPS, compared with 10 and 9 bands in PS and SS, where the peptides numbers were 8, 7, 9, 7, 7, 6, 8, 8, 6, 8 and 6 peptides in protein

profiles of GPS of G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11}, G_{15}, G_{16} and G_{18}, respectively. Results showed that the peptide P_{18} (84 -88 KDa) was detected only in protein profiles of the GPS generations of G_5, G_7, G_{11}, G_{16} and G_{18}, while the peptide P_{19} (79 -83 KDa) was detected in protein profiles of the generations of G_1, G_2, G_3, G_4 and G_{15} as well as PS and SS (Table, 1). The peptide P_{20} (74 -78 KDa) was detected in protein profiles of the generations of G_3, G_{11}, G_{16} and G_{18} as well as PS. The peptide P_{21} (69 -73 KDa) was detected in protein profiles of the generations of G_1, G_2, G_3, G_4, G_5 and G_7 as well as PS and SS. The peptide P_{22} (64 -68 KDa) was detected in protein profiles of the generations of G_{11}, G_{15}, G_{16} and G_{18}. The peptide P_{23} (59 -63 KDa) was detected in protein profiles *B. tabaci* adults, except G_{11}, G_{15}, G_{16} and G_{18}. The peptide P_{24} (54 -58 KDa) was detected in protein profiles of the GPS generation of G_6 only as well as PS and SS.

Results also showed that the peptide P_{25} (49 -53 KDa) was detected in protein profiles of the generations of G_1, G_2, G_3, G_7, G_{11}, G_{16} and G_{18} as well as PS and SS. The peptide P_{26} (44 -48 KDa) was detected in protein profiles of the generations of G_1, G_3, G_4, G_5, G_6, G_7, G_{11} and G_{15} as well as PS and SS. Results indicated that the peptides P_{27} (39 -43 KDa) and P_{29}(29 -33 KDa) were detected in protein profiles of all tested *B. tabaci* adults, while the peptide P_{28}(34 -38 KDa) was not detected in protein profiles of the generation of G_{18} only (Table, 1).

Peptides of low molecular weight:

The peptides are of a fast mobility (9 -28 KDa) comprising 4 groups showing differences among the tested *B. tabaci* adults (Table, 1 and Fig, 2). The numbers of low molecular weight peptides were in the range of 1 to 3 peptides in the protein profiles of GPS, compared to 1 and 3 bands in PS and SS, while no low proteins was detected in the profiles of the generations of G_2 and G_6. Results showed that the peptide P_{30} (24 -28 KDa) was detected in protein profiles of the generations of G_1, G_3, G_4, G_5, G_7, G_{11}, G_{15}, G_{16} and G_{18} as well as SS and PS. The peptide P_{31} (19 -23 KDa) was detected in protein profiles of the generations of G_4, G_5, G_{11}, G_{16} and G_{18}. The peptide P_{32} (14 -18 KDa) was detected in protein profiles of the generations of G_1, G_4, G_5, G_7, G_{11}, G_{16} and G_{18} as well as SS. The peptide P_{33} (9 -13 KDa) was detected in protein profiles of the generation of G_1 as well as SS (Table, 1).

Clustering of peptides (Dendrogram):

Clustering of the detected peptides presented in Table (1) and Fig (2) was illustrated in the dendogram in Fig. (2). The dendogram placed the detected peptides of SS, PS and the generations of G_1, G_3 and G_7 in the tested *B. tabaci* adults treated with lambda-cyhalothrin in one cluster (Cluster I) with high similarity of 0.85 between PS and G_3. Clustering SS, PS and the generation of G_3 in one category and G_1 showed the similarity level of 0.63 clustering between SS, PS, G_1, G_3 in one category and G_7 decreased the similarity level to 0.56 (Fig, 2).

The dendogram showed that the detected peptides in protein profiles of GPS of G_2, G_4, G_5, G_{11}, G_{16} and G_{18} formed categories of one cluster (Cluster II) with similarity between them. The high similarity level of 0.73 was recorded between the generations of G_{16} and G_{18}, following by the similarity level of 0.71 between the generations of G_4 and G_5 and similarity level of 0.68 between the generations of G_2 and G_{11}. Clustering G_2 and G_{11} in one category and the generations of G_{16} and G_{18} in another category the similarity level was 0.63. Clustering the generations of G_2, G_{11}, G_{16} and G_{18} in one category and the generations of G_4 and G_5 in another category showed less similarity level of 0.56.

Generations of parent strain

The dendogram placed the detected peptides in protein profiles of the generations of G_6 and G_{15} in one cluster (Cluster III) showed the similarity level of 0.70. Results showed the similarity level between the cluster I and cluster II in one category and cluster III in another category decreased the similarity level to 0.51 (Fig,2).

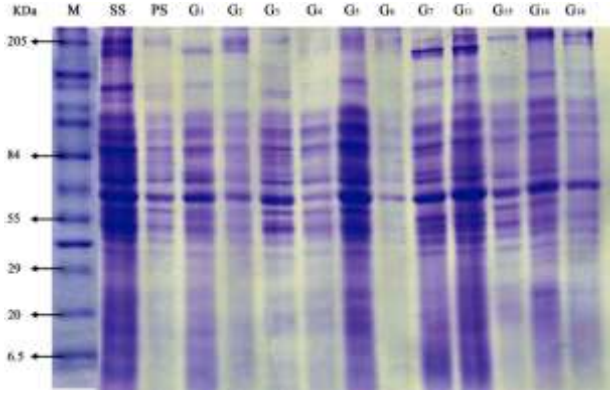

Fig (1): Protein profiles of peptides in the generations of parent strain (GPS) [G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_{11}, G_{15}, G_{16} and G_{18}] of *B. tabaci* treated with insecticide of lambda-cyhalothrin (Karate), compared with parent strain (PS) and susceptible strain (SS), detected by SDS-PAGE.

Table (1): Molecular weight (KDa) and amount (%) of protein peptide in susceptible strain (SS), parent strain (PS) and parent strain generation (G_1-G_{18}) [GPS] of *Bemisia tabaci* adults treated with insecticide of lambda-cyhalothrin (Karate) detected by SDS-PAGE.

Peptide Code	M.W. (KDa)	peptide detected in *Bemisia tabaci* adults ○Amount (%) of protein												
		Susceptible strain (SS)	Parent strain (PS)	Generations of parent strain (GPS)										
				G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_{11}	G_{15}	G_{16}	G_{18}
High molecular weight of peptides														
P_1	169 - 173	-	-	-	-	-	-	2.03	3.16	-	2.02	-	-	-
P_2	164 - 168	-	-	-	-	-	-	-	-	-	-	-	3.73	-
P_3	159 - 163	-	-	-	-	-	-	-	-	-	-	-	-	-
P_4	154 - 158	1.64	5.02	-	4.23	3.07	-	1.93	-	-	2.05	-	4.53	-
P_5	149 - 153	-	3.23	-	-	4.79	1.93	1.47	-	10.42	1.68	7.08	5.39	7.26
P_6	144 - 148	1.57	-	2.20	2.75	-	1.47	0.80	-	-	-	-	-	-
P_7	139 - 143	-	-	2.40	2.43	2.77	0.80	1.82	8.71	5.34	3.43	-	-	2.79
P_8	134 - 138	-	-	-	-	-	1.82	-	-	4.05	3.20	-	-	-
P_9	129 - 133	1.72	-	-	-	-	-	-	-	-	-	-	-	-
P_{10}	124 - 128	1.65	-	-	-	-	-	-	-	-	-	-	-	-
P_{11}	119 - 123	-	-	-	-	-	-	-	-	-	-	-	-	-
P_{12}	114 - 118	-	-	-	-	-	-	-	-	-	-	-	-	-
P_{13}	109 - 113	-	-	-	6.17	-	-	5.33	-	-	-	-	6.81	-
P_{14}	104 - 108	4.86	4.74	3.72	-	-	-	-	12.16	-	5.64	6.37	-	3.03
P_{15}	99 - 103	-	2.91	-	-	4.34	-	-	-	5.88	-	-	-	-
P_{16}	94 - 98	2.82	2.31	-	-	2.15	-	-	-	-	-	-	-	-
P_{17}	89 - 93	-	-	-	-	-	-	-	-	-	-	-	-	-
Moderate molecular weight of peptides														
P_{18}	84 - 88	-	-	-	-	-	-	7.38	-	5.79	6.20	-	9.93	8.48
P_{19}	79 - 83	6.56	7.05	5.70	7.07	6.57	7.68	-	-	-	-	12.60	-	-
P_{20}	74 - 78	-	2.82	-	-	3.27	-	-	-	-	8.84	-	8.41	9.33
P_{21}	69 - 73	7.82	8.56	7.52	9.10	6.39	12.66	8.83	-	9.97	-	-	-	-
P_{22}	64 - 68	-	-	-	-	-	-	-	-	-	6.65	11.17	7.33	6.86
P_{23}	59 - 63	7.56	9.56	8.59	9.90	11.31	14.07	11.21	35.60	10.85	-	-	-	-
P_{24}	54 - 58	5.16	4.58	-	-	-	-	-	3.68	-	-	-	-	-
P_{25}	49 - 53	2.75	3.13	4.58	11.98	4.52	-	-	-	4.88	4.89	-	6.96	5.77
P_{26}	44 - 48	5.27	6.23	5.24	-	7.13	11.58	6.31	8.81	5.42	5.66	10.20	-	-
P_{27}	39 - 43	8.63	12.57	10.68	15.86	14.90	10.32	10.54	11.29	13.04	13.29	17.82	13.40	11.71
P_{28}	34 - 38	4.50	7.32	7.59	9.87	11.33	8.54	5.57	4.82	8.69	5.50	10.51	5.81	-
P_{29}	29 - 33	11.66	15.66	11.07	15.85	15.60	15.38	13.16	4.41	13.16	13.61	18.45	11.78	18.07
Low molecular weight of peptides														
P_{30}	24 - 28	11.98	4.31	4.67	-	4.71	4.69	4.75	-	4.07	3.52	5.80	7.42	12.36
P_{31}	19 - 23	-	-	-	-	-	8.82	2.83	-	-	5.26	-	3.12	3.48
P_{32}	14 - 18	6.25	-	5.62	-	-	6.27	16.04	-	5.81	8.56	-	5.39	7.95
P_{33}	9 - 13	12.47	-	20.42	-	-	-	-	-	-	-	-	-	-

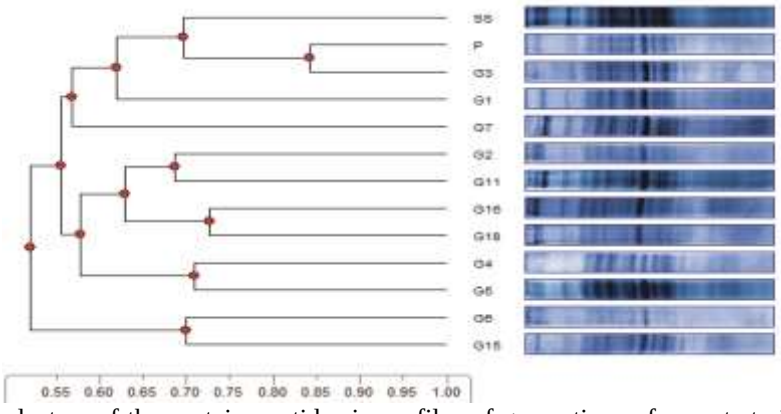

Fig (2): Dendrogram clusters of the protein peptides in profiles of generations of parent strain (GPS) [G_1, G2, G_3, G_4, G_5, G_6, G7, G_{11}, G_{15}, G_{16} and G_{18}], compared with parent strain (PS) and susceptible strain (SS) of *B. tabaci* adults that treated with insecticide of lambda-cyhalothrin (Karate), detected by SDS-PAGE.

4. Discussion

According to biochemical markers were widely used to study genetic variation within insect species, the development of insecticide resistance also was confirmed by electrophoretic (PAGE) studies. Results of electrophoretic analysis revealed that the protein profiles of generations of parent strain (GPS) populations that selected with lambda- cyhalothrin, showing clear differences in separated peptides, compared with protein profiles of both parent strain (PS) and susceptible strain (SS) (**Ying *et al.*, 2007 and Abou-Youssef *et al,* 2010)**. The results showed that the protein profiles of *B. tabaci* were distinguished into 9- 16 peptides for GPS, compared with 16 and 18 peptides in both PS and SS, respectively. Results also revealed that the protein profiles of the populations of GPS at 18^{th} generation were distinguished KDa range into some specific protein peptides i.e. (P_{11}) (P_{24}) (P_{26}) and (P_{37}) where its were not detected in protein profiles of PS and SS.

The peptides are slow mobility (89- 178 KDa) showing the clear differences among the protein profiles of *B. tabaci* populations. Accordingly, the absence or occurrence some peptides in protein profiles could be considered as evidence for appearance of differences caused by lambda-cyhalothrin programs. Our results revealed that the disappearance or occurrence of peptides in protein profiles associated to the quantitative differences in their protein amount (%).The present dendogram study for peptides detected in protein profiles of *B. tabaci*, i.e. GPS, PS and SS, showed the clear differences between them. The similarity between them placed those of *B. tabaci* (PS and SS) in a sole category showing the similarity level (0.63) (0.70) from one side and GPS on the other side, where the similarity was (> 0.65).

Our results are agreement with those recorded by **Omer *et al.* (2005).** They reported that SDS- PAGE analysis revealed large of peptides ranging between 11- 120 KDa, in protein patterns of the greater wax moth larvae, *Galleria mellonella L.,* induced by *Bacillus thuringienis kurstaki*. They were arranged in 22 peptide groups in descending order of 5 KDa. The obtained results are very important because this form of PAGE is the most widely used method for analysis protein mixture qualitatively or quantitative based on the separation of proteins according to their molecular mass **(Wilson and Walker, 1995)**. In addition, protein electrophoresis is a powerful, relatively simple and inexpensive **(Millership, 1993)**. Our results are agreement with those recorded by **Sethi *et al.*, (2002).** They reported that the mode of inheritance of insecticide resistance in *B. tabaci* was found to be controlled by almost completely recessive, more than one gene having additive effects and segregating in the base population under continuous selection.

The obtained results indicated that the differences of separated protein peptides of tested *B. tabaci* evidence the genotype variation of insect; especially the gene is the units of heredity. The sequence of nucleotides in a gene determines the amino acid sequence and hence, the structure of discrete proteins since protein synthesis as gene expression. Insecticide resistance is a consequence of the selection of resistance genes through insecticide applications. Resistance genes may already be present in a population, or may arise through mutation.

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