EFFECT OF SOME PESTICIDES ON THE SARCOPLASMIC PROTEIN FRACTIONATION OF COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

Studying the genotoxic effects of different doses (1/10, 1/5 and 1/2 LC_{50}) of organophosphorus compound (Hinosan) and carbamate (Sevin) for 30 days on the electropherograms of sarcoplasmic proteins of common carp (Cyprinus carpio) with regard to the number, mobility and density of fractions revactions that :

1. Ten percent polyacrylamide gel electrophoresis of proteins extracted from untreated normal specimens yielded 14 bands. These bands were to be : the fastest mobile band, 4 distal bands, 5 mid bands, 3 proximal bands and the least mobile band. Fractions 1 and 4a showed the highest and lowest density percentage of all bands, respectively.
2. Hinosan exposed specimens yielded 13-15 bands. The percentage of appearance (Polymorphism) ranged from 33.3 - 100 %. A new band 8b appeared with 33.3 % polymorphism at 1/10 LC_{50} concentration. In addition, band 4b only appeared in 1/2 LC_{50} dose with 50% polymorphim. On the other hand, at 1/5 and 1/2 LC_{50} band 10 disappeared completely.
3. The differences in number of protein bands were marked in sevin treated specimens. 13-16 fractions were scannered with the three doses studied. At 1/2 LC_{50} dose, fractions 4b and 8b appeared newly. On the other hand, fractions 6 and 10 completely disappeared at both 1/10 and 1/5 LC_{50} doses. Percentage of polymorphism ranged from 33.3-100 %.
4. Comparison of the relative mobilities of bands between control and exposed Hinosan and Sevin specimens showed some amount of variations specially in the proximal fractions.
5. Studying the effects of te different concentrations of Hinosan and Sevin on changes in the relative proportions of protein fractions detected that, band 9 was the most strengthen fractions appeared in the current investigation. However, total distal fractions recorded a significant variation when fish were exposed to both pesticides.

INTRODUCTION

The herbivorous fishes are undoubtedly the most important species of food-fish in the world for aquaculture, yet, they have received far less attention in studies than the carnivorous. In particular, the families Cyprinidae (carps), Cichlidae (tilapias) and Mugilidae (mullets) form the basis of much of the world production (Bardach *et al.* 1972) .

Agricultural waste water and drainage water are always loaded with the residues of chemicals used for pest control and fertilizers. Fish and other aquatic animals living in this water are unable to escape from pesticides once it has reached the water, and have to submit to its environmental hazards until it has been removed by adsorption, sedimentation and degradation or other mechanisms.

Hinosan (Edifenphos) is an organophosphorus fungicide which is extensively used for specific action against rice blast (Worthing and Hance 1991). Also, Carbaryl is an importhnt insecticide commonly sold under the trade name of "Sevin". This compound is used to control a variety of insects and acts as both stomach and contact poison (Neumeyer 1969).

Banding patterns of proteins drown by the electrophoresis techniques are the wide spread metnods helped to formulate a better idea about the physiological and genetic effects and the action of chemicals such as pesticides. Manna (1986 and 1989) used tilapia fish as a model for testing genotoxic agents and studied the changes in mobility, density and number of polyacrylamide gel electrophoretic bands of total proteins. Manna and Mukherjee (1986) and Manna and Sadhukhan (1992) reported the number of protein bands extracted from dorsal trunk muscles of malathion and aldrex treated male and female Oreochromis mossambicus, respectively. They showed some amount of variations in the number, density and mobility of protein bands between and within the two sexes. They concluded also that, the number of bands were affected by the time of exposure. Also, that number of bands were affected by the time of exposure. Also, the genotoxic effect of sodium arsenite on tilapia were studied by Manna and Mukherjee (1989a). Rashed *et al.* (1992) described some variations in the number and location of muscle protein bands of four tilapia species subjected to organophosphoro us insecticide tamaron.

The aim of the present study was to investigate the electrophoretic changes (either polymorphic or densitometric) of different sarcoplasmic protein fractions of common carp (Cyprinus carpio) as a result of exposure to different doses of Hinosan

and Sevin pesticides. The importance of this point is related to the fact that our fish farms are mostly supplied by polluted drainage water with such pesticides.

MATERIALS AND METHODS

Common carp (Cyprinus carpio L.) used in the present study were artificially reproduced in Abbassa Fish Hatchery. Seventy healthy juveniles (40 g) were chosen from the same generation. The experimental fish were transported to the laboratory (Central Laboratory for Aquaculture Research) using transportation track to get acclimatized (10 days). Fish were kept in 100 litre holding glass aquaria which were filled with chlorine-free water, and dissolved oxygen was kept always at saturation level. Water temperature was within 20-25 ^{o}C. They were fed twice/day using artificial fish pellts (25 % protein) with feeding rate 3% of body weight .

The experimental fishes were divided into seven groups, each group comprised 10 fishes. The first group was kept in aquarium containing well aerated chlorine free water. The other six groups were kept in water containing 1/10, 1/5 and 1/2 LC_{50} of both Hinosan (O-ethyl SS-diphenyl phosphodithioate, $C_{14}H_{15}O_2PS_2$) and Sevin (Naphthyl N-Methyl carbamate, $C_{12}H11No_2$) pesticides as determined by Ramadan (1996).

Fish were kept in aquaria for 30 days. The exposure to the tested concentrations of the pesticides in aquarium was repeated daily. The aquarium water was changed daily.

Half gramme of fish muscle was homogenized with 1.5 ml of sample buffer (Laemmli 1970) using a teflon-coated tissue grinder. Samples were shaken, then, centrifuged for 15 minutes at 12, 000 rpm. Supernatants were transferred to Eppindorf tubes. Samples were kept in deep freezer at -20^{o}C until electrophoretic separation.

Vertical tube Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Separating gel (10% acrylamide) and Stacking gel (5% acrylamide), was performed according to the method of Payne (1976). Gels were stained by 0.1 % Amido Black 10 B (in 7% acetic acid) and scanned by a photoelectric densitometer (Helena model 1402) at wave length 525 mm. Both the relative areas percentage (intensity) of each protein fraction and its relative mobility were calculated and

statistically analyzed by microstate software version 2.01.

RESULTS

The genotoxic effect of Hinosan and Sevin on the sarcoplasmic proteins of Cyprinus carpio was tested electrophoretically with regared to the number and density of fractions .

a) Number of fractions :

The 10% polyacrlamide gel electrophoresis of proteins extracted from muscles of normal specimens yielded 14 bands. These bands were identified according to their mobility as : one fastest mobile band, 4 distal bands, 5 mid bands, 3 proximal bands and one least mobile band. In the exposed specimens, another two bands appeared in the mobility areas of band 4 and 8. So, in the treated specimens the number of protein fractions varied considerably and the response was not uniform in the two pesticides .

1. Hinosan exposed specimens :

Number of protein fractions from elecrophoregrams were 15 at 1/10 LC_{50}, 13 at 1/5 LC_{50} and 14 at 1/2 LC_{50}. The percentage of appearance (Polymorphism) were summerized in Table 1. At 1/10 LC_{50}, a new fraction 8b appeared with 33.3% polymorphism. The same polymorphic apperance was detected in both 2 and 10 fraction. Another polymorphism (66.7 %) was shown in fraction 11. At 1/5 and 1/2 LC_{50}, band 10 disappeared completely. On the other hand, band 4_b appeared only in 1/2 LC50 dose with 50% polymorphism. In 1/5 LC_{50} dose, band4_a showed 66.7% polymorphism compared to 100% in conrol group .

2. Sevin exposed specimens :

From Table 4, 13-16 fractions were scannered from the three doses studied. Fraction 4b appeared absolutely at both 1/10 and 1/2 LC_{50} doses. Also, fraction 8b showed 100% appearance at 1/5 LC_{50} dose and polymorphic (33.3 %) at 1/2 LC_{50} dose. On the other hand, at both 1/10 and 1/5 LC_{50} doses, fractions 6 and 10 completely disappeared, while, 6 at 1/2 LC_{50} dose showed 33.3% polymorphism. Fractions 2,3 (1/2 LC_{50}), 4a (1/5 LC_{50}), 7 and 11 (1/10 LC50) demonstrated different percentages of polymorphism .

Table 1. Number and polymorphism of the polyacrylamide gel fractions of the sarcoplasmic proteins in the control and exposed *Cyprinus carpio* with different doses of Hinosan herbicide.

Fraction	Control		Hinosan 1/10 LC50		Hinosan 1/5 LC50		Hinosan 1/2 LC50	
	No. (6)	%	No. (6)	%	No. (6)	%	No. (6)	%
1	6	100	6	33.3	6	100	6	100
2	6	100	2	100	6	100	2	100
3	6	100	6	100	6	66.7	6	100
4a	0	0	6	0	6	0	6	0
4b	6	100	0	100	3	100	0	100
5	6	100	6	100	6	100	6	100
6	6	100	6	100	6	100	6	100
7	6	100	6	100	6	100	6	100
8a	0	0	2	33.3	6	0	2	0
8b	6	100	6	100	0	100	6	100
9	6	100	2	33.3	6	0	2	100
10	6	100	4	66.7	0	100	4	100
11	6	100	6	100	6	100	6	100
12	6	100	6	100	6	100	6	100
13	6	100	6	100	6	100	6	100
14	6	100	6	100	6	100	6	100
Total number	14		15		13		14	

( ) : Number of samples.

Table 2. Relative mobility values of polyacrylamide gel sarcoplasmic protein fractions in the control and exposed *Cyprinus carpio* with different doses of Hinosan herbicide.

Fraction	Control		Hinosan 1/10 LC50		Hinosan 1/5 LC50		Hinosan 1/2 LC50	
	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D
1	100	0.00	100	0.00	100	0.00	100	0.00
Distal fractions :								
2	88.88	4.70	93.21	0.38	90.36	2.11	92.24	2.49
3	84.78	5.95	90.42	2.79	84.36	3.17	87.28	3.05
4a	79.05	6.83	85.91	3.24	79.85	2.61	83.59	2.80
4b							79.37	2.97
5	74.39	7.89	81.58	1.54	78.38	3.80	77.24	4.13
Mid fractions :								
6	67.41	8.02	71.65	2.58	69.37	3.71	67.44	4.63
7	61.62	7.44	65.69	3.57	64.50	3.43	62.65	4.28
8a	55.42	7.79	61.72	2.22	59.00	5.71	56.63	5.71
8b			53.53	0.38				
9	40.66	3.83	50.89****	3.10	50.27**	7.33	49.26***	5.32
10	33.46	7.27	38.86	0.38				
Proximal fractions :								
11	25.54	4.18	30.16	5.33	32.29*	5.44	27.86	8.35
12	20.07	2.29	28.86**	6.86	28.51***	5.30	21.84	8.67
13	13.09	1.74	17.70**	3.56	18.12*	4.76	16.15	6.94
14	6.84	1.00	9.74****	0.92	7.05	0.57	7.16	3.58

( ) : Number of samples. S.D. : Standard deviation.
* : Significant at P < 0.05. * : Significant at P < 0.02.
*** : Significant at P<0.01. *** : Significant at P<0.0 .

Table 3. Percentage of sarcoplasmic protein fractions of *Cyprinus carpio* after exposure to different doses of Hinosan herbicide.

Fraction	Control		Hinosan 1/10 LC50		Hinosan 1/5 LC50		Hinosan 1/2 LC50		P value for F-test
	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	
1	16.471	0.614	16.80	7.859	12.51	1.747	9.88	3.391	N.S.
Distal fractions :									
2	4.80	2.014	5.52	0.014	9.06****	0.707	6.93	1.505	<0.02
3	4.51	0.494	5.33**	0.526	6.25	2.793	5.23	1.388	N.S.
4a	3.85	0.687	5.00	3.061	5.11***	0.010	6.42	3.816	N.S.
4b	-	-	-	-	-	-	3.43	1.739	N.S.
4 total	3.85	0.687	5.00	3.061	5.11***	0.010	8.49***	3.126	N.S.
5	6.35	3.302	13.36****	1.462	14.11****	2.076	15.58***	4.710	< 0.02
Total	19.51	3.336	25.53*	4.281	31.97****	2.777	36.22****	5.286	< 0.02
Mid fractions :									
6	5.54	4.534	5.04	0.601	6.16	0.378	4.80	1.292	N.S.
7	5.87	4.137	2.63	0.596	7.11	3.401	5.78	2.523	N.S.
8a	13.17	6.796	6.63*	2.162	11.21	4.502	6.55*	0.840	N.S.
8b	-	-	2.80	0.007	-	-	-	-	N.S.
8 total	13.17	6.796	7.57	2.050	11.21	4.502	6.55*	0.840	N.S.
9	10.11	7.940	16.64	6.555	16.81	0.887	19.35	9.705	N.S.
10	9.71	3.441	3.50***	0.007	-	-	-	-	N.S.
Total	44.401	6.032	33.04	7.454	41.28	8.413	36.48	5.980	N.S.
Proximal fractions :									
11	4.98	1.106	3.45*	1.212	1.85****	0.602	4.05	2.261	<0.05
12	5.21	1.991	4.93	2.682	1.95***	0.274	3.88	1.534	N.S.
13	4.29	2.789	6.76	4.633	4.20	2.520	5.58	3.020	N.S.
Total	14.48	3.707	13.99	7.838	8.00***	1.643	13.50	4.678	N.S.
14	5.14	1.916	10.63***	2.367	6.25	2.246	3.92	1.108	< 0.02

( ) : Number of samples.
\- : Fraction not detected.
* : Significant at P < 0.05.
*** : Significant at P<0.01.

S.D. : Standard deviation.
N.S. : Not significant.
* : Significant at P < 0.02.
*** : Significant at P<0.01 .

Table 4. Number and polymorphism of the polyacrylamide gel fractions of the sarcoplasmic proteins in the control and exposed *Cyprinus carpio* with different doses of Hinosan herbicide.

Fraction	Control		Sevin 1/10 LC50		Sevin 1/5 LC50		Hinosan 1/2 LC50	
	No. (6)	%	No. (6)	%	No. (6)	%	No. (6)	%
1	6	100	6	100	6	100	6	100
2	6	100	6	100	6	100	3	50
3	6	100	6	100	6	100	5	83.3
4a	0	0	6	100	3	50	6	100
4b	6	100	6	100	0	0	6	100
5	6	100	6	100	6	100	6	100
6	6	100	0	0	0	0	2	33.3
7	6	100	4	66.7	6	100	6	100
8a	0	0	6	100	6	100	6	100
8b	6	100	0	0	6	100	2	33.3
9	6	100	6	100	6	100	6	100
10	6	100	0	0	0	0	6	100
11	6	100	4	66.7	6	100	6	100
12	6	100	6	100	6	100	6	100
13	6	100	6	100	6	100	6	100
14	6	100	6	100	6	100	6	100
Total number	14		13		13		16	

( ) : Number of samples.

Table 5. Relative mobility values of polyacrylamide gel sarcoplasmic protein fractions in the control and exposed *Cyprinus carpio* with different doses of Sevin herbicide.

Fraction	Control		Sevin 1/10 LC50		Sevin 1/5 LC50		Sevin 1/2 LC50	
	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D
1	100	0.00	100	0.00	100	0.00	100	0.00
Distal fractions :								
2	88.88	4.70	91.33	0.89	90.29	1.46	91.91	1.69
3	84.78	5.95	86.99	2.58	84.89	2.21	89.65	0.85
4a	79.05	6.83	84.29	2.18	83.77	0.52	86.12*	0.89
4b			79.77	1.93			81.62	0.96
5	74.39	7.89	76.88	2.50	80.63	0.57	78.77	0.97
Mid fractions :								
6	67.41	8.02						
7	61.62	7.44	65.76	4.78	64.04	2.47	73.07	0.42
8a	55.42	7.79	59.04	4.99	59.18	1.74	69.30*	0.23
8b					54.06	1.30	61.78	0.97
9	40.66	3.83	47.86	7.38	48.93****	0.26	56.06	3.66
10	33.46	7.27					37.87	3.86
Proximal fractions :								
11	25.54	4.18	29.40	11.48	30.67**	0.90	26.76	2.78
12	20.07	2.29	26.19	8.23	25.80****	0.42	21.32	1.87
13	13.09	1.74	13.38	4.46	17.74****	0.07	13.34	1.96
14	6.84	1.00	7.23	1.51	8.86***	0.62	6.68	1.24

( ) : Number of samples. S.D. : Standard deviation.
* : Significant at P < 0.05. * : Significant at P < 0.02.
*** : Significant at P<0.01. *** : Significant at P<0.0 .

Table 6. Percentage of sarcoplasmic protein fractions of Cyprinus carpio after exposure to different doses of Servin herbicide.

Fraction	Control		Servin 1/10 LC50		Servin 1/5 LC50		Servin 1/2 LC50		P value for F-test
	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	Mean (6)	S.D	
1	16.47	10.614	12.89	1.663	14.48	3.073	11.95	2.259	N.S.
Distal fractions:									
2	4.80	2.014	4.46	1.129	10.29*	4.601	3.52	1.938	<0.05
3	4.51	0.494	2.83*	1.454	4.60	1.419	3.42*	0.855	N.S.
4a	3.85	0.687	4.67	1.067	4.00	0.006	4.70	1.113	N.S.
4b	-	-	2.70	0.498	-	-	2.56	0.546	N.S.
4 total	3.85	0.687	7.37****	1.346	4.00	0.006	7.26****	1.577	< 0.01
5	6.35	3.302	14.33****	2.133	22.73****	1.479	11.25**	2.570	< 0.01
Total	19.51	3.336	28.99***	4.057	39.61****	5.309	23.35	3.731	< 0.01
Mid fraction :									
6	5.54	4.534	-	-	-	-	2.70	1.414	N.S.
7	5.87	4.137	6.40	1.963	7.39	2.081	6.97	2.844	N.S.
8a	13.17	6.796	8.63	0.590	4.35***	0.274	5.88*	2.695	N.S.
8b	-	-	-	-	4.75	0.378	4.50	3.536	N.S.
8 total	13.17	6.796	8.63	0.588	9.10	0.652	7.68	1.896	N.S.
9	10.11	7.940	21.86***	2.051	16.74	4.431	14.13	3.906	N.S.
10	9.71	3.441	-	-	-	-	12.33	2.850	N.S.
Total	44.40	16.032	34.75	4.399	33.22	5.861	42.18	6.873	N.S.
Proximal fraction :									
11	4.98	1.106	7.70*	2.250	1.35****	0.055	5.55	1.216	< 0.01
12	5.21	1.991	8.03	5.498	2.90	1.205	7.45	3.476	N.S.
13	4.29	2.789	4.60	2.656	3.25	0.274	5.23	1.775	N.S.
Total	14.48	3.707	18.80	9.244	7.50***	1.534	18.23	3.416	N.S.
14	5.14	1.916	4.57	2.288	5.19	0.988	4.28	2.039	N.S.

( ) : Number of samples.
\- : Fraction not detected.
* : Significant at P < 0.05.
*** : Significant at P<0.01.

S.D. : Standard deviation.
N.S. : Not significant.
* : Significant at P < 0.02.
*** : Significant at P<0.01 .

B) Relative mobility of fractions :

It appeared from the electrophorograms that, the relative mobility of bands of the control group was restricted to limited values. The comparison of the relative mobilities of bands between control and exposed specimens showed some amount of variation (Tables 2 and 5) .

1. Hinosan exposed specimens :

As shown from Table 2, the less mobile sarcoplasmic protein fractions were afftected by the different doses of Hinosan than more mobile ones. The relative mobility of band 9 significantly increased (50.89 ± 3.10, $P<0.001$; 50.27 ± 7.33, $P<0.02$ and 49.26 ± 5.32, $P< 0.01$ for 1/10, 1/5 and 1/2 LC_{50}, respectively) compared to the control one (40.66 ± 3.83). There were also significant increase values due to the mobility of resolved bands 11 (1/5 LC_{50}), 12 and 13 (1/10 and 1/5 LC_{50}) and 14 (1/10 LC_{50}) .

2. Sevin exposed specimens :

From Table 5, the response of the relative mobility of sarfcoplasmic protein fractions was not uniform in the three doses of Sevin. There was no significant changes at 1/10 LC_{50} dose. At 1/5 LC_{50}, the less mobile fractions (9,11, 12, 13 and 14) were significantly increased in mobility compared to te control group. At 1/2 LC_{50}, distal (4a) and mid (7 and 9) fractions were significantly increased compared to the control group .

c) Density of fractions :

The densitometric studies of the control group revealed that, fractions 1 and 4a showed the highest (16.47 ± 10.614%) and lowest (3.85 ± 0.687%) percentage of all bands. In treated groups, the different bands were also variable densitometrically within the same gel, and between corresponding bands of different gels (Tables 3 and 6 and Figures 1 and 2).

1. Hinosan exposed specimens :

From Table 3, one can easily deduce that, band 9 showed the highest density fraction among the three doses studied. Also, at 1/10 LC_{50}, fraction 1 recorded the same highest density. On the other hand, the lowest density bands were detected in fractions 7 at 1/10 LC_{50}, 11 and 12 at 1/5 LC_{50} and 12 at 1/2 LC_{50} .

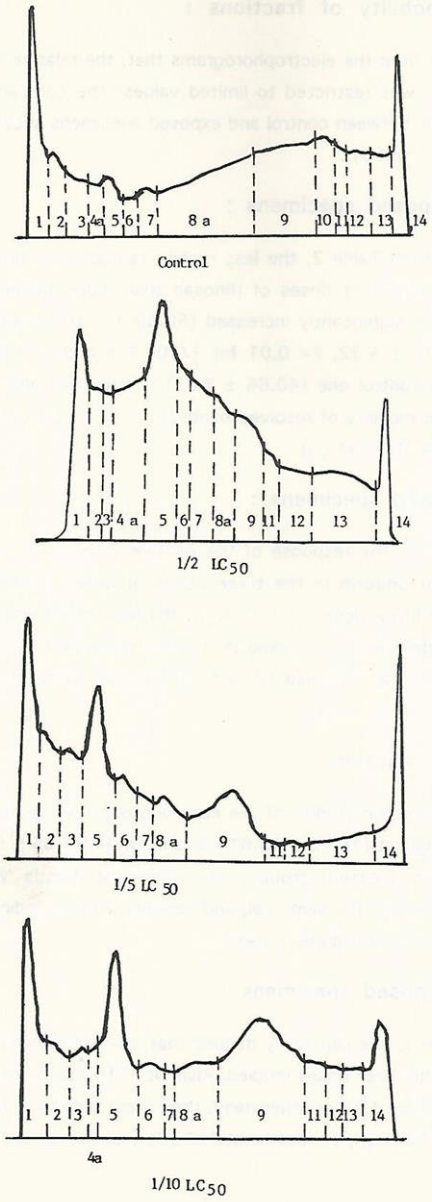

Fig.1. Scanning and calculation curve of sarcoplasmic protein fractions of *Cyprinus carpo* after exposure to Hinosan.

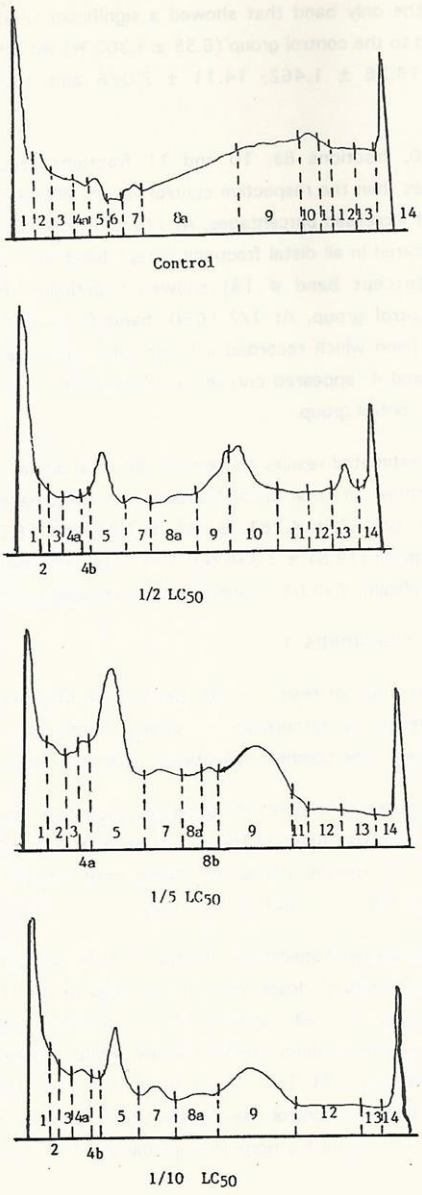

Fig. 2. Scanning and calculation curve of sarcoplasmic protein fractions of *Cyprinus carpo* after exposure to sevin .

Fraction 5 is the only band that showed a significant proprtional increased percentage compared to the control group (6.35 ± 3.302 %) with the 1/10, 1/5 and 1/2 LC50 doses (13.36 ± 1.462, 14.11 ± 2.076 and 15.58 ± 4.710 %, respectively).

At 1/10 LC50, fractions 8a, 10 and 11 fractions recorded significant decreased percentages than the respective control values, Whereas, fractions 3 and 14 showed significant increased percentages. At 1/5 LC50, the significant increased values were demonstrated in all distal fractions except band # 3. On other hand, all proximal fractions (except band # 13) showed significant decreased values compared to the control group. At 1/2 LC50, band 8a is the only detectable significant ($P<0.05$) band which recorded a lower value compared to the control group. The sum of band 4- appeared only in this dose-gives a significant ($P<0.01$) higher value than the control group.

The significant estimated results concerning the total distal, mid and proximal fractions were only shown in total distal fractions which increased proportionally with increased doses (25.53 ± 4.281, 31.97 ± 2.77 and 36.22 ± 5.286 %) compared to control group (19.51 ± 3.336 %). Also, total proximal fractions at 1/5 LC_{50} calculated a significant ($P<0.01$) reduced value compared to control value.

2. Sevin exposed specimens :

Table 6 contains a list of results of the percentage of protein fractions and their accumlative values in the sarcoplasm of *Cyprinus carpio* exposed to different doses of Sevin insecticide. The scanner presentation is given in Figure 2.

As in Hinosan exposed specimens, the greatest percentage band at both 1/10 LC_{50} and 1/2 LC_{50} doses was found in fraction 9, while, at 1/5 LC_{50} dose, it was detected in fraction 5. The smallest percentage bands were noted in fractions 3 and 4_b at 1/10 LC_{50}, 11 at 1/5 LC_{50} and 6 at 1/2 LC_{50}.

Similar to Hinosan exposed specimens, fraction 9 is the only band that showed a significant elevated values in all doses studied compared to the control group. At 1/10 LC_{50}, total fraction 4, had bands 9 and 11 recorded significant higher percentages than the corresponding control values, while, fraction 3 showed a significant lower percentage. At 1/5 LC_{50}, fraction 2 indicated a significant ($P<0.05$) higher value than the control one. On the other hand, as demonstrated in Hinosan exposed fish, all proximal fractions (except band # 13), in addition to band

8_a, showed significant decreased values compared to control group. At 1/2 LC_{50}, fractions 3 and 8a recorded significant ($P<0.05$) lower values than the control ones. The total of bands 4 gave a significant ($P<0.001$) higher value than te control group.

Total distal fractions showed statistically significant higher values than the control group in both 1/10 ($P<0.01$) and 1/5 ($P<0.001$) LC_{50} doses, whereas, total proximal fractions showed reduced value ($P<0.01$) only at 1/5 LC_{50}.

The effect of different doses of each pesticide on the sarcoplasmic protein fractions are statistically calculated as "F-test" and summarized in Tables 3 and 6. The remarkable point is that, the same significance appeared in both pesticides with slight difference in its degree. Fractions 4 total and 14 showed exception from this observation. The former band significantly affected with dose ($P<0.01$) in case of Sevin only and the opposite was demonstrated in the fraction 14 ($P<0.02$).

DISCUSSION

Since the first extensive and comparative work on myogen proteins of fish (Connell 1953), several electrophoretic studies have been carried out by different workers (Thompson 1960, Cowie 1968, Whitmore 1986, Basaglia and Marchetti 1990, 1991, Basaglia 1992, El-Saied 1993 and Macaranas *et al.* 1995) in finding out the genetic variability and classification of fish; knowledge in this regard is still fragmentary .

The 10% gel electrophoresis results in the resolution of 13-16 fractions with different polymorphic characters mainly in the three zones (Tables 1 and 4). Kirpichnikov (1981) resoluted 15-20 fractions from the fish muscle proteins. Many of them are monomorphic and species-specific within a species or a population (Huntsman 1970 and Mckenzie 1973), but, in certain fish species the variation in one or two zones of muscle proteins could be observed (Grag and Mckenzie 1970 and Utter and Hodgins 1971). Also, marked ontogentic changes with gradual transitions in muscle protein patterns, as well as, a marked increase in the number of protein patterns, as well as, a marked increase in the number of bands were detected by Nyman (1967) when Atlantic salmon (*Salmo salar*) reached sexual maturity. Herzberg and Pasteur (1974) mentioned that, juvenile Mugilidae differ from the adults of their species in the fast moving region by addition or strengthening of a band.

When studying effects of the different concentrations of Hinosan and Sevin in water on changes in the relative proportions of protein fractions, a fluctuation in all bands under study was registered. The most strength bands appeared in the current investigation were 1 (control); 5 (1/5 LC_{50} Sevin) and 9 (other doses in both compounds). Fractions 3 (1/10 LC_{50} Sevin), 4a (control); 6 (1/2 LC_{50} Sevin), 7 (1/10 LC_{50} Hinosan), 11 (1/5 LC_{50} Hinosan and sevin) and 12 (1/2 LC_{50} Hinosan) showed the least density bands in the groups studied (Tables 3 and 6).

On the basis of the changes observed, disturbances in the important functions of myogen proteins and, consequently, in the physiological processes in the carp fingerling exposed to the different concentrations of Hinosan and Sevin in water may be expected. The fastest fraction (band # 1) having important and carrier functions, participates in the osmoregulation of blood, and is a protein reserve of the organism (Ipatov and Lukjanenko 1982). Also, it is considered to be an important criterion of the state of health and condition of fish (Rehulka 1989). Our data showed that, fraction 1 insignificantly decreased when fish were exposed to both pesticides (Table 3 and 6). Low concentrations of this fraction were found in diseased and starved individuals. On the other hand, an intensive supply of food resulted in high levles of this fraction (Rehulka 1989). Arunachalam *et al.* (1980) found that a 27-day exposure of freshwater catfish *Mystus vittatus* to sublethal concentration of Sevin led to a decrease in feeding rate and growth rate. The reduced growth and conversion efficiency may be due to the expenditure of more energy for the purpose of maintenance. Therefore, this insecticide is considered to be a metabolic stressor. Also, the effect of four concentrations of Sevin on the rate of uptake of two sugars, and an amino acid by the intestine of the freshwater snake-head fish (*Channa punctatus*) has been investigated by Sastry and Siddiqui (1985). All four concentrations of the pesticide decreased the rate of transport of the three nutrients. No direct relationship could be established between the concentration of Sevin in the medium and decrease in the rate of transportation of nutrients, although, increase in the concentration of Sevin reduced the absorption of nutrients by the intestine to a greater extent. Khiliare and Wagh (1989) and Palanichamy *et al.* (1989) reported in their studies on different pesticides that, Sevin (carbamide group) is less toxic to fish *Puntius stigma* and *Mystus vittatus* than organochlorine and organophosphorous compounds. This conclusion was supported by the minimum reduction of muscle free amino acids and protein content and minimum increase in muscle acid and alkaline protease activity when exposed to Sevin as compared to others.

A significant increase in the total distal fractions in both pollutants was measured in the present work (Tables 3 and 6). Different responses of the total proximal fractions to the two studied compounds were recorded in the present investigation. The percentages at 1/5 LC_{50} of both Hinosan and Sevin significantly ($P<0.01$) decreased, whereas, non-significant decrease and increase were observed in the other doses of Hinosan and Sevin, respectively (Tables 3 and 6).

Ramalingam (1982) and Rashed *et al.* (1992) reported that the changes detected in the muscle protein electrophoresis of Tilapia species after exposure to organophosphoous insecticides might be a result of the pesticide stress, and some unexpressed genes might have come to be expressed to contradict such effect. On the other hand, Manna and Mukherjee (1986), Badr and Humar (1987),Manna and Sadhukhan (1991) and Rashed *et al.* (1992) showed that time of exposure and concentration of compounds had enhanced the protein content .

The genotoxic potentiality of each of the compounds studied was supported by the present study on the muscle proteins with respect to their number and density as compared to control. The precise mechanism of induction of genotoxic effects for the treatment of the two compounds has not been resolved. Manna and Mukherjee (1989b) discussed that, malathion, mercuric chloride and sodium arsenite produced palpable effects, possibly due to the disturbances in the nucleo-protein moiety of chromosomes. Since protein synthesis is controlled by DNA, the marked variation in protein bands-the functional aspect of genetic material-for the treatment of malathion, mercuric chloride, aldrex 30 (organochlorine insecticide) and sodium arsenite to *Oreochromis mossambicus* would very likely imply that these chemicals had affected the RNA which impaired genetic function and led to the variation in bands revealed by the gel electrophoretic study (Manna and Mukherjee 1986 and 1989a,b and Manna and Sadhukhan 1992). However, the biochemical mechanisms for the induction of genotoxic effects, as usually forwarded by many workers seemed not tenable for the general principle. Therefore, it was suspected by Manna (1975) that some physico-chemical stress could be responsible for the induction of chromosome aberration .

The study of protein band by gel electrophoresis indicated at least indirectly the genotoxic changes electrophoresis indicated at least indirectly the genotoxic changes caused by Hinosan and Sevin compounds. It should be noted that, while the results of the present study could be used to provide information about the genetic variation within and between the two pesticides, the biochemical markers cannot be

expected to give any information in culture.

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تأثير بعض المبيدات على أجزاء بروتين اللحم فى سمكة المبروك العادى

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أظهرت دراسة التاثيرات السمية الوراثية لتركيزات مختلفة (١ / ١٠ ، ١ / ٥ ، ١ / ٢ من الجرعة النصف مميته) لكل من المركب الفوسفورى (هينوزان) والمركب الكربامیتى (سينفين) لمدة شهر على صورة اجزاء بروتين اللحم لسمكة المبروك العادى من خلال التاثير على عدد وحركة وكثافة الاجزاء المختلفة النتائج الآتية :

١) اظهر الفصل الكهربائى الجيلاتينى لاجزاء البروتين فى العينات الغير معاملة وجود ١٤ جزء تمثل كل من : الجزء الأول السريع فى الحركه – ٤ أجزاء تتحرك لمسافة بعيده – ٥ أجزاء تتحرك لمسافة متوسطه – ٣ أجزاء تتحرك لمسافه قريبه بالأضافه الى جزء واحد بطى جدا فى الحركه. ولقد وجد ان الجزئين الأول و ٤ أ هما الاكثر والاقل كثافة على التوالى بين الاجزاء المفصولة.

٢) تتراوح عدد اجزاء البروتين فى المعاملة بالتركيزات الثلاثة للهينوزان بين ١٣ – ١٥ جزء بنسبة ظهور ما بين ٣٣,٣ – ١٠٠ ٪. كما ظهر جزء جديد (٨ب) بنسبة ظهور ٣٣,٣ ٪ فى تركيز ١ / ١٠ فقط. هذا بالاضافة الى ان جزء ٤ ب ظهر فقط فى تركيز ال ١ ٢/ بنسبة ظهور قدرها ٥٠٪. اما فى المعاملة بتركيزى ١ / ٥ و ١ / ٢ فقد اختفى الجزء العاشر كاملا.

٣) بالنسبة للمعاملة بالمبيد الكربامیتى سيفين . كان التغير فى عدد الاجزاء البروتينية واضحا . فلقد بلغ عدد الاجزاء فى التركيزات الثلاثة ما بين ١٣ – ١٦ جزء. حيث ظهر جزآن جديدان (٤ ب و ٨ ب) فى التركيز ١ / ٢. بينما اختفى الجزآن السادس والعاشر كلية فى كل من التركيزين ١ / ١٠ و ١ / ٥ . هذا وقد تراوحت درجة ظهور الاجزاء المختلفة للبروتينات ما بين ٣٣,٣ ، ١٠٠ ٪.

٤) أوضحت مقارنة الحركه النسبيه للأجزاء المختلفه فى عينات المجموعه الضابطه وعينات المجموعه المعرضه للمبيدين تحت الدراسة بعض التغير خاصة فى الأجزاء ذات الحركه لمساقه قريبه.

٥) اوضحت دراسة تأثير التركيزات المختلفة لكل من الهينوزان والسيفين على النسبة المئوية لكثافة اجزاء البروتين المختلفة ان الجزء التاسع هو اكثر الاجزاء كثافة. هذا بالاضافة الى ان مجموع الأجزاء المتحركه لمسافه بعيده فقط فى كل من المبيدين قد اختلفت اختلافا معنويا فى التركيزات الثلاث المستخدمة.