

DETERMINATION OF SOME PESTICIDE RESIDUES EXTRACTED FROM WATERMELON AND THEIR EFFECTS ON ALBINO RATS ENZYME ACTIVITIES

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ABSTRACT

Pesticide residues of (malathion, pirimiphos methyl, chlorpyrifos methyl, carbosulfan and methomyl) were extracted from watermelon (seeds, peel and plup). These residues were determined and their effects on the enzyme activities of albino rats were measured. Results revealed that Plasma ChE activity was significantly decreased in all treated rats groups with all pesticides tested and their residues in all experimental period. Carbosulfan and chlorpyrifos methyl exhibited the most effective compounds in the ChE activity. The inhibition percent were 23.5 % and 17.33 % respectively .Plasma AST activity was decreased after 48 hours of treatment with the organophosphorus pesticides tested while with the carbamate compounds it was increased. On the other hand, these activities were increased after 21 days of treatment with malathion and all carbamate compounds tested compared with that of control. Plasma ALT activity was decreased after 48 hours from treatment with malathion, and chlorpyrifos methyl. Contrarily, ALT activity was increased after 21 days of treatment with malathion, pirimiphos methyl, carbosulfan and methomyl compared with that of control. Plasma alkaline phosphatase activity was significantly increased with all tested pesticides and their residues in all experimental period compared with that of control. The maximum percent of increasing was found with the carbamate pesticide, carbosulfan. Total protein concentration in plasma was decreased with all tested pesticides and their residues after 48 hours from treatments and the maximum percent of decreasing was found with malathion (70.49 %) . Also, total protein concentration was increased with all tested pesticides and their residues after 21 days from treatments compared with that of control. Albumin concentration in plasma was increased in all experimental period with malathion, pirimiphos methyl, carbosulfan and methomyl compared with that of control. In contrary, albumin concentration was decreased with chlorpyrifos methyl pesticide in all experimental period. Plasma cholesterol concentration was also decreased with malathion, pirimiphos methyl, carbosulfan and methomyl. The maximum percent of decreasing was occurred with carbosulfan (56.57 %). Only chlorpyrifos methyl insecticide showed an increasing the cholesterol concentration compared with that of control.

INTRODUCTION

The use of the synthetic pesticides has rapidly increased during the last few years because of their fast action and prolonged protection against different pests . However, this increasing has begun to receive much attention because residues in food commodities may be hazardous to human health and created pollution problem to the environment . Hence, this study has interested to appear the pesticides pollution sides and their hazards on human health especially with a daily commodity food like watermelon plant (fruits and seeds) .So, this study was directed to focus the following points: Determination of some pesticide residues such as, the organophosphorus

pesticides (malathion, pirimiphos methyl, chlorpyrifos methyl) and carbamate pesticides (carbosulfan and methomyl) in watermelon (peel, pulp and seeds) at different intervals after treatments in field. The side effects of the tested pesticides and their residues on some enzyme activities (cholinesterase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase). Also, to determine total protein, albumin and cholesterol in white rats to appear their effects on human and animal health were also studied.

MATERIALS AND METHODS

Insecticides use:

Malathion: Malathion, E.C. 57% (S-1,2-bis (ethoxy carbonyl) ethyl O,O-dimethyl phosphorodithioate) Recommended rate of application: 1.5 l/feddan (watermelon crop).

Pirimiphos-methyl: (Actellic), E.C. 50%. (0-2-diethylamino-6-methylpyrimidin-4-yl 0,0-dimethyl phosphorothioate., Recommended rate of application: 1.5 L/feddan (watermelon).

Chlorpyrifos-methyl: Reldan, E.C. 50%. (0,0-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate.) Recommended rate of application: 1L/feddan (watermelon).

Carbosulfan: Marshal, W.P. 25%. (2,3-dihydro -2,2-dimethylbenzofuran -7-yl (dibutylaminothio) = methyl carbamate.). Recommended rate of application: 0.8 kg/feddan (watermelon).

Methomyl: Lannate, WP. 90%. (S-methyl N-(methylcarbamoyloxy) thioacetimidate Recommended rate of application: 0.3 kg/feddan (watermelon).

Rearing of animal

A pure strain of healthy white albino male and female rats were purchased from Medicine Faculty, Tanta Univ., and reared under the laboratory conditions of $25 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH. The rats were housed in metallic cages $45 \times 35 \times 20$ cm, fed daily with balanced ratio consisted of bread, lettuce, snake cucumber and soaked wheat in water. Male rats of 3-4 months old with the mean average weight of 180-200 gm were used in the current study.

Treatments of white rats:

Five groups of male rats (3 rats / group) equal in old age and weight as described above were used in the present experiment for each pesticides. Different pesticides concentrations for this experiment were prepared by dissolving the desired concentrations in watermelon seeds oil. The first group was given a single oral dose of the pesticides concentrations that exist in the watermelon seeds using special stomach tube, the second group was given a single oral dose of the medium concentration that exist in the peel and pulp of watermelon, the third group was given a single oral dose of $1/20$ LD₅₀ (These concentrations were 20.87, 20.50, 30.0, 2.50, and 0.24

mg/200gm for malathion, pirimiphos methyl, chlorpyrifos methyl carbosulfan and methomyl as described in The Pesticides Manual. 1994), the fourth group was given a single oral dose of watermelon oil which was used as control with that of the fifth group (untreated animals). The rats were returned to their cages and supplied with food and water.

1- Chemical analysis

1-1. Field pesticides treatments:

Watermelon *Citrullus lanatus* var. *colocynthoides* was planted on April, 27th 1997, under the normal field conditions and agricultural practice at Sedi Salem center, Kafr El-Sheikh Governorate. The crop was planted in six plots 175 m² area for each. The crop was sprayed with the recommended rates of each insecticide reported by the Ministry of agriculture and land reclamation recommendation (1996). A plot for each insecticide was treated with one of the tested insecticides. The northern plot was left as control. The insecticidal formulations were diluted with water (200 litre/feddan) and applied on July 22th 1997 (87 days after planting).

1-2. Sampling:

Three fruits of watermelon, with the mean weight of 1200 gm were collected randomly from each insecticide treatments at intervals of one hour after application (zero time), 1, 3 and 5 days. Clean new polyethylene bags were used for preservation of the collected samples. The samples were stored at -20°C in a deep-freezer until time of the analysis.

1-3. Extraction and clean up procedures:

Different methods of extraction and clean up were used for peel, pulp and watermelon seeds that (according to Bayoumi *et al.*, 2003) depending on the chemical structure of the tested pesticides.

1-4. Pesticides determination

1-4-1. Recoveries percentage

Rate of recoveries of the insecticides on peel, pulp and seeds of watermelon were determined by adding known amounts (100 ug a.i.) of each insecticide to portions (50 gm) of untreated samples from peel and pulp, (40 ug a.i.) of each insecticide to portions (20 gm) of untreated samples from seeds. The average recovery values of each insecticide in each sample were used to correct all obtained values of each insecticide residues. These percentage of recoveries were ranged between (78.65 to 114.12)

1-4-2. Determination of pesticides residue by GLC

Shimadzu chromatographic GC-4CM equipped with flame photometric detector (FPD) with phosphorus filter was used to determine malathion, pirimiphos-methyl and chlorpyrifos-methyl. The calibrated conditions of the gas chromatographic were carefully checked before injecting any sample. (Bayoumi *et al.*, 2003)

1-4-3. Colorimetric methods:

Carbosulfan insecticide was determined according to the method of Rangaswamy *et al.* (1976) While methomyl pesticide was determined according to the method of Meagher *et al.* (1967).

2- Biochemical determination:

The activity of cholinesterase (ChE) was determined according to the method of Ellman *et al.* (1961).

Transaminases activity were determined by kits. The colorimetric method according to Reitman and Frankel (1957)

Alkaline phosphatase activity was determined according to kind and king (1954) as modified by Belfield and Goldberg (1971) by kits.

Total protein was Determined by kits. The method carried out by Gornall *et al.* (1949).

Cholesterol concentration was determined by the cholesterol kits Watson (1960).

RESULTS AND DISCUSSION

Table (1) show the mean extracted concentration of the different pesticides used (malathion, pirimiphos methyl, chlorpyrifos methyl, carbosulfan and methomyl).

These concentrations were used to obvious the hazards of these insecticides residues on human health. White rats were used in these experiments to focus their effects by using the change in the enzymes activities as indicators .

Table (1): Mean pesticides residues(ppm) extracted from watermelon parts(peel, pulpand seeds) determined using GLC and spectrophotometric method

Pesticides samples	Malathion	Pirimifos methyl	Chlorpyrifos methyl	Carbosulfane	methomyl
seeds	6.50	2.08	3.68	2.50	0.24
Peel and pulp	16.88	6.33	5.00	10.60	1.13
1/20 LD ₅₀	20.87	20.5	30.00	24.70	2.68

As described of the results obtained before by Bayoumi *et al.* (2003) it could be concluded that, at the end of the experimental period chlorpyrifos methyl showed the minimum residue concentration in watermelon peel and pulp, however methomyl showed the highest residue concentration in watermelon peel. On the other hand, carbosulfan showed the highest residue concentration in watermelon pulp. The minimum concentration of pesticide residues in watermelon seeds was showed with pirimiphos methyl, while the highest residue concentration was showed with the carbamate insecticide carbosulfan.

1- Biochemical determination

1-1. Diagnostic use of enzymes

Human plasma contains a remarkable range of enzymes which serve no obvious function there; they are presumably derived either from leakage from living cells or from the debris of dead or dying cells. Several diseases are accompanied by marked changes in the levels of certain enzymes in the plasma. For many years alkaline phosphatase activity in the plasma has been measured in the investigation of liver and bone diseases. Other plasma enzymes now estimated for diagnostic purposes include: amino transferases (transaminases), total protein, albumin, cholinesterase and cholesterol in liver diseases.(Harper *et al.*, 1979).Some correlation between the changes in plasma enzyme activity and liver damage has shown to exist after treatment with insecticides (Krample. 1970 ; Grice *et al.*, 1971 ;Korsurd *et al.*, 1972 ;Abbassy *et al.*, 1988 and Bayoumi *et al.*, 1995-a, 1997). So, this part of study was directed to reveal the relationship between the tested pesticides and their effect combined with the watermelon oil on some important enzymes activities and some biochemical parameters which affect on the liver functions and other important organs in the body. Hence, the activities of cholinesterase, transaminases (AST&ALT), alkaline phosphatase and the concentrations of total protein, albumin and cholesterol were determined in treated white rats plasma.

1-2. Effects on cholinesterase activity

This enzyme has been measured in plasma in a number of disease states. In general, low levels are found in patients ill with liver disease, malnutrition, chronic debilitating and anemias. The effects of a single oral doses of tested pesticides and their residues extracted from different parts of watermelon on plasma ChE activity in treated male rats were summarized in Table (2). The results showed that, plasma ChE activity was significantly decreased with all tested pesticides after 48 hours from treatment and maximum percent of decreasing was occurred with carbosulfan and chlorpyrifos methyl 23.5 % and 17.33 % respectively .The enzymatic activity of ChE were recovered to approximately the initial values after 21 days from administration. Through the duration of the study, mortality was occurred with two of the pesticides concentrations which represent the peel and pulp medium of watermelon, and watermelon seeds in carbosulfan and methomyl. These results are in agreement with those findings by Gladenko *et al.* (1984); Saleh. (1990) Akay *et al.* (1992); Fayez and kilgore (1992); Kumar *et al.* (1993); Bayoumi *et al.* (1995-b); Tag El-Din *et al.* (1996); Bayoumi *et al.* (1997) and Abd-Allah. (1998).Who concluded that, ChE activity was significantly decreased after exposure to organophosphorus or carbamate insecticides in white rats.

1-3. Effects on transaminases activity (AST & ALT):-

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group of aspartic acid to α -ketoglutaric acid, forming glutamic and oxaloacetic acids. Alanine aminotransferase (ALT) transfers the amino group of alanine to α -ketoglutaric acid, forming glutamic and pyruvic acids.

Table(2): Effect of a single oral doses of tested pesticides on plasma ChE^{''} of treated white rats after 48 hours and 21 days of treatments.

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days
6.50	0.969 ±0.037	1.377 ±0.016	2.08	1.546 ±0.028	1.780 ±0.055	3.68	1.586 ±0.045	1.790 ±0.055	2.50	0.604 ±0.004	0.544 ±0.018	0.24	1.255 ±0.022	1.127 ±0.037
16.88	0.870 ±0.043	1.223 ±0.061	6.33	1.194 ±0.043	1.530 ±0.260	5.00	1.457 ±0.051	1.660 ±0.021	10.64	0.489 ±0.021	-----	1.13	0.992 ±0.023	-----
20.87	0.840 ±0.04	1.044 ±0.106	20.5	0.636 ±0.009	1.040 ±0.118	30.0	0.293 ±0.007	0.744 ±0.023	24.73	0.323 ±0.017	-----	2.68	0.849 ±0.061	-----
Control (oil)	1.022 ±0.132	1.591 ±0.085	Control oil	1.022 ±0.132	1.591 ±0.085	Control Oil	1.022 ±0.132	1.591 ±0.085	Control oil	1.022 ±0.132	1.591 ±0.085	Control oil	1.022 ±0.132	1.591 ±0.085
Control (water)	1.550 ◊ ±0.028	1.609 ±0.005	Control water	1.690 ±0.020	1.850 ±0.025	Control water	1.690 ±0.020	1.850 ±0.025	Control water.	1.374 ±0.013	1.620 ±0.068	Control water.	1.374 ±0.013	1.620 ±0.068

* All concentrations were prepared by dissolving in watermelon seeds oil .

** activity = u moles ASCh / min. / mg protein.

◊ Mean ± S.D.

Serum transaminase levels in normal subjects are low, but after extensive tissue destruction these enzymes are liberated into the serum (Harper *et al.* 1979). Transaminases are important and critical enzymes in the biological processes. They play a role in amino acids metabolism and biosynthesis. Consequently they are considered as specific indicators of liver damage. ALT is more specific than AST in this respect (Wilkinson. 1970). Also, (Hayes. 1989) reported that, the activity of hepatic enzymes (e. g. AST, ALT and alkaline phosphatase) released into the blood by the damaged liver is one of the most useful tools in the study of hepato-toxicity. Plasma AST and ALT activities of male rats given a single oral doses of the tested pesticides and their residues extracted from different parts of watermelon fluctuated between increasing and decreasing. Results recorded in Tables (3, 4) showed that, plasma AST activity was decreased with malathion, pirimiphos methyl and chlorpyrifos methyl after 48 hours from treatment and the maximum percent of decreasing was occurred with pirimiphos methyl 5.48 % . On the other hand, AST activity was increased with malathion (21 days after treatment), carbosulfan and methomyl, and maximum percent of increasing was occurred with malathion 233.3 % . Also, ALT activity was decreased with malathion (after 48 hours from treatment) and chlorpyrifos methyl, and maximum percent of decreasing was occurred with malathion 23.82 % . while, ALT activity was increased with malathion (after 21days from treatment), pirimiphos methyl, carbosulfan and methomyl, and maximum percent of increasing was occurred with pirimiphos- methyl 262.98 % .The aforementioned results were in agreement with those of many authors, Kiran *et al.* (1988); Saleh. (1990); El-Harrawie *et al.* (1991); Hanafy *et al.* (1991); Mostafa *et al.* (1992) and Tag El-Din *et al.* (1996). Who reported that, transaminases activity was increased after exposure to organophosphorus or carbamate insecticides in white rats. In contrary of these investigations, other investigators observed decreasing in levels of transaminases activity after exposure to organophosphorus or carbamate insecticides in white rats, Akay *et al.* (1990); Pawlowska *et al.* (1991); Zidan *et al.* (1991) and Bayoumi *et al.* (1995-a).

1-4. Effects on alkaline phosphatase

Results in Table (5) showed that, alkaline phosphatase activity was significantly increased in the treated male rats for all tested pesticides and their residues extracted from different parts of watermelon, and maximum percent of increasing was happened with carbosulfan 359.67 % . Similar effects were obtained by many authors, Abbassy *et al.* (1989); El-Harrawie *et al.* (1991); Hanafy *et al.* (1991); Akay *et al.* (1992); Zayed *et al.* (1992); Tag El-Din *et al.* (1996) and Abd-Allah. (1998). who illustrated that, there were significantly increasing in the alkaline phosphatase activities in the treated white rats. The increasing in plasma alkaline phosphatase activity may be due to two factors ; increasing the enzyme synthesis and releasing of the enzyme by the damaged hepatic cells [Murphy. (1966); Enan *et al.* (1982) and Enan. (1983)].

Table(3): Effect of a single oral doses of tested pesticides on plasma AST** of treated white rats after 48 hours and 21 days of treatments.

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21 days
6.50	58.52 ±0.294	96.67 ±0.637	2.08	32.59 ±0.243	37.41 ±0.241	3.68	24.07 ±0.302	30.74 ±0.157	2.50	46.29 ±0.295	61.48 ±0.207	0.24	52.22 ±0.257	62.59 ±0.412
16.88	53.70 ±0.100	105.9 ±0.043	6.33	28.15 ±0.150	32.96 ±0.563	5.00	16.59 ±0.614	19.26 ±0.502	10.64	69.63 ±0.170	-----	1.13	59.26 ±0.308	-----
20.87	48.88 ±0.053	108.9 ±0.060	20.5	3.33 ±0.060	15.56 ±0.162	30.0	11.85 ±0.452	17.41 ±0.298	24.73	81.85 ±0.153	-----	2.68	73.70 ±0.313	-----
Control oil	34.44 ±0.136	77.78 ±0.100	Control oil	34.44 ±0.136	77.78 ±0.100	Control oil	34.44 ±0.136	77.78 ±0.100	Control oil	34.44 ±0.136	77.78 ±0.100	Control oil	34.44 ±0.136	77.78 ±0.100
Control water	60.74 ◊ ±0.072	46.67 ±0.163	Control water	60.74 ±0.070	55.19 ±0.066	Control water	60.74 ±0.071	55.19 ±0.066	Control water	43.33 ±0.334	41.85 ±0.105	Control water	43.33 ±0.334	62.59 ±0.412

* All concentrations were prepared by dissolving in watermelon seeds oil .

** activity = U / L.

◊ Mean ± S.D.

Table(4): Effect of a single oral doses of tested pesticides on plasma ALT^{} of treated white rats after 48 hours and 21 days of treatments.**

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days
6.50	20.79 ±0.118	28.77 ±0.11	2.08	44.54 ±0.057	47.14 ±0.471	3.68	17.68 ±0.121	20.27 ±0.357	2.50	16.31 ±0.281	26.52 ±0.240	0.24	16.48 ±0.148	27.90 ±0.230
16.88	6.58 ±0.125	35.00 ±0.508	6.33	49.57 ±0.217	51.13 ±0.388	5.00	9.36 ±0.252	14.90 ±0.160	10.64	31.37 ±0.124	-----	1.13	18.89 ±0.175	-----
20.87	4.68 ±0.064	38.99 ±0.256	20.5	51.65 ±0.335	52.51 ±0.106	30.0	7.28 ±0.308	11.61 ±0.159	24.73	38.47 ±0.250	-----	2.68	28.59 ±0.104	-----
Control oil	20.97 ±0.248	26.34 ±0.205	Control oil	20.97 ±0.248	26.34 ±0.205	Control oil	20.97 ±0.248	26.34 ±0.205	Control oil	20.97 ±0.248	26.34 ±0.205	Control oil	20.97 ±0.248	26.34 ±0.205
Cont.rol water	19.64 ◊ ±0.080	22.70 ±0.090	Cont.rol water	19.64 ±0.120	21.32 ±0.285	Cont.rol water	19.64 ±0.120	21.32 ±0.285	Cont.rol water	14.85 ±0.036	15.89 ±0.080	Cont.rol water	14.85 ±0.036	15.89 ±0.080

* All concentrations were prepared by dissolving in watermelon seeds oil

** activity = U / L.

◊ Mean ± S.D.

Table (5): Effect of a single oral doses of tested pesticides on plasma alkaline Phosphatase of treated white rats after 48 hours and 21 days of treatments.**

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21 days
6.50	73.28 ±0.416	69.12 ±0.248	2.08	29.50 ±0.306	27.04 ±0.026	3.68	26.62 ±0.166	25.91 ±0.115	2.50	44.04 ±0.344	56.11 ±0.186	0.24	33.48 ±0.173	50.81 ±0.410
16.88	75.35 ±0.267	69.23 ±0.057	6.33	41.55 ±0.055	38.55 ±0.136	5.00	36.13 ±0.420	34.08 ±0.291	10.64	61.62 ±0.143	---	1.13	38.76 ±0.115	-----
20.87	86.97 ±0.015	80.44 ±0.063	20.5	50.07 ±0.283	47.04 ±0.479	30.0	51.62 ±0.135	47.55 ±0.259	24.73	87.22 ±0.545	---	2.68	41.88 ±0.375	-----
Control oil	48.20 ±0.053	63.96 ±0.205	Control oil	48.20 ±0.053	63.96 ±0.205	Control oil	48.20 ±0.053	63.96 ±0.205	Control oil	48.20 ±0.053	63.96 ±0.205	Control oil	48.20 ±0.053	63.96 ±0.205
Cont.rol water	27.78 ◊ ±0.205	29.13 ±0.157	Cont.rol water	24.72 ±0.125	25.53 ±0.530	Cont.rol water	24.72 ±0.125	25.53 ±0.530	Cont.rol water	24.25 ±0.185	25.19 ±0.345	Cont.rol water	24.25 ±0.185	50.81 ±0.410

* All concentrations were prepared by dissolving in watermelon seeds oil .

** activity = kind and king U / 100 ml.

◊ Mean ± S.D.

1-5. Effects on total protein

The proteins of the plasma are actually a very complex mixture which includes not only simple proteins but also mixed or conjugated proteins such as glycoproteins and various types of lipoproteins. Thus, it is customary to separate the proteins of the plasma into 3 major groups (fibrinogen, albumin and globulin) by the use of varying concentrations of sodium or ammonium sulfate (Harper *et al.*, 1979). Results presented in Table (6) indicated that total plasma protein concentrations were decreased with all tested pesticides and their residues extracted from different parts of watermelon after 48 hours from treatments, and maximum percent of decreasing was occurred with malathion 70.49 % .These results are in agreement with those of, Rao. (1989); Saleh (1990) and Bayoumi *et al.* (1995-b). Who reported that, total protein concentrations were decreased after exposure to different groups of pesticides. On the other hand, total plasma protein concentrations were increased with all tested pesticides and their residues extracted from different parts of watermelon after 21 days from treatments, and maximum percent of increasing was happened with malathion 113.81 % . The aforementioned results were in agreement with those of many investigators who observed increasing in total protein concentrations after exposure to different groups of pesticides, El-Harrawie *et al.* (1986 & 1991) and Fujitani *et al.* (1993).

1-6. Effects on albumin

This fraction of the plasma proteins, is the most abundant of the proteins, synthesized by the liver. Results concerning the effects of the tested pesticides and their residues extracted from different parts of watermelon on albumin concentration are illustrated in Table (7). The data showed that, albumin concentrations were decreased with chlorpyrifos methyl, and maximum percent of decreasing was 6.19 % . While, albumin concentrations were increased with malathion, pirimiphos methyl, carbosulfan and methomyl, and maximum percent of increasing was occurred with methomyl 159.72 % . The obtained results are in harmony with those findings by Saleh. (1990) and El-Hamady. (1997) who observed a decreasing in albumin concentrations in white rats exposed to various groups of insecticides. On the other hand, Abd-Allah. (1998) found that, albumin concentration was increased in treated white rats with chlorpyrifos methyl and pirimicarb after 60 day from treatment with daily oral dose.

1-7. Effects on cholesterol

As known cholesterol is widely distributed in all cells of the body, but particularly in nervous tissue . Many reports have involved cholesterol as a causative agent for a considerable number of diseases. The works of Abd Elkader (1960), Saarivirta (1974), Frohlich (1976), Johnson and Somkuti (1989), Boyd and McGuire (1990), and Bosinger *et al.* (1993); are some instances of these reports. Breast cancer in women, cancerous growths in tissues , atherosclerosis, hypertension, biliary cirrhosis, liver necrosis, gallstones, cardiovascular disorders, and other adverse biological effects; are the diseases referred by these instances of reports.

Table(6): Effect of a single oral doses of tested pesticides on plasma total Protein^{''} of treated white rats after 48 hours and 21 days of treatments.

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21 days
6.50	6.58 ±0.151	7.27 ±0.164	2.08	8.54 ±0.505	8.07 ±0.297	3.68	8.60 ±0.152	8.15 ±0.287	2.50	7.82 ±0.315	8.24 ±0.444	0.24	7.89 ±0.241	8.36 ±0.420
16.88	5.64 ±0.133	7.33 ±0.11	6.33	8.14 ±0.230	8.11 ±0.309	5.00	7.98 ±0.289	8.19 ±0.198	10.64	7.07 ±0.022	----	1.13	7.46 ±0.127	----
20.87	4.85 ±0.224	7.58 ±0.106	20.5	7.85 ±0.075	8.32 ±0.408	30.0	7.67 ±0.159	8.67 ±0.251	24.73	6.90 ±0.056	----	2.68	7.30 ±0.116	----
Control oil	6.91 ±0.049	6.67 ±0.105	Control oil	6.91 ±0.049	6.67 ±0.105	Control oil	6.91 ±0.049	6.67 ±0.105	Control oil	6.91 ±0.049	6.67 ±0.105	Control oil	6.91 ±0.049	6.67 ±0.105
Control water.	6.88 ◊ ±0.066	6.66 ±0.060	Control water.	8.78 ±0.124	8.78 ±0.124	Control water.	8.78 ±0.124	8.03 ±0.037	Control water.	8.26 ±0.247	7.46 ±0.209	Control water.	8.26 ±0.247	7.46 ±0.209

* All concentrations were prepared by dissolving in watermelon seeds oil .

**concentration = g / l.

◊ Mean ± S.D.

Identification of cholesterol as a contributing factor in developing diseases has created reiterated calls to reduce its levels in the dietary intakes for diminishing blood cholesterol, which is the essentially responsible for these diseases. As early; Pyle *et al.* (1966), Hurt (1972), Reiser (1973), Saarivirta (1974), and Punwar (1975) were amongst those who emphasized the momentousness of reducing cholesterol levels in diets. The recalls of Johnson and Somkuti (1989), Boyd and McGuire (1990), Sieber and Eyer (1990), Oakenfull *et al.* (1991) and Peter (1993) are more recent in this connection .

The effects of a single oral doses of tested pesticides on plasma cholesterol concentration in treated male rats were summarized in Table (8). The results showed that, plasma cholesterol concentrations were decreased with malathion, pirimiphos methyl, carbosulfan and methomyl, , and maximum percent of decreasing was occurred with carbosulfan 56.57 %, but with chlorpyrifos methyl cholesterol concentration was increased and maximum percent of increasing was 107.89 % . Decreasing of cholesterol concentration in treated animal with different groups of pesticides was reported before by Ali and Shakoori. (1990) and Zaidi *et al.* (1990) .On the other hand, elevation in cholesterol concentration in white rats was found by Reena *et al.* (1989); Ghosh. (1990) ; Ogata and Izushi (1991) ; Fujitani *et al.* (1993) and Katayama. (1993) . Also, our results revealed that, watermelon seeds is rich in its content from oil, whereas the percent of the extracted oil was 30.92 % . This oil contain a high concentration of plant sterols (1749.01 mg /dl). However, cholesterol concentration was decreased in blood when white rats were given the watermelon seeds oil which indicate that, the plant sterols concentration in oil do not affect of the cholesterol level of blood plasma. The reducing in cholesterol in blood plasma in all treated rats was due to the pesticides residues as indicated in our previous results. So, from the previous results we can say that, watermelon seeds oil can be use safely on human diet without any hazards from the increasing in cholesterol level in blood.

From the previous results it could be concluded that, when white rats were given a single oral doses of the residues extracted from different parts of watermelon fruits which represent the least and highest residues concentrations in watermelon peel, pulp and seeds, fluctuated results were obtained and can be summarized as follows :

Rats which were given chlorpyrifos methyl residue extracted which represent the least concentration in watermelon peel and pulp exhibited decreasing in plasma ChE, AST, ALT activity, total protein and albumin concentration while, increasing was occurred in plasma alkaline phosphatase activity and cholesterol concentration.

Rats which were given pirimiphos methyl residue extracted which represent the least concentration in watermelon seeds exhibited decreasing in plasma ChE, AST activity, total protein and cholesterol concentration On the other hand, increasing was occurred in plasma ALT, alkaline phosphatase activity and albumin concentration.

Table(7): Effect of a single oral doses of tested pesticides on plasma albumin of treated white rats after 48 hours and 21 days of treatments.**

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21 days
6.50	2.87 ±0.125	3.26 ±0.220	2.08	2.76 ±0.105	2.87 ±0.064	3.68	0.132 ±0.011	0.512 ±0.038	2.50	2.61 ±0.104	3.32 ±0.101	0.24	2.35 ±0.338	3.20 ±0.115
16.88	2.92 ±0.284	3.32 ±0.060	6.33	2.79 ±0.100	3.06 ±0.035	5.00	0.147 ±0.039	0.637 ±0.031	10.64	3.27 ±0.165	----	1.13	3.19 ±0.136	----
20.87	3.47 ±0.217	3.40 ±0.100	20.5	3.02 ±0.267	3.28 ±0.031	30.0	0.176 ±0.007	1.180 ±0.191	24.73	3.34 ±0.096	----	2.68	3.45 ±0.200	----
Control oil	2.46 ±0.210	2.61 ±0.055	Control oil	2.46 ±0.210	2.61 ±0.055	Control oil	2.46 ±0.210	2.61 ±0.055	Control oil	2.46 ±0.210	2.61 ±0.055	Control oil	2.46 ±0.210	2.61 ±0.055
Control water	2.35 ◊ ±0.157	2.19 ±0.437	Control water	2.13 ±0.252	2.36 ±0.323	Control water	2.13 ±0.252	2.36 ±0.323	Control water	2.16 ±0.148	2.08 ±0.086	Control water	2.16 ±0.148	2.08 ±0.086

* All concentrations were prepared by dissolving in watermelon seeds oil.

** concentration = g / dl.

◊ Mean ± S.D.

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Table(8): Effect of a single oral doses of tested pesticides on plasma cholesterol of treated white rats after 48 hours and 21 days of treatments.**

Pesticides														
Organophosphorous compounds									Carbamate compounds					
Malathion			Pirimiphos methyl			Chlorpyrifos methyl			Carbosulfan			Methomyl		
Conc.*	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days	Conc.	48hrs	21days
20.87	71.05 ±0.268	90.19 ±0.043	20.5	86.84 ±0.272	88.88 ±0.479	30.0	107.89 ±0.01	111.11 ±0.066	2.50	56.57 ±0.088	73.20 ±0.160	0.24	75.00 ±0.005	86.23 ±0.020
Control water	100.0 ◊ ±0.099	104.80 ±0.1	Control water	100.00 ±0.099	104.80 ±0.1	Control water	100.00 ±0.099	104.80 ±0.1	Control water	100.00 ±0.099	104.80 ±0.1	Control water	100.00 ±0.099	104.80 ±0.1
Control oil	93.42 ±0.045	97.78 ±0.052	Control oil	93.42 ±0.045	97.78 ±0.052	Control oil	93.42 ±0.045	97.78 ±0.052	Control oil	93.42 ±0.045	97.78 ±0.052	Control oil	93.42 ±0.045	97.78 ±0.052

* All concentrations were prepared by dissolving in watermelon seeds oil.

**concentration = mg / dl.

◊ Mean ± S.D.

Rats which were given carbosulfan and methomyl residues extracted which represent the highest concentrations in watermelon peel, pulp and seeds exhibited decreasing in plasma ChE activity, total protein and cholesterol concentration while, increasing was occurred in plasma AST, ALT, alkaline phosphatase activity and albumin concentration after 48 hours from treatments. On the other hand, through the duration of the experiment (within 21 days), mortality was occurred with carbosulfan and methomyl residues extracted which represent the highest concentrations in watermelon peel, pulp and seeds.

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تقدير متبقيات بعض مبيدات الآفات المستخلصة من بطيخ اللب على نشاط الانزيمات فى الفئران البيضاء (البيينو)

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تم دراسة تأثير بعض المبيدات الفوسفورية والكرباماتية (الملاثيون - البيريثروس - الميثيل - الكلوربيرفوس ميثيل - الكاربوسلفان - الميثوميل) و المستخدمة فى حقول بطيخ اللب و متبقياتهما فى كلا من قشر و لب و بذور البطيخ على بعض الانزيمات فى الفئران البيضاء (البيينو) المعاملة حيث قسمت الفئران الى خمس مجموعات المجموعة الاولى أعطيت جرعة واحدة تمثل التركيز الموجود من المبيد فى بذور البطيخ و المجموعة الثانية عوملت بمتوسط التركيز الموجود فى كلا من قشر و لب البطيخ و المجموعة الثالثة تم معاملتها بتركيز يمثل 1/20 LD₅₀ و المجموعة الرابعة تم معاملتها بزيت بذور البطيخ الغير معاملة و المجموعة الخامسة لم تعامل و استخدمت كمقارنة . و تم ذبح الفئران بعد ٤٨ ساعة و ٢١ يوم و تمت عمليات التقدير للانزيمات المختلفة.

الأعراض الاكلينيكية : لم تظهر أى أعراض مع مبيدات الملاثيون و البيريثروس ميثيل و الكلور بيرفوس ميثيل . أما بالنسبة لمبيد الكاربوسلفان و الميثوميل حدث احمرار للاعين مع ظهور احتقان للدم حول الاعين و حول الانف و حدوث بعض الارتجافات و قد اختفت هذه الاعراض بعد فترة زمنية وجيزة تتراوح من ساعة الى ثلاث ساعات تلى ذلك حدوث موت خلال فترة الـ ٢١ يوم مع التركيزين اللذين يمثلان التركيز الموجود فى بذور البطيخ وكذلك التركيز الذى يمثل متوسط الموجود فى كلا من قشر و لب البطيخ .

القياسات البيوكيميائية : فى بلازما دم الفئران المعاملة لوحظ حدوث تغير فى مستوى انزيم الكولين استراز و كذلك حدوث تغيرات فى مستوى بعض القياسات البيوكيميائية التى تمثل وظائف

الكبد مثل الترانس أمينيز ، و الفوسفاتيز القلوى ، و البروتين الكلى ، و الالبومين ، و الكوليسترول مشيراً بذلك الى احتمال حدوث ضرر في وظائف الكبد حيث وجد الآتى :-

- ١- انزيم الكولين استراز (ChE) cholinesterase : حدث انخفاض فى نشاطه مع كل المبيدات المختبرة و قد حدثت أقصى نسبة انخفاض مع مبيد الكاربوسلفان و الكلوربيرفوس ميثيل حيث كانت ٢٣,٥% ، ١٧,٣٣% على التوالي وذلك بالمقارنة بالكونترول.
- ٢- (AST) aspartate aminotransferase : حدث انخفاض فى نشاطه بعد ٤٨ ساعة مع المبيدات الفوسفورية المختبرة بينما مع المبيدات الكاربماتية المختبرة حدث ارتفاع فى نشاطه. وعلى الجانب الاخر حدث ارتفاع فى نشاطه بعد ٢١ يوم مع الملاثيون والمبيدات الكاربماتية المختبرة وذلك بالمقارنة بالكونترول.
- ٣- (ALT) alanine aminotransferase : حدث انخفاض فى نشاطه بعد ٤٨ ساعة مع الملاثيون و الكلوربيرفوس ميثيل . وعلى الجانب الاخر حدث ارتفاع فى نشاطه بعد ٢١ يوم مع الملاثيون و البيريمفوس ميثيل و الكاربوسلفان و الميثوميل وذلك بالمقارنة بالكونترول.
- ٤- الفوسفاتيز القلوى alkaline phosphatase : حدث ارتفاع فى نشاطه مع كل المبيدات المختبرة و حدثت أقصى نسبة ارتفاع مع مبيد الكاربوسلفان وذلك بالمقارنة بالكونترول.
- ٥- البروتين الكلى total protein : حدث انخفاض فى تركيزه مع كل المبيدات المختبرة بعد ٤٨ ساعة وقد حدثت أقصى نسبة انخفاض مع مبيد الملاثيون حيث كانت ٧٠,٤٩% ثم حدث ارتفاع فى تركيزه مع كل المبيدات المختبرة بعد ٢١ يوم وذلك بالمقارنة بالكونترول.
- ٦- الالبومين albumin : حدث ارتفاع فى تركيزه مع كل المبيدات المختبرة وذلك بالمقارنة بالكونترول. أما مع الكلوربيرفوس ميثيل فقد حدثت معه انخفاض فى تركيزه.
- ٧- الكوليسترول cholesterol : حدث انخفاض فى تركيزه مع كل المبيدات المختبرة و قد كانت أقصى نسبة انخفاض مع مبيد الكاربوسلفان حيث كانت ٥٦,٥٧% أما مع الكلوربيرفوس ميثيل فقد أحدث ارتفاع فى تركيزه وذلك بالمقارنة بالكونترول.