



EFFECT OF CHLORPYRIFOS ON LIVER FUNCTION OF ALBINO RATES

Y. Ashoush⁽¹⁾, M. Abozid⁽¹⁾, S. Mansour⁽¹⁾ and A. Morgan⁽²⁾

⁽¹⁾ Biochemistry department, Faculty of Agriculture, Menoufia University.

⁽²⁾ Central public health laboratories, Ministry of Health, Egypt.

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ABSTRACT: *This study was conducted with the aim of studying the survival of chlorpyrifos on cucumber fruits. The fruits were applied by the recommended doses of chlorpyrifos. Residues of pesticides in fruits were determined used liquid chromatography.*

The results showed that the residual chlorpyrifos decreased gradually due to time factor and the process of the fruit washing with water had a clear effect in reducing the concentration of the presence of chlorpyrifos in the applied fruits.

Chronic toxicity of chlorpyrifos on liver function after the pesticide application showed that pesticides induced some effects on different biological functions of experimental animals applied with pesticide.

Key words: *Chlorpyrifos – liver function – cucumber – pesticides residues.*

INTRODUCTION

Pesticides are one of the few toxic substances released deliberately into the environment to kill living organisms e.g., weeds (herbicides), insects (insecticides), fungus (fungicides), and rodents (rodenticides). Although the term pesticide is often misunderstood to refer only to insecticides, it is also applicable to herbicides, fungicides, and various other substances used to control pests' (Matthews, 2006). Agriculture is the largest consumer (around 85% of world production) of pesticides to chemically control various pests. Moreover, pesticides are also used in public health activities to control vector-borne diseases (e.g., malaria and dengue) and unwanted plants (e.g., grass and weeds) in ornamental landscaping, parks, and gardens. They are also useful in suppressing or avoiding the proliferation of insects, pests, bacteria, fungi, and algae in electrical equipment, refrigerators, paint, carpets, paper, cardboard, and food packaging materials (Gilden *et al.*, 2010). However, unintended

exposure to pesticides can be extremely hazardous to humans and other living organisms as they are designed to be poisonous (Sarwar, 2015). They may also be harmful to people who are exposed to pesticides through occupational (or home) use, eating foods or liquids containing pesticide residue, or inhalation (or contact) of pesticide-contaminated air (Pimentel *et al.*, 2013). The physical makeup, behavior, and physiology of children make them more susceptible to pesticides than adults (Mascarelli, 2013). Pesticide exposure is linked with various diseases including cancer, hormone disruption, asthma, allergies, and hypersensitivity (Van Maele-Fabry *et al.*, 2010). A line of evidence also exists for the negative impacts of pesticide exposure leading to birth defects, reduced birth weight, fetal death, etc. (Baldi *et al.*, 2010 and Meenakshi *et al.*, 2012 and Wickerham *et al.*, 2012). In people, short-run oral exposure (one day) to low (milligrams) levels of organophosphate will cause a symptom, fatigue, runny nose or eyes, salivation, nausea, intestinal discomfort,

sweating, and changes in heart rate. Short-term oral exposure to abundant higher (grams) levels of organophosphate might cause dysfunction, seizures, loss of consciousness, and death. Reports in folks conjointly show that short-run exposure to organophosphate might cause muscle weakness weeks once the first symptoms have disappeared. Other effects of exposure to organophosphate embrace changes in behavior or sleeping pattern, mood changes, and effects on the nerves and/or muscles in the limbs which may appear as odd sensations such as numbness or tingling or as muscle weakness (Solati *et al.*, 2012).

MATERIALS AND METHODS.

Materials

1. Chlorpyrifos

Product name: pyrifos 48% EC Family: organophosphorous.

2. Pesticides application.

Cucumber fruits were planted in farm in Wardan Village in Giza governorate, Egypt. These plants were sprayed with recommended doses for chlorpyrifos according to the ministry of agriculture recommendation 2016.

3. Kits.

ALT, AST and ALP kits that used in analysis purchased by Spain React Company from Spain.

4. Collection of plant fruits samples.

Samples from cucumber was taken at random one hour and then after 1, 3, 7 and 14 days after the pesticides spraying.

The effect of washing process with water to eliminate pesticide residues from cucumber and, which was taken one hour and one day after the application, was studies.

Methods.

1. Extraction and clean up.

The technique extraction is QUECHERS method (AOAC, 2007).

- Ten g. of the sample were added to ten ml of acetonitrile then Shake for one min. Then mixture powder of 4 g. MgSO₄ + 1 gm sodium citrate dehydrate + 0.5 g. sodium sesquihydrate was added to the vortex for one min. Then the mixture centrifuged for 5 min at 4000 xg then 6 ml from upper layer were transferred and put in dispersive tube which contain 150 mg primary secondary amine (PSA) + 900 g MgSO₄. Then vortexed 30 seconds. The mixture was centrifuged for 5 min at 4000 xg after purification 1 ml in vial was taken to inject.

2. Determination.

Chlorpyrifos determined by using Sciex 5500 Qtrap liquid chromatography equipped with triple mass spectrometer detector (MSD).

3. Toxicological experiments.

Animals of this investigation were a total of 50 healthy and sexually mature albino rats and concentrations of chlorpyrifos were 5.769 ppm, 3.436 ppm, 1.896 ppm and 1.588 ppm.

4. Chronic toxicity (4- months).

Randomized groups of rats housed in cages were allocated into 5 intoxicated groups, each having 10 male rats, and induced by chlorpyrifos that determined on fruits after an hour of the pesticide application and after a day and after washing those fruits at the same times of the application through drinking water for 4 successive months .

5. Biochemical analysis (Determination of liver functions in plasma).

Aspartate aminotransferase (AST) was determined according to the method of Murray (1984)a. and alanine aminotransferase (ALT) activities in plasma was determined according to the method of Murray (1984)b. While, alkaline phosphatase (ALP) activity in plasma was determined according to the method of Wenger (1984).

6. Statistical analysis procedures.

All data were subject to statistical analysis by one-way ANOVA test using SPSS software for windows version 22.

RESULT AND DISCUSSION

Results.

1. Persistence of tested insecticides residues.

Residues of chlorpyrifos of cucumber fruits at different intervals from insecticides application were shown in Table (1). The data showed that the concentrations of the initial deposits were 5.769 ppm after one hour from application. These amounts were rapidly declined to 3.436 ppm after one day from application. These residues were dissipated to different degrees with the

time elapsed after 3, 7 and 14 days after insecticides application to reach 1.248 ppm, 0.051 ppm and 0.019 ppm for chlorpyrifos.

The data indicated that the loss of the residues was found to be continued on prolonging the time, where the percent loss rate amounted to 40.44, 78.36, 99.11 and 99.67 % for chlorpyrifos after 1, 3, 7 and 14 days after insecticides application, respectively.

2. Removal of tested insecticides residues from cucumber fruits by washing process.

The results in Table (2) showed levels at ppm and the percent of removal of each tested insecticide after treatment by washing process after one hour and one day of pesticide application. These results showed that chlorpyrifos residues on and in cucumber fruits were 5.769. These amounts were reduced to 4.257 by washing process. With corresponding percent or removal was 26.20 % after one hour of application.

The residues were 3.436 for chlorpyrifos after one day of pesticide application. These amounts were reduced to 2.962 after washing with tap water with corresponding percent of 48.65% removal rate.

Table (1): Residues and percentage rates of loss of chlorpyrifos in cucumber fruits.

Pesticides		Time after application (days)				
		Initial**	1	3	7	14
Chlorpyrifos	Residue* (ppm)	5.769	3.436	1.248	0.051	0.019
	% loss	00	40.44	78.36	99.11	99.67

Values represent means obtained from 3 samples.

Initial: samples were taken one hour after pesticide application.

Table (2): Effect of washing process on residues and percentage rates of loss of chlorpyrifos in cucumber fruits.

Pesticides		Time after application (days)			
		Initial**	After washing	1	After washing
Chlorpyrifos	Residue* (ppm)	5.769	4.257	3.436	2.962
	% loss	00	26.20	40.44	48.65

Values represent means obtained 3 samples.

Initial: samples were taken one hour after pesticide application.

All of present results confirm and agree the data Abou-Arab (1999), Ismail, *et al.*, (1993), Nabarawy, *et al.*, (1992) and Soliman (2001) they reported that the effects of washing tap water for 5, 10 and 20min. on pesticide residues in cabbage had effect in reducing some pesticides. Washing with 10% acetic acid solution for 20 min. caused loss in chlorpyrifos, p,p-DDT, cypermethrin and chlorothalonil. They reported also that partial removal was effected by the washing.

Washing was found effective in dislodging the residues as it depends on a number of factors like location of residues, age of residues, water solubility, temperature and type of washing. In earlier studies also showed effects of these factors were observed in different vegetables by various researchers (Sarode and Lal, 1982 and Dikshit *et al.*, 1986).

Washing with tap water, had an effect in reducing trichlorfon, dimethoate, dichlorvos, fenitrothion and chlorpyrifos (Zhang *et al.*, 2007 and Abou-Arab, 1999).

A gradual reduction was also observed when increasing the time at the same concentration (Radwan *et al.*, 2005). It has been recommended that cucumber

should be washed with tap water and/or detergent solutions carefully to decrease the intake of pesticide residues before raw eating (Kaushik *et al.*, 2009).

3. Biochemical evaluation harmful effects in male albino rats caused by chlorpyrifos on plasma liver functions.

3.1. Effect of chlorpyrifos on plasma aspartate aminotransferase (AST)

From the results presented in Table (3) chlorpyrifos effect showed, that there were insignificant changes in the AST activity between the albino rats control group and the albino rats in the 1st, 2nd, 3rd and 4th study group.

Inside 1st, 2nd and 3rd month the AST was elevated in the male albino rats with significant changes between the male albino rats control group and in the male albino rats 1st, 2nd, 3rd, and 4th study group, respectively.

Moreover, during the recovery period, the AST was reduced in the male albino rats with significant changes between the male albino rats control group and in the male albino rats 1st, 2nd, 3rd and 4th study group, respectively.



Table 3: Effect of chlorpyrifos on plasma aspartate amino transferase AST activity in albino rats.

AST U/L	Chlorpyrifos				
	Control Group 0ppm	1 st Study Group 5.769ppm	2 nd Study Group 3.436ppm	3 rd Study Group 1.896ppm	4 th Study Group 1.588ppm
Initial	46.29 ±1.61	46.64 ±0.74	47.06 ±0.77	47.09 ±1.18	47.28 ±1.03
1 st month	43.45 ±3.77	*38.90 ±1.01	*37.45 ±1.26	*36.40 ±2.74	**35.39 ±1.32
2 nd month	63.17 ±1.73	**74.44 ±3.82	**73.16 ±2.15	**73.52 ±1.85	**80.23 ±2.16
3 rd month	82.06 ±3.36	**101.4 ±4.84	**94.64 ±1.07	**93.37 ±1.81	**91.33 ±2.19
Recovery period	81.96 ±2.69	**49.55 ±1.29	**51.62 ±1.89	**51.20 ±1.48	**51.41 ±1.91

Values represent means ± S.D obtained from 5 animals. Significant level: * p ≤ 0.05; ** p ≤ 0.01; compare with control.

3.2. Effect of chlorpyrifos on plasma alanine aminotransferase (ALT).

From the results presented in Table (4) chlorpyrifos effect showed that there were insignificant changes in the ALT activity between the albino rats control group and the 1st, 2nd, 3rd and 4th study group.

Within one-month ALT activity was elevated with significant changes between the albino rats in the control group and the albino rats in the 1st and 3rd study group. There were insignificant changes between the albino rats in the control group and the 2nd and 4th study group.

Within two month can observe that, the ALT activity was elevated with significant changes between the albino rats in the control group and the albino rats in the 1st, 2nd, 3rd and 4th study group.

Within three month can observe that ALT activity was elevated with significant changes between the male albino rats in

the control group and the 1st, 2nd, 3rd and 4th study group.

Furthermore, during the recovery period the ALT activity was returned to the normal with significant changes between the male albino rats in the control group and the 1st, 2nd, 3rd and 4th group.

3.3. Effect of chlorpyrifos on plasma alkaline phosphatase (ALP).

The results presented in Table (5) chlorpyrifos effect showed, there were insignificant changes in ALP between control group and the 1st, 2nd and 4th study group. Meanwhile observe that, a significant change in the ALP activity between the albino rats in the control group and the albino rats in the 3rd study group.

Within one-month ALP activity was elevated with significant changes between the albino rats in the control group and the albino rats in the 1st, 2nd and 3rd study group. Except there was

the insignificant change in the ALP activity between the albino rats in the

control group and the albino rats in the 4th study group.

Table 4: Effect of chlorpyrifos on plasma alanin amino transferase ALT activity in albino rats.

Pesticides		Chlorpyrifos			
ALT U/L	Control Group 0ppm	1 st Study Group 5.769ppm	2 nd Study Group 3.436ppm	3 rd Study Group 1.896ppm	4 th Study Group 1.588ppm
Initial	38.03 ±0.82	38.89 ±1.97	38.32 ±1.29	37.94 ±1.35	38.33 ±0.95
1 st month	26.31 ±0.76	**34.73 ±4.03	32.40 ±6.33	*32.29 ±4.04	27.02 ±4.50
2 nd month	47.35 ±1.16	**64.46 ±4.40	**64.93 ±4.02	**62.69 ±4.85	**58.85 ±5.62
3 rd month	52.29 ±1.32	**83.22 ±4.26	**80.96 ±5.48	**85.0 ±7.02	**78.38 ±5.98
Recovery period	45.26 ±1.98	**49.57 ±1.34	**50.66 ±2.10	**53.65 ±1.71	**52.46 ±1.40

Values represent means ± S.D obtained from 5 animals. Significant level: * p ≤ 0.05; ** p ≤ 0.01; compare with control.

Table 5: Effect of chlorpyrifos on plasma alkaline phosphatase ALP activity in albino rats.

Pesticides		Chlorpyrifos			
ALP U/L	Control Group 0ppm	1 st Study Group 5.769ppm	2 nd Study Group 3.436ppm	3 rd Study Group 1.896ppm	4 th Study Group 1.588ppm
Initial	86.98 ±1.53	88.12 ±1.32	87.12 ±0.85	*89.28 ±0.85	85.80 ±1.53
1 st month	120.52 ±2.41	**137.70 ±2.01	**132.30 ±5.57	**139.93 ±5.98	126.09 ±5.2
2 nd month	137.26 ±1.46	**155.10 ±1.94	**153.96 ±5.57	**153.90 ±5.69	**150.90 ±3.4
3 rd month	159.22 ±1.33	**180.34 ±5.28	**179.56 ±7.57	*172.83 ±8.46	**173.65 ±6.8
Recovery period	167.12 ±1.21	**128.40 ±1.71	**123.02 ±1.10	**109.09 ±1.17	**97.89 ±1.7

Values represent means ± S.D obtained from 5 animals. Significant level: * p ≤ 0.05; ** p ≤ 0.01; compare with control.

Inside 2nd and 3rd month, the ALP activity was elevated in the albino rats with significant changes between control group and the 1st, 2nd, 3rd and 4th study group, respectively.

Furthermore, during the recovery period the ALP activity was returned to the normal in the albino rats in the 1st,

2nd, 3rd study group and 4th group with significant changes.

Discussion.

The pesticidal effect on the liver function of experimental animals various periods revealed significant changes in the liver functions parameters. The changes in biochemical parameters are

important to indicate the susceptibility of organ system to pollutants. In an organism toxic substances (such as pesticides) brings about a kind of stress and an organism responds to that by developing necessary potential.

Chlorpyrifos, which is an organophosphate insecticide, gets inside the cytoplasm from cells as a lipophilic molecule and harms cellular molecules inside the cell (Uzun *et al.*, 2010). Oxidative damage first starts with the production of reactive oxygen types and causes damage in macromolecules such as lipid, DNA and protein (Ambali *et al.*, 2011). Free radicals that occur as a result of oxidative stress, especially DNA, protein and cell phospholipids have an ability to react with a great number of organic and inorganic compounds, mainly multiple unsaturated fat acids (Bagchi *et al.*, 1995 and Lodovici *et al.*, 1997).

Mansour *et al.*, (2009) assessed the effects of chlorpyrifos in rat liver histopathologically and showed that chlorpyrifos increased lipid peroxidation; decreased plasma superoxide dismutase (SOD), glutathione-S-transferase and serum acetyl choline esterase activities, also caused histopathological changes such as hepatocyte degeneration, focal inflammatory cell infiltrations and diffuse Kupffer cell proliferation.

The study of Celik and Suzek (2008) revealed that chlorpyrifos-treatment caused an increase in the activities of ALP, AST and ALT in serum of male rats. The increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place. Moreover, chlorpyrifos is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. Researchers reported that enzymatic activities of AST

and ALT are sensitive serological liver toxicity (Messerah *et al.*, 2010).

High elevation of the liver enzymes (AST, ALT, ALP) are in agreement with findings of many researchers on experimental animals exposed to several OP insecticides including chlorpyrifos(CPF) and dizbean (DIZ) (Mansour *et al.*, 2009 and Mansour and Mossa 2010). Moreover, altered liver enzyme activities have been previously reported among pesticide workers exposed to an OP pesticide alone or in combination with other pesticides (Kalender *et al.*, 2010).

Conclusion

In spite of applied chlorperifos according to the recommendations, the residues of the pesticide in the fruits caused a significant increase in liver function. While, washing process leads to a decrease in the presence of chlorpyrifos residues in the fruits, which helps to reduce the risks to the body.

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تأثير مبيد الكلوربيريفوس على وظائف كبد الجرزان البيضاء

يوسف عشوش⁽¹⁾، مدحت ابوزيد⁽¹⁾، صلاح منصور⁽¹⁾، أيمن مرجان⁽²⁾

⁽¹⁾ قسم الكيمياء الحيوية - كلية الزراعة - جامعة المنوفية .

⁽²⁾ الإدارة المركزية للمعامل - وزارة الصحة .

المُلخَص العَرَبِي

اجريت هذه الدراسة بهدف دراسة بقاء مبيد الكلوربيريفوس على محصول الخيار ودراسة سمية المبيد على وظائف الكبد عند معاملة حيوانات التجارب (الجرزان البيضاء) بتركيزات المبيد المختلفة على مدار مدة زمنية محددة . حيث تم رش محصول الخيار بالمعدلات الموصى بها من الكلوربيريفوس، وتم تقدير بقايا المبيد بالمحصول محل الدراسة الخيار باستخدام الكروماتوجرافي السائل . ودراسة تأثير عاملي الوقت والغسيل على بقاء المبيد في المحصول تبين أن عامل الوقت يؤثر على بقاء المبيد حيث كلما زادت الفترة الزمنية لتواجد المبيد قل التركيز وكذلك وجد أن الغسيل بالماء كان له تأثير واضح في الحد والتقليل من تركيز وجود الكلوربيريفوس في النبات . و أظهرت دراسة السمية المزمنة للكلوربيريفوس تأثيرا واضحا على وظائف الكبد وتبين أن المبيد يؤدي الي ارتفاع وظائف الكبد بشكل مرضي ويزيادات معنوية .

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السادة المحكمين

أ.د/ إمام عبدالمبدئ عبدالرحيم كلية الزراعة - جامعة القاهرة
أ.د/ خالد مأمون طه كلية الزراعة - جامعة المنوفية



ملخص عربي

