DETERMINATION OF DELTAMETHRIN RESIDUES IN MANDARIN ORANGE AND STUDYING ITS PHYSIOLOYICAL EFFECTS ON MALE MICE BLOOD PROFILE

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ABSTRACT

Deltamethrin insecticide was highly toxic to mammals and had long-term residual effects and easily causing environmental pollution. The dependence concentration of deltamethrin (25 ppm) was used to treat unripe mandarin orange fruits through a field experiment. A daily and weekly fruit samples, up to 35 days, were examined for deltamethrin residues. The concentrations of deltamethrin which immigrated via peel into flesh of mandarin orange were 3.4, 3.9, 4.5 and 5.2 ppm after 1, 2, 3 and 4 days respectively. The levels of deltamethrin residues were decreased (due to many degradation reasons) from the maximum amount (after 4 days) to undetectable amount after 35 days. The field experiment showed that the fruits are ready to consume (matured) after 21 days, at this time, the deltamethrin residue was 2.3 ppm in mandarin fruit flesh, while the Maximum Residue Level of deltamethrin recommended is 0.02 mg/kg. Dealing with the peel part of fruits, at zero time, the mandarin orange peel trapped more than 40% of the initial concentration (25 ppm) of deltamethrin. The effect of 0, 2, 4, 6 and 8 ppm deltamethrin, on blood profile of male mice was determined.

*Keywo*rds: Citrus, insecticide, field experiment, blood parameters.
Part of M.Sc. thesis of the first author

تقدير متبقيات الدلتامثيرين في اللالنكي ودراسة تأثيراتها الفسيولوجية على صورة الدم في ذكور الفئران

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المستخلص

يعد الدلتامثيرين من المبيدات الحشرية عالية السمية للثديات وله أثار طويلة الأمد كمتبقي أضافة الى سهولة تلويثه للبيئة. من خلال تجربة حقلية تم أستخدام تركيز الدلتامثيرين الموصى به (25 جزء في المليون) لمعاملة ثمار اللالنكي غير الناضجة. تم سحب نماذج يومية وأسبوعية صعودا الى 35 يوما لتقدير متبقيات الدلتامثرين. كان أنتقال الدلتامثيرين عبر القشرة الى داخل الثمرة بواقع 3.4، 3.9، 4.5، 5.2 جزء في المليون بعد 1، 2، 3، 4 يوم على التوالي. أن مستويات متبقيات الدلتامثيرين أنخفضت (بسبب عوامل تحطم متعددة) من أعلى مستوى لها (بعد 4 أيام) الى مستويات غير محسوسة بعد 35 يوم من بدأ عملية الرش. أظهرت التجربة الحقلية بأن الثمار كانت ناضجة جاهزة للأستهلاك بعد مرور 21 يوم من الرش وكان نسبة المبيد المتبقي في لب ثمارالالنكي انذاك بحدود 2.3 جزء في المليون، في حين أن أقصى حد مسموح كدلتامثيرين متبقي بحدود 0.02 ملغم/ كغم. أما في حالة قشور اللالنكي، وفي الوقت صفر، فأنها أحتجزت اكثر من 40% من التركيز الأولي للدلتامثيرين (25 جزء في المليون). تمت دراسة تأثير التراكيز صفر، 2، 4، 6، 8 جزء في المليون من الدلتامثيرين على صورة الدم لذكور الفئران.

كلمات مفتاحية: حمضيات، مبيد حشري، تجربة حقلية، ثوابت الدم.

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INTRODUCTION

Residues of pesticides are ranked very high as an important risk factor in society. Pesticide residues in food are under strong legislation and regulation in most countries, and strict rules are a means of reducing the use of these poisonous chemicals. Usually the authorities set up a maximum residue limit (tolerance limit) based on toxicological data or on the expected residue level obtained when good agricultural practice has been followed. Acceptable daily intake is the key concept for determining tolerance levels of residues (17). Deltamethrin (below structure) is a synthetic, type II pyrethroid insecticide, used to control numerous insect pests of field crops, potted plants, and ornamentals.

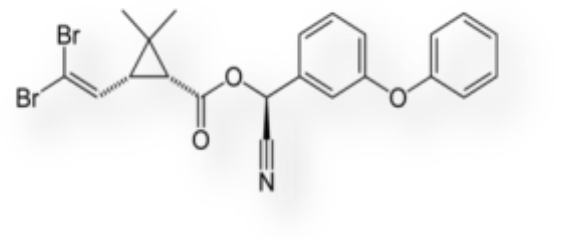

Fig1. Deltamethrin structure

Type II pyrethroids cause a dramatic prolongation and enhancement of sodium tail currents in voltage-clamped nerve axons (11) and have an α-cyano group that induces "long-lasting" inhibition of the sodium channel activation gate. This results in prolonged permeability of the nerve to sodium and produces a series of repetitive nerve signals in sensory organs, sensory nerves, and muscles (21). Researchers observed that deltamethrin and other Type II pyrethroids may also affect ion channels other than sodium channels, possibly due to their phosphorylation state (24). Paresthesia was the most commonly reported symptom from dermal exposure in occupational studies involving pyrethroids. The signs of toxicity typically associated with deltamethrin are include characteristic effects of choreoathetosis (sinuous writhing) and salivation (3). In rats, this presents as pawing and burrowing behavior followed by salivation and tremors, progressing to choreoathetosis and clonic seizures may occur in the final stage. Summarizing the overall effects of deltamethrin, the National Pesticide Information Center reports that rats exhibited motor incoordination, salivation, respiratory defects, spasms involving the limbs and tail, and clonic seizures when administered deltamethrin orally. The present study is investigating effect of deltamethrin residues in and on mandarin orange fruits and their effects on blood profile of male mice.

MATERIALS AND METHODS

An adequate number of fruit trees at Al-Rashdiah area (at Baghdad province) were chosen to carry out this experiment. The trees were selected (on Nov.01, 2016) with unripe fruits. The trees of mandarin orange fruits were sprayed by 25 ppm concentration of deltamethrin insecticide using a 10 liters sprayer holder.

Preparation of deltamethrin concentration

Deltamethrin of 25gm/l (registration certificate, appendix No.1) of Bayer Company has been used to prepare 25 ppm of emulsified solutions using 1 mL of the original concentration (25000 ppm deltamethrin) and completed to one liter by deionized water. The trees were sprayed by professional farmer in a sunny unrainy day.

Sampling:

The treated samples of mandarin orange fruits peeled (peels and fruit flesh) and stored under freezing in sterilized zipper polyethylene bags. The peels and fruit fleshes samples were analyzed for their deltamethrin residues by HPLC.

Sample preparation for analysis

The extraction of deltamethrin residues from mandarin orange samples were carried out by using quick, easy, cheap, effective, rugged and safe (QuEChERS) Dispersive Solid Phase Extraction procedure as below: 50 gr. of fruit flesh or peels were sliced separately into small pieces. A mixture of 90 mL hexane and 10 ml acetone were added to the sliced sample and blending by 3000 rpm blender for 5 min. The filtrate was centrifuge under 8000 rpm for 10 min at room temperature. The filtrate was separated into three layers, low aqueous layer, intermediate impurities layer and upper organic layer (deltamethrin layer). The upper organic layers from each centrifuge tube were withdrawn by disposable syringes and collected together (about 30 to 40 ml). 1.5 gm. sodium chloride and 5 gr. anhydrous sodium

sulphate were added to get rid of water residue that will be present in organic layer. A Buchner filtration was done to clarify the organic layer. The organic layer with its deltamethrin content was prepared for HPLC analysis

HPLC analysis

A Shimadzu High Performance Liquid Chromatography (HPLC) type LC-20 AD was used to analyze the samples (mandarin orange, fleshes and peels) for their content of deltamethrin residues according to the conditions below:

Column : Zorbax SB-C18 (250 mm x 4.6).
Mobile phase : Acetonitrill: Water (98: 2).
Flow rate : 0.5 ml/min.
njection volume: 5 µL.
UV Detector : 230 nm
Oven temp. : 40^{o} C

Preparation of standard deltamethrin for HPLC analysis

Standard deltamethrin (0.0125 gm) was dissolved in a limited volume of acetonitrill and completed to 25 ml in volumetric flask. The prepared standard has a concentration of 500 ppm deltamethrin. 2 ml of the standard deltamethrin was placed in the HPLC tray vial. A duplicate run was achieved to determine the retention time (8.279 min) of the standard deltamethrin.

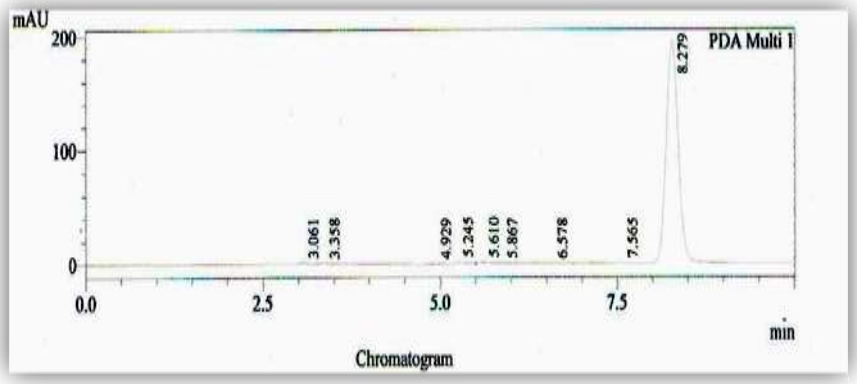

Fig2. The HPLC standard diagram of deltamethrin insecticide

The experiment of animal house

The experiment on the laboratory mice has been carried out at the center of biotechnology – University of Al-Nahrain, Baghdad-Iraq.

The laboratory mice

Fifty male mice have been supplied by the animal experimental unit of The National Center of Biological Control / Ministry of Health. Mice weighing about (23-28 gm.) and all are allowed to acclimatize for one week in animal house conditions (22 ± 3^{o} C, relative humidity 50-55%, and 12 h light/dark cycle) prior to the experiment. Mice were separated into 5 groups in cages of 10 mice capacity. A standard nutritionally balanced diet (manufactured by Grain & Flour Mills Organization, India) was supplied by Al-Nahrain Research Center and according to (25).=

The blood profile measurement

Five concentrations (0, 2, 4, 6 and 8 ppm) of deltamethrin have been studied for their effect on mice blood profile after been administrated orally by 0.2 ml/daily for 45 days under controlled conditions

Blood collection and serum separation

One mL disposable syringe (insulin syringe) was inserted in a live mouse heart area under a supervision of professional (5-8 syringes may require).The blood volume from each mouse was collected in Eppendrof tube. Eppendrof tubes were centrifuged at 30^{o} C and 3000 rpm for 10 min. The blood sample was separated into two layers, upper serum layer and lower rejected layer. The serum layer from each mouse was stored under freezing condition till the biochemical lipid blood profile measurements. Ready kits were used to determine the effects of different concentrations of deltamethrin on triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), catalase, malondialdehyde (MDA), glutathione (GSH), urea, creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), luteinizing hormone

(LH), follicle stimulating hormone (FSH) and testosterone.

RESULTS AND DISCUSSION

Results of deltamethrin field spraying

A field spraying experiment using 25 ppm of deltamethrin has been carried out to monitor the actual levels of residues that were trapped on ripe fruits and which were ready(mature) to consume.Table.1 shows that the deltamethrin migration into mandarin orange flesh throughout peel is not follows sharp relationship. Due to loosen peel of mandarin orange (porosity) comparing with other citrus fruits, the active ingredient (deltamethrin) was freely immigrating into fruit flesh especially at the first stages (up to 4 days) of the field experiment. The same explanation may serve to illustrate, the mandarin orange peel may catching more deltamethrin. Pesticide residues, especially in vegetables and fruits, are a reason for concern with respect to the health of consumers and workers. The World Health Organization and United Nations Environmental Program have estimated one to five million cases of pesticide poisoning among agricultural workers each year with about 20,000 fatalities, mostly reported from developing countries (20). Deltamethrin is an exceptionally potent insecticide which is used at very low application rates against a spectrum of stored-product pests (16). Many studies have been made in many countries on the distribution and fate of deltamethrin residues found at the various steps from the day on which the fruits treated until it consumed by human beings. The major concerns with pesticides residues are their toxic effects. The three major routes of entry for pesticides include contamination of the skin, mouth and the nose. The public health issue of pesticide exposure is further complicated by the presence of impurities in so-called, inert-ingredients such as solvents, wetting agents and emulsifiers (2, 13).=

Table 1. Mandarin orange(Field experiment)

Time/ day	Deltamethrin concentration	
	25 ppm	Peel (25 ppm)
Control	0	0
Zero	0	15.8
1 day	3.4	14.1
2 day	3.9	12.45
3 day	4.5	10.9
4 day	5.2	9.4
5 day	4.75	7.9
6 day	4.4	6.25
7 day	4.0	4.7
14 day	3.1	3.2
21 day	2.3	1.75
28 day	1.5	0.3
35 day	0	0
42 day	0	0
LSD 0.05	1.82 *	2.62 *

Experiments of animal house

As a food science concerned, and since the consumer is our target, we must take in mind what are the possible damages from consuming such fruits holding these levels of deltamethrin? In the previous field experiment we allowed the fruits to be going over ripening (35 days), while the mandarin orange fruits are ready to consume after 21 days from the stage we have started. The deltamethrin residue in mandarin orange flesh after 21 days using 25 ppm was 2.3 ppm is higher than the Maximum Residue Levels (MRLs) of deltamethrin which it is 0.02 mg/kg. We know that sometimes these fruits are available in our markets prior to complete ripening, which are mean high deltamethrin residues. So in order to deal with these two cases (mature and immature fruits) we have suggested a series of deltamethrin concentrations (2, 4, 6 and 8 ppm) to be served (orally) to experimental animals to discover the harms it's caused to male mice

The blood profile analysis

Many blood parameters have been examined after iserving oral male mice 0.2 ml/day daily by deltamethrin suspension of 25 ppm for 45 days under controlled conditions. Table .2 shows that there was a reverse relationship between deltamethrin concentrations and triglycerides levels. The primary look may refer to the existence of advantage relationship, since the high levels triglycerides causes many diseases, such as obesity, kidney disease, alcohol consumption and hypothyroidism. But on the other side, there were disadvantages appeared by consuming foods with high deltamethrin concentration

residues such as very low carbohydrates, inherited conditions, fat metabolism disorders, liver or thyroid diseases and medication (4). Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones. The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemia. High blood cholesterol is one of the major risk factors for heart disease (6). there was a reverse relationship between total cholesterol and deltamethrin concentration. The first impression at cholesterol, that it is a harmful component in the body, but if we survey its necessity and the healthy problems associated with its abnormal decreasing level we can discover how much wanted it is. The overall relationship between the HDL level and deltamethrin concentration. The relationship tends to be reverse and this refers to a bad effect of deltamethrin on human health because HDL represents the good cholesterol. However, the three chosen male mice not refer obviously there was a reverse relationship between HDL level and uptake deltamethrin, but there was such relashionship between total cholesterol and deltamethrin concentration. A zigzag relationship between LDL levels and deltamethrin concentrations. No obvious relationship has been discovered, 2 ppm deltamethrin concentration reduced LDL level in male mouse blood serum comparing with the control. The three other concentrations of deltamethrin, 4, 6 and 8 ppm, using three replications of male mouse showing randomly relationship with LDL. LDLs carry cholesterol from the liver to other parts of the body where it can cause atherosclerotic disease. High levels of LDL are associated with an increased risk for coronary artery disease. A clear reverse relationship between VLDL levels and deltamethrin residue concentrations. More than 50% of VLDL was reduced by using 8 ppm deltamethrin. Very low density lipoprotein(VLDL) cholesterol is produced in the liver and released into the bloodstream. High levels of VLDL cholesterol have been associated with the development of plaque deposits on artery walls, which narrow the passage and restrict blood flow.

Table 2. Effect of different concentrations of deltamethrin on lipid profile

Conc. (ppm)	Mean				
	Cholesterol (mg/ld)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
ontrol : 0	118.67	291.67	41.00	20.00	57.67
2	99.33	275.00	37.67	10.00	55.33
4	96.33	214.00	37.67	16.00	42.67
6	90.00	164.33	36.00	21.33	32.67
8	79.33	125.00	34.33	21.67	27.33
LSD 0.05	6.214 *	25.685 *	5.923 *	8.442 *	5.498 *

A clear direct relationship is shown between blood serum catalase and deltamethrin concentration (table 3.) About 50% increasing in blood serum catalase was observed by using 8 ppm deltamethrin. Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (8). It catalyzes the decomposition of hydrogen peroxide to water and oxygen A proportional relationship between blood serum malondialdehyde levels and concentration of deltamethrin residues. The increasing in malondialdehyde is more than 7-fold by using 8 ppm deltamethrin comparing with its initial concentration in control. Biomonitoring of MDA has been used in both *in vivo* and *in vitro* studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer. Higher levels of MDA are reported in patients of various categories including lung cancer patients, complex regional pain syndrome patients and glaucoma patients (34). No effect of 2 ppm deltamethrin on blood serum glutathione comparing with control. A reverse relationship appeared between glutathione and deltamethrin concentrations higher than 2 ppm (4, 6 and 8 ppm). Different kinds of tightly associated reactions occur in the human organism, the purpose of which is to guarantee homeostasis. Reactive species (including free radicals) participate in some physiological oxidative reactions. However, when these reactions cross threshold levels, damaging factors will prevail and lead to oxidative stress (OxS) (10). In order to prevent OxS, there is

an elaborate antioxidant defense system consisting of enzymatic antioxidants (including glutathione). Thus, it is important that the antioxidant defense system in blood, especially in red blood cells, is effective and recovers properly after exhaustive physical load. As compared with male mice control group, the increasing of deltamethrin in the feed of other male mice groups show increasing in blood serum urea . Urea is an end product of metabolism: it does not participate in any synthetic reactions. In the kidneys, the contribution of urea to medullary hypertonicity is an important determinant of the ability of the kidneys to generate concentrated urine. Urea is recycled in this process but does not underg any metabolic conversion (33). A clear increasing was obtained in creatinine levels associated with increasing in deltamethrin concentrations. The 3-fold increasing in blood serum creatinine by using 8 ppm deltamethrin. Creatinine is a heterocyclic nitrogenous produced from creatine in muscle at a rate dependent on muscle bulk and is excreted unchanged by the kidneys, mainly by glomerular filtration but to a small extent by active secretion. A small amount of creatinine (approximately 10%) is derived from dietary sources (particularly cooked meat) (33). Because the kidneys are responsible for clearing creatinine from blood, measurement of serum creatinine levels is useful indicator of renal function (7).

Table 3 . Effect of different concentrations of deltamethrin on level of enzyme

Conc. (ppm)	Mean				
	Cat. (u/ml)	MDA (UM)	GSH (UM)	Urea (mg/dl)	Creat. (mg/dl)
Control : 0	11.53	0.630	4.00	38.00	0.240
2	12.36	1.466	3.83	35.66	0.303
4	13.93	1.833	3.70	41.00	0.356
6	18.33	2.866	3.266	45.67	0.446
8	19.73	3.833	2.000	58.00	0.650
LSD 0.05	2.731 *	0.400 *	0.567 *	4.505 *	0.105 *

Table 4. shows a proportional relationship between blood serum glutamic oxaloacetic transaminase (GOT) and deltamethrin concentration. To some extent, a similar relationship was found with glutamic pyruvic transaminase (GPT) and deltamethrin . The results refer to liver function disorder by increasing deltamethrin concentrations up to 8 ppm. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are formerly called serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), respectively. AST or ALT levels are a valuable aid primarily in the diagnosis of liver disease. When body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage. The ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged (15, 28).

Table 4. Effect of different concentrations of deltamethrin on level of hormones

Conc. (ppm)	Mean	
	GOT (IU/L)	GPT (IU/L)
control : 0	27.67	19.00
2	32.67	23.66
4	29.67	29.00
6	39.66	40.00
8	46.33	40.33
LSD 0.05	10.21 *	6.88 *

Table 5. shows a stair-like increasing in luteinizing hormone associated with deltamethrin increasing. Luteinizing hormone (LH) is a reproductive regulator also called "Lutropin". Luteinization is limited to female mammals, however, the name "LH "is used for wide range of animal species, as well as males. LH has a heterodimer structure consisted of α-subunit and β-subunit. α-Subunit is a glycoprotein which is common to other pituitary hormones follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH), while β-subunit is specific to LH, and is also a glycoprotein (18). Table 5.shows none reguler elevating in Human Follicle Stimulating Hormone (FSH) by

increasing deltamethrin concentration. A real elevating was observed by using 6 and 8 ppm deltamethrin. Human follicle stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. FSH has an alpha and beta subunit. The subunit contains 92 amino acids while the β subunit of in FSH contains 115 amino acids. The FSH hormone functions differently in females and males. It is to be noted that in women the growth and maturation of the ovarian follicle is dependent on FSH, while in men FSH act on the testes. There was a direct relationship between testosterone quantity and deltamethrin concentrations of 2, 4 and 6 ppm , the amount been reduced at 8 ppm deltamethrin (but not less than control). It is unexpected result whether it is good or not. Clinical guidelines and position statements recommend testing for testosterone to aid in diagnosing diseases and disorders or monitoring treatments (1). Testosterone measurements are used in patient care for the diagnosis of hypogonadism in men (1) and androgen excess in women with polycystic ovary syndrome being one of the conditions causing androgen excess (22, 32). Research found that testosterone levels are associated with various diseases and conditions, such as metabolic syndrome (27), diabetes (29), cardiovascular disease (12, 26), fractures (14, 31), neurodegenerative disorder (9, 23), and higher mortality in men with lower testosterone levels (19,30).

Table 5. Effect of difference groups (conc.) in Level of hormones

Conc. (ppm)	Mean		
	LH (mlu/ml)	FSH (mlu/ml)	Testo. (ng/ml)
Control : 0	4.36	4.13	1.366
2	4.00	3.63	1.433
4	4.06	3.33	1.500
6	4.60	4.67	1.466
8	6.46	4.67	1.566
LSD 0.05	1.964 *	1.194 *	0.393 NS

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