EFFECT OF ACETAMIPRID RESIDUES IN TOMATO FRUITS ON SOME BLOOD PROFILE PARAMETERS OF MALE MICE

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ABSTRACT

Acetamiprid is one of the most wide used insecticides over the entire world. Three acetamiprid concentrations, the dependence one (200 ppm) and two other high concentrations (300 and 400 ppm) were used to treated tomato fruits at the final stage of ripening. A daily samples up to 15 days, were examined for their acetamiprid residues. The maximum migration of acetamiprid (systemic) into tomato fruit was observed after three days (1.7, 1.89 and 2.27ppm) of spraying by 200, 300, and 400 ppm. After 7 days of application, the acetamiprid residues are more than five-fold of the Maximum Residue Limit (acetamiprid MRL=0.2 mg/kg). Four groups of male mice (each of 10, control and three treatments) were used to study the effects of acetamiprid on some blood profile parameters. Three selected concentrations of acetamiprid 32, 64 and 128 ppm were used to orally administrate the male mice (0.0064, 0.0128 and 0.0256 mg/mouse daily respectively) under controlled conditions for 28 days. It was found that there was a direct relationship between acetamiprid concentrations and triglycerides, total cholesterol, LDL, VLDL, catalase, malondialdehyde, urea, creatinine, AST, ALT and superoxide dismutase level. While, reverse relationships were observed with HDL, LH, FSH, testosterone and acetyl cholinesterase

Keywords: Vegetable, insecticide, field experiment, physiological effects.

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تأثير متبقيات الأسيتامبرد في ثمار الطماطة على بعض ثوابت صورة الدم في ذكور الفئران

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المستخلص

الأسيتامبرد واحد من أكثر المبيدات الحشرية انتشارا في العالم. تم أستخدام ثلاثة تراكيز من الأسيتامبرد، الموصى به (200 جزء بالمليون) وتركيزين أخرين أعلى (300 و 400 جزء بالمليون) لرش ثمار الطماطة في المراحل النهائية من النضج. تم أخذ نماذج يومية صعودا الى 15 يوم للكشف عن متبيقيات الأسيتامبرد. أن أقصى هجرة للأسيتامبرد (جهازي) الى داخل ثمار الطماطة حصلت بعد ثلاثة ايام من الرش (1.70، 1.89و 2.27 جزء بالمليون عند استخدام 200، 300 و 400 جزء بالمليون). أزدادت متبقيات الأسيتامبرد بعد 7 أيام من عملية الرش بمقدار خمسة أضاف الحدود المسموح بها (0.2 ملغم/ كغم). تم تقسيم الفئران الى أربعة مجاميع (كل مجموعة 10، سيطرة وثلاث معاملات) لدراسة تأثير الأسيتامبرد على بعض ثوابت صورة الدم. تم أختيار ثلاثة تراكيز من الأسيتامبرد، 32، 64 و 128 جزء بالمليون لتجريع ذكور الفئران (0.0064، 0.0128 و 0.0256 ملغم/ فأر يوميا على التوالي) تحت ظروف مسيطر عليها ولمدة 28 يوما. وجد ان هنالك تناسب طردي بين تراكيز الأسيتامبرد والكليسيريدات الثلاثية، الكوليستيرول الكلي، LDL، VLDL، أنزيم الكاتليز، مالوندايالديهايد، اليوريا، الكرياتنين، ALT، AST، وانزيم Superoxide distumtase، بينما وجدت علاقة عكسية مع HDL، LH، FSH، التيستستيرون وأنزيم Acetyl cholinesterase.

الكلمات المفتاحية: خضروات، مبيد حشري، تجربة حقلية، تأثيرات فسيولوجية.

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INTRODUCTION

Pesticide is any substance or a mixture of substances intended for preventing, destroying, repelling or mitigating any pest.Pesticides vary greatly in toxicity, persistence in the environment, and ability to bioaccumulate up the food chain. All pesticides are regulated by the Environment Pesticides Agency (EPA) which regulates the sale and use of pesticides through product registration and labeling so as to prevent unreasonable adverse effects on people and environment (15). Most pesticides can cause severe illness or even death if misused, but every registered pesticide can be used safely.Two types of pesticide toxicity (or damage) often are mentioned: acute and chronic. Acute effects are those resulting from a single exposure. They usually are noted within a few minutes to several days. Chronic effects result from repeated low-level exposure and often do not manifest themselves for decades (13)**.** Acetamiprid is a neonicotinoid compound (Fig.1) shows excellent efficacy against aphids, leaf hoppers, white flies, thrips, leaf beetles, leaf miner moth, termites, etc. in various crops (1, 18, 20). Acetamiprid group of insecticides are the most highly effective and largest selling insecticides worldwide for crop protection.

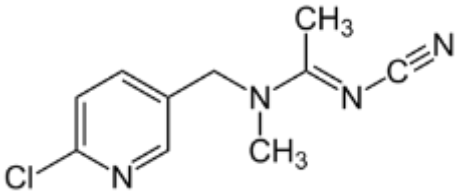


Fig. 1. Acetamiprid structure

Acetamiprid is a systemic insecticide with trans-laminar action which has a contact and stomach action. Although, acetamiprid is highly used abroad but there are still many doubts related to its toxicity and health hazards. Exposure of animals and birds to insecticides for a short duration induces a state of stress leading to decrease in production and behavioral as well as biochemical changes. The continuous use of pesticide imposes hazardous effect on the physiological function of various body systems (7)**.** In the present paper the effect of acetamiprid residues in tomato fruits on some parameters of blood profile in male mice were studied.

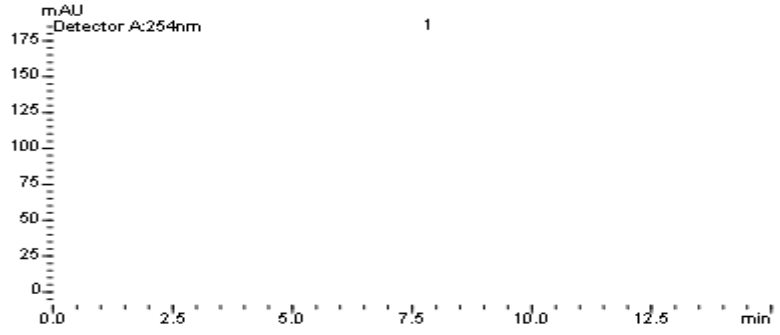
MATERIALS AND METHODS

An acetamiprid suspension of 20% active gradient has been used to prepare 200, 300 and 400 ppm concentrations. The 200 ppm is the concentration that was recommended by the company as an effective concentration against the insect infection. The mature tomato plants with unripe fruits have been divided into four groups (Control and three groups of 200, 300 and 400 ppm).A sample of approximately 500 gm was taken from each treatment at 0, 1, 2, up to 15 days. Each sample was blended separately and stored in sterilized zipper polyethylene bag at freezing point.

Acetamiprid extraction

QuEChER's Method was used to extract acetamiprid residues as described by (10): A Shimadzu High Performance Liquid Chromatography (HPLC) model LC-2010 A HT, was used to determine acetamiprid residues under the conditions below:

Column : Orbit C18 (250 X 4.6 mm)
Mobile phase : Methanol: Water (60: 40 v/v)
Flow rate: 0.5 ml/min
Injection volume : 10 µl
UV Detector : 254 nm
Oven temperature : 30Ċ
Time (min)



Time (min)

Fig. 2. HPLC diagram of the standard acetamiprid

Experiment of animal house

Forty healthy male mice were selected. The mice weighing from 23 to 28 gm, all are allowed to acclimatize for one week in animal house conditions (22 ± 3^{o}C, relative humidity 50-55%, and 12 hour light/dark cycle). The mice were separated into 4 groups. A standard nutritionally balanced diet (manufactured by Grain & Flour Mills Organization, India) was supplied by Al-Nahrain Research Center and according to Reeves (16) was used to feed the mice for 28 days. Four concentrations of acetamiprid (0, 32, 64 and 128 ppm) have been prepared. This range of acetamiprid was associated with its residues in tomato fruits at the end of the field experiment.

Blood profile measurements

Four concentrations of acetamiprid (0, 32, 64 and 128 ppm) have been studied for their effects on male mice blood profile after have been administrated orally by 0.2 ml/daily for 28 days.The blood sample of male mice 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 acetamiprid on triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), catalase, malondialdehyde (MDA), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and acetyl cholinesterase (AchE). A statistical analysis system (SAS) was used to analyze data in order to study the effect of various factors according to complete randomly design (CRD). The significance differences between the means were tested by using least significant difference (LSD).

RESULTS AND DISCUSSION

A field spraying experiment using certain concentrations of acetamiprid have been carried out to restrict the actual levels of residues that were retained on ripe and ready to consume fruits. Three levels of acetamiprid were prepared, a recommended concentration (200 ppm) and two other concentrations which were higher than the recommended one (300 and 400 ppm). The two higher concentrations were proposed to discover the side effects of acetamiprid on human beings if the farmers were restored to this application in order to protect their crops away from spoilage (tomato were conducted under greenhouse conditions).

Acetamiprid disappearance curves

Fig.3 shows the disappearance of 200, 300 and 400 ppm concentration of acetamiprid as a function of time. There was a direct relationship between the concentrations of acetamiprid residues within tomato fruits and the time up to three days of application. A decline in acetamiprid residue concentrations (of 200 and 300 ppm) was observed after the third day up to 11 and 13 days respectively (the concentration is less than the MRL). By using 400 ppm acetamiprid the residues, even after 14 days are more than MRL. A higher migration of acetamiprid was taken place during the first 3 days of application. The acetamiprid was subsequently degraded due to many internal and external factors. As shown in Fig.3, the migration was occurred through the tomato peel and was reached its higher value at the third day of field spraying. As shown in the Fig. 3, a remarkable migration of acetamiprid (0.48 ppm), through the peel, was observed after 1 hour of application (high initial acetamiprid concentration on tomato fruits) as compared with the next periods (0.48 ppm comparing with about 0.025 ppm per hour during the first day of application by using 200 ppm).

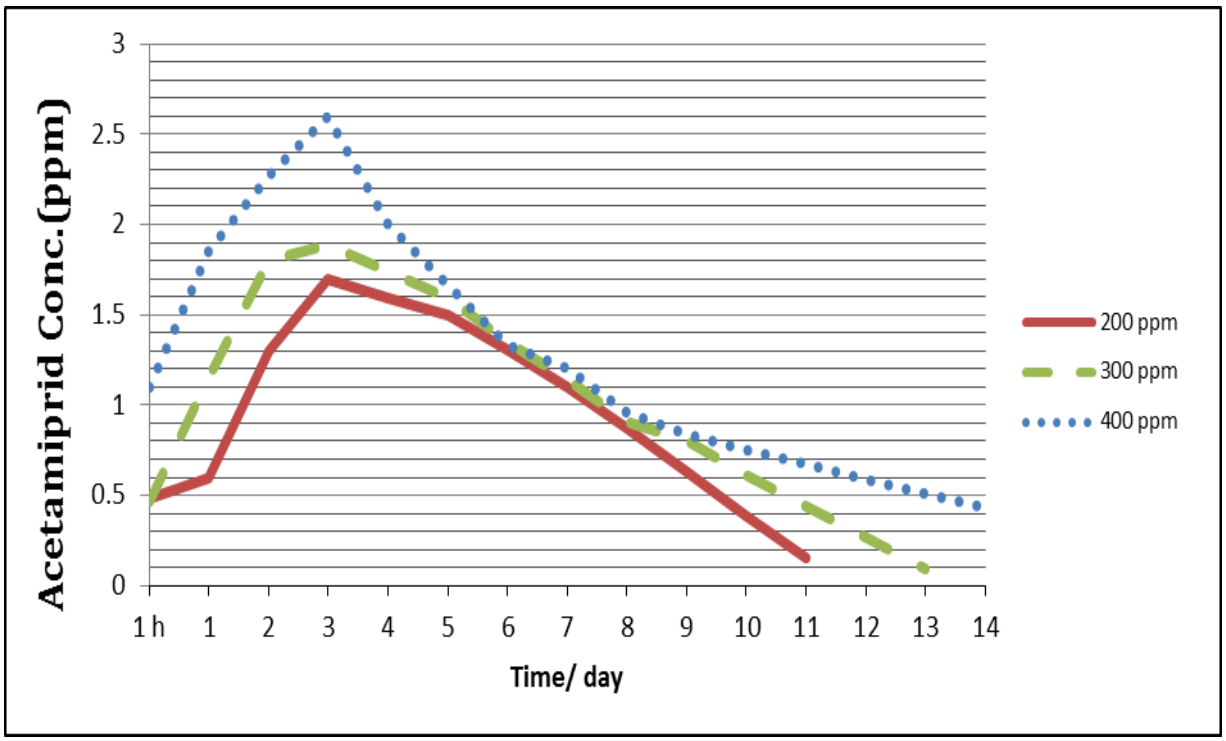


Fig. 3. Disappearance of acetamiprid as a function of time

Fig. 3 showed that after 7 days of application, the acetamiprid residues within tomato fruits were more than the MRL. Thus we are not recommending consuming fresh ripening tomato fruits after 7 days of application. A pesticide's fate is affected by the pesticide's physical and chemical properties and the natural processes that occur in the environment.Fate refers to the pattern of distribution of an agent, its derivatives or metabolites in an organism, system, compartment or (sub) population of concern as a result of transport, partitioning, transformation or degradation (14). Although degradation of pesticides is influenced by different environmental processes (4), but under natural field conditions, volatilization is the main process that affects pesticides

Animal house results

One of the most important targets in this study is to illustrate the bad effects of consuming tomato fruits with acetamiprid residues. The present study proposed to determine the effect of orally ingestion different concentrations of acetamiprid (32, 64 and 128 ppm) on the blood profile of male mice. Table 1, shows a direct relationship between acetamiprid concentrations and triglycerides levels, total cholesterol, LDL and VLDL in male mouse blood (Normal triglycerides in mice 45-65 mg/dL).Increase in the triglyceride levels indirectly indicating to liver damage **(**11). The table also shows a direct relationship between acetamiprid concentrations and total cholesterol levels. The effect of acetamiprid on total cholesterol levels revealed dose dependent increase in levels as compared to control group (rats) (22, 6). A direct relationship between LDL levels and acetamiprid concentrations is observe in Table 1. LDL represents the bad part of cholesterol which contributes mainly inhuman being CHD. The results are in agreement with Roy(17) when used thiacloprid (Neonicotinoid pesticides) for 28 days in rats. Table 1 shows a reverse relationship between the HDL levels in male mice blood and acetamiprid concentrations. The relationship refers to a bad effect of acetamiprid on human health, because HDL represents the good cholesterol. A low HDL-cholesterol concentration is considered to be a value below 35 mg/dL, and high HDL, ≥60 mg/dL (12). Table 2 shows a direct relationship between the concentrations of acetamiprid and urea and creatinine levels. The results were agreed with what was found by (2), that urea was significantly increased in fish blood serum treated with acetamiprid after 28 days of exposure which in turn refers to a physiological damage occurred in kidney tissue.

Table 1. Effect of acetamiprid on some blood profile parameters in male mice

Group	Mean ± SE				
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
32 ppm	85.00 ± 2.88 b	89.00 ± 1.73 bc	21.33 ± 0.88 b	45.87 ± 2.34 b	17.80 ± 0.34 bc
64 ppm	94.00 ± 1.52 b	110.33 ± 9.06 ab	17.67 ± 0.67 bc	54.27 ± 1.62 b	22.07 ± 1.81 ab
128 ppm	131.00 ± 9.54 a	127.33 ± 14.62 a	14.33 ± 1.20 c	85.20 ± 7.63 a	25.47 ± 2.92 a
Control	79.00 ± 1.00 b	57.50 ± 2.50 c	26.50 ± 2.50 a	41.00 ± 1.00 b	11.50 ± 0.50 c
LSD value	19.148 *	32.891 *	4.132 *	2.598 *	6.578 *

* (P<0.05).
Means having with the different letters in same column differed significantly

Table 2. Effect of acetamiprid on urea and creatinine of male mice blood

Group	Mean ± SE	
	Urea (mg/dl)	Creatinine (U/L)
Control	29.50 ± 2.50 c	0.285 ± 0.03 c
32 ppm	44.00 ± 2.31 b	0.640 ± 0.06 b
64 ppm	48.00 ± 1.15 b	0.943 ± 0.04 a
128 ppm	57.60 ± 2.33 a	1.130 ± 0.10 a
LSD value	7.402 *	0.241 *

* (P<0.05).
Means having with the different letters in same column differed significantly

Table 3 shows the effect of acetamiprid on liver enzymes. There was a direct relationship between acetamiprid concentrations and AST, ALT, Superoxide dismutase (SOD), catalase activities but there was a reverse relationship with AchE activity. Many researchers (5, 19, 25) found, there was significant increases in mice plasma levels of AST and ALT at higher doses of acetamiprid. Many authors (5) reported that the activity of SOD enzyme was significantly increased after 90 days of exposure to acetamiprid as compared with control group. This is explained by the depletion of this enzyme by following repeated exposure to acetamiprid which generates ROS during its metabolism (including quinones derivatives). The increasing in SOD (a parameter that is not considered here) leads to an increase of H_2O_2 production and also causes reduction in catalase (8). MDA was as a marker of lipid peroxidation (LPO) of liver in treated mice. As shown in table 3, a significant increase in hepatic level of MDA in animals treated with higher doses of acetamiprid. These results were associated with what was found by (5, 9, 22). An article (24) mentioned was investigated that catalase was significantly increased (dose-dependent manner) after the application of acetamiprid in certain bacterial strains of Gram negative and Gram positive suggested probable cause of certain oxidative stress. A reverse relationship was observed between acetamiprid concentrations and AchE activity (Table 3). The activity of AchE was inhibited in mice brain. The results were in agreement with the study results by (3,23) whom found that the activity of AchE was decreased in rats brain administered orally with imidacloprid (neonicotinoid insecticide as acetamiprid) by 20 ppm/day for 90 days.

Table 3. Effect of acetamiprid on blood enzymes of male mice

Group	Mean ± SE					
	AST (U/L)	ALT (U/L)	SOD U/L	CAT mg/dl	MDA U/L	AchE U/L
32 ppm	70.66 ± 1.61 c	22.67 ± 2.73 c	1.83 ± 0.04 c	2.12 ± 0.11 b	1.89 ± 0.24 c	488.33 ± 10.27 b
64 ppm	84.67 ± 2.90 b	38.00 ± 2.64 b	2.98 ± 0.32 b	2.58 ± 0.11 b	2.86 ± 0.07 b	459.33 ± 16.25 b
128 ppm	114.00 ± 6.65 a	48.67 ± 3.75 a	4.23 ± 0.21 a	3.90 ± 0.40 a	4.01 ± 0.33 a	345.33 ± 19.23 b
Control	34.50 ± 0.74 d	15.00 ± 3.00 c	1.70 ± 0.40 c	2.05 ± 0.45 b	1.83 ± 0.13 c	600.00 ± 32.00 a
LSD value	11.52 *	10.65 *	0.853 *	0.957 *	0.802 *	62.32 *

* (P<0.05).

Means having with the different letters in same column differed significantly

Table 4 discusses the effect of acetamiprid on the hormones levels. There was a reverse relationship between the concentrations of acetamiprid and FSH, LH and testosterone levels. The reduction in LH levels due to the damage in testes tissue, because the spermatogenesis is a process under hypophyseal hormonal control that involves gonadotropin synthesis of LH and FSH, and they act on Leydig and Sertoli cells, respectively (21). The study showed that acetamiprid treatment caused altered or arrested spermatogenesis with degeneration of seminiferous tubules of testes. The results were associated with what was found by (22, 26). The levels of testosterone in serum and testicular homogenate were significantly declined in acetamiprid treated groups as compared with control which was agreed with (22, 26).

Table 4. Effect of acetamiprid on blood hormone levels in male mice

Group	Mean ± SE		
	FSH (mlU/ml)	LH (mlU/ml)	Tes.. (mlU/ml)
32 ppm	2.213 ± 0.16 a	1.510 ± 0.04 b	3.243 ± 0.21 a
64 ppm	1.810 ± 0.34 ab	1.051 ± 0.08 c	2.313 ± 0.17 b
128 ppm	1.1266 ± 0.21 b	0.9466 ± 0.06 c	2.115 ± 0.10 b
Control	2.395 ± 0.16 a	1.915 ± 0.13 a	3.830 ± 0.49 a
LSD value	0.911 *	0.283 *	0.884 *

* (P<0.05).

Means having with the different letters in same column differed significantly

Acetamiprid residues were remained in tomato fruits (more than MRL) even after 7 days of application. A remarkable migration of acetamiprid was occurred via tomato skin into tomato flesh after 3 days of application (systemic insecticide). When the farmers using more than recommended concentration of acetamiprid (such as 400 ppm), the residues detected even after 15 days of spraying. Acetamiprid residues have bad effects on blood profile of the male mice.

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