

ADVERSE EFFECTS OF FENVALERATE ON SOME BLOOD HEMATOLOGICAL PARAMETERS AND THE DEVELOPMENT OF CHICKEN EMBRYOS

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ABSTRACT

The side effects of fenvalerate as a pyrethroid insecticide on some blood parameters of chicken embryos injected with doses of 1/10, 1/50, 1/100, 1/250, 1/500 and 1/1000 of the LD₅₀ were investigated. Results showed that fenvalerate caused significantly ($P < 0.05$) decrease of packed cell volume (PCV %), hemoglobin concentration (Hb) and red blood cell count (RBCs). The treatment with 1/500 dose from fenvalerate was most effective in the reduction of RBC counts and increased mean cell volume (MCV), mean cell hemoglobin (MCH). Chicken embryos injected by 1/500, 1/1000 of the LD₅₀ of fenvalerate caused a significant ($P < 0.05$) increase in the activities of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which considered as biochemical indicators of liver damage, heart and brain. Mean values of early dead embryo and develop abnormality of chicken embryos percentages were increased with the treatment by 1/10, 1/50, 1/100, 1/250, LD₅₀ fenvalerate doses. Hatchability percentage was reduced. In general, the results indicated that the injection of embryos by fenvalerate increased the embryos develop abnormally and mortality rate and changes in some blood parameters, which included the suppression of erythropoiesis and hemoglobin synthesis. Also, fenvalerate caused significant ($P < 0.05$) increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are considered as biochemical indicators of liver, heart and brain damage.

INTRODUCTION

The loss of food grain during storage due to various insect pests is a very serious problem. Climate and storage conditions, especially in the tropics and sub-tropics, are often highly favorable for insect growth and development. In most countries, post – harvest losses in cereals and pulses due to attack by insects have been estimated at 20-30% (Niber et al., 1992).

In recent years there has been an increasing concern over the possible environmental hazards posed by chemical pollutants particularly pesticides and heavy metals on wildlife WHO (1984) and El-Gendy et al. (1988). Pesticides are broad spectrum biocides toxic not only to target pests, but also to no targets including wildlife; Hill and Mendenhall (1980), Deweese et al. (1983), Wiemeyer et al (1984) and Littrell (1988).

Pyrethroids were used in many countries throughout the world to control insect pests in stored grains (Solyman, 2003).

Mohamed (1988) found that daily doses of fenvalerate and deltamethrin caused a significant fall in hemoglobin content and a reduction in the number of red blood cells in male albino rats. Shakoori et al (1990) when administered female rabbits to 10 mg a.i./kg body weight for 7 days of bifenthrin in order to evaluate the toxicity in non- target organisms 29, 42, 5, 3, and 30% decreased in the total erythrocyte count, total leukocyte, packed cell volume and hemoglobin content respectively, after 4 days of bifenthrin administration. Also 54 and 34% increased in the mean of corpuscular hemoglobin and the mean of corpuscular hemoglobin concentration were found respectively. Essawy et al. (1994) administered male albino rats with the synthetic pyrethroid lambda-Cyhalothrin (175-200 mg) in doses of 1.0 and 4.0 mg/ kg body weight daily for 65 successive days. A decrease in erythrocyte counts, packed cell volume and hemoglobin concentrations and an increase in white blood cell counts due to increased neutrophils and lymphocytes were reported.

Therefore, the aim of the present study was to study the adverse side effects of sub- lethal doses from fenvalerate on some blood hematological parameters on the development of chicken embryos.

MATERIALS AND METHODS

The experimental work was carried out in the Animal and poultry production Department, Faculty of Agriculture (Damanhour), Alexandria University through 2004.

FENVALERATE:

a- Chemical name: (RS)- α - cyano- 3- phenoxybenzyl (RS)- 2-(4-chlorophenyl)- 3- methyl butyrate.

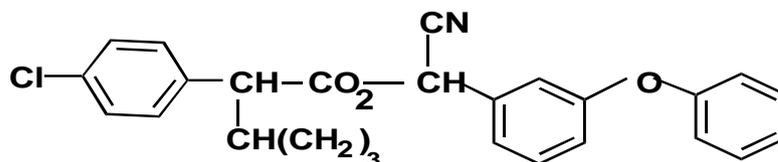
b- Common name: fenvalerate.

c- Trade name: Sumicidin and Somi-alpha.

d- Molecular formula: $C_{25}H_{22}ClNO_3$.

e- Molecular weight: 419.9

f- Structural formula:



g- Formulations: Somi-alpha 5%EC was used, UL, SC, and WP.

h- Toxicity: Acute oral LD_{50} for rats is 451 mg/kg.

A total number of 350 fertile eggs from Golden Montazah Strain were randomly selected and divided into 7 groups of 50 eggs were incubated in incubators, with an average weight of 57.4 ± 1.56 g. For each the third day of incubation, 50 fertile eggs from each group were injected with 1/10, 1/50, 1/100, 1/250, 1/500 and 1/1000 LD_{50} fenvalerate, while, the control group was injected with buffer solution by the same volume, using a hypodermic needle, through a hole puncture in the outer shell, the needle was inserted through the air sac. The holes were sealed with liquid paraffin after inoculation, then returned to the incubator. All unhatched eggs were opened at 21st day of incubation to be examined for evidence of stage of embryonic mortality, which were classified as early dead that died before 7 days and late dead embryos (7-21days). External pipped (beak penetrated the egg shell and dead in shell) were calculated. Hatchability was calculated as a percentage of fertile eggs.

After hatching, the blood samples from slaughtered chickens were taken randomly from five chickens from each group to determine some of their blood haematological parameters. Red blood cells (RBC) were counted on an AO Bright line hemocytometer using a

light microscope at 400x magnification. Hemoglobin (Hb) concentration was determined by the cyanomethemoglobin procedure (Eilers, 1967). Wintrobe hematocrit tubes were used for determination of hematocrit value (HV).

The mean cell volum (MCV) , mean cell hemoglobin (MCH), and the mean cell hemoglobin concentration (MCHC) were referred as absolute values. These values were calculated from the results of red blood cell count, hemoglobin concentration and hematocrit value, respectively. These values have been widely used in the classification of anemia.

Mean cell volume (MCV):

$$\text{MCV} = \frac{\% \text{ Hematocrit} \times 10}{\text{Number of RBC}} \quad (\text{micron}^3 / \text{red blood cell}).$$

Mean cell hemoglobin (MCH):

$$\text{MCH} = \frac{\text{Hemoglobin concentration (g/dl)} \times 10}{\text{Number of RBC}} \quad (\mu\text{g})$$

Mean cell hemoglobin concentration (MCHC):

$$\text{MCHC} = \frac{\text{Hemoglobin (g/dl)}}{\% \text{ of hematocrit}} \times 100 \quad (\%)$$

After blood samples were taken, liver, heart, gizzard, yolk sac and brain were removed and weighed, individually, for the nearest 0.01 gm. Data were statistically analyzed by analysis of variance and significant difference of means were tested using Duncan's multiple range test described by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

The mean values of early dead embryo, pipped embryo, hatchability percentage and relative hatchability values to the control percentages for different fenvalerate doses are presented in Table (1). The mean values of early dead embryo percentages were an increased

with the treatment by 1/10, 1/50, 1/100, 1/250, LD₅₀ fenvalerate doses. The percentages of increase ranged between 60 to 100.

The observed increasing of early embryonic mortality at 1/10: 1/250 LD₅₀ fenvalerate doses may be due to the increased fenvalerate doses. These results agreed with the findings of Varnagy et al.(2002) who observed that the increase incidence rate of mortality when the pesticide was injected into the eggs air space in a volume of 0.1 ml/egg of Ross breed.

On the other hand, the mean values of hatchability percentages were 70.0, 40.0 and 50.0% for control groups and 1/500, 1/1000 fenvalerate dose, respectively. Injected eggs with 1/10, 1/50, 1/100, 1/250, and 1/500 significantly reduced hatchability (0.00%) and increased develop abnormalty of the embryos. The similar results was obtained by Elawar (1990). Who found that the hatchability was reduced to 0.0% as compered to 80% when carbaryl pesticide was injected in fertile egg on day 15 of incubation.

Results in Table (2) showed that the tested fenvalerate significantly affect the percentage of hematocrit value (HV). The treatment of 1/500 LD₅₀ fenvalerate was significantly decreased HV with the percentage (8.39%) as comperad with the control group. The decrease in HV is obviously contributed by the decrease of cellular count in blood of chicken embryos that given sub- lethal doses of fenvalerate. The obtained findings were agree with that of many authers. Nassar (2001) showed that the values of PCV% in blood of white male rats given repetitive oral doses of λ -cyhalothrin, cypermethrin, deltamethrin and fenvalerate were 33.25, 36.33, 31.5, and 24.0% respectively. It was noticed that the PCV values in the blood of pyrethroid – treated rats were decreased as compered with untreated rats . The percentages of decrease were 13.6, 5.6, 18.2 and 37.7 %, respectively. The percentage of decrease was more pronounced in the fenvalerate treatment.

On the other hand, Abbassy *et al.*, (2002) showed that the tested pyrethroids did not significantly affect the percentage of PCV, except the repetitive dose treatments with both forms of cypermethrin or deltamethrin and the formulated form of cyhalothrin which decreased it in percentage ranged from 10.9 to 25.2 %.

Data in Table (2) show that treatment of chicks with repeated doses of the fenvalerate decreased the count of red blood cells (RBCs) significantly ($P < 0.05$). The treatment with a dose of 1/500 of LD_{50} from fenvalerate was most effective in the reduction of RBC counts. The administration of fenvalerate in a dose of 1/500 LD_{50} dose resulted in suppression of erythropoiesis and hemoglobin synthesis. This results agree with the results of Essawy *et al.*, (1994) and Saxena (1997). They found decrease in erythrocyte in the blood of albino rats after cypermethrin treatment. Shakoori *et al.* (1992) suggested that the reduction in RBC and Hb content could be probably due to the blockage of protein synthesis and histogenesis.

Results recorded in Table (2) show that the fenvalerate doses did not significantly affected the percentage of the hemoglobin concentration (Hb) and mean cell hemoglobin concentration (MCHC). Nassar (2001) revealed that the concentration of hemoglobin was decreased in the blood of cypermethrin, deltamethrin and fenvalerate – treated rats. The percentages of the decrease of Hb, when calculated relative to Hb concentration in the blood of untreated rats, were 6.7, 29.7, and 5.5% respectively. Also, no significant changes were recorded in hemoglobin content, hematocrit value and RBCs counts in mice treated with repeated sublethal doses of cypermethrin (EL-Gendy *et al.*, 1999).

Data in Table (2) revealed that mean cell volume (MCV) increased in the fenvalerate treated chicks. The increased in MCV and MCH were more significant in the 1/500 LD_{50} fenvalerate treated chicks.

The previous effect of pyrethroids, decrease and / or increase in hemoglobin concentration (Hb), hematocrit value (HV), red blood cell counts (RBC's), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in blood of pyrethroids treated embryos were in agree with the results of other several authors. Mohamed (1988) found that daily doses of fenvalerate and deltamethrin caused a significant fall in hemoglobin content and a reduction in number of RBC's, of treated rats. Shakoori *et al.*, (1990) found decrease in erythrocyte, PCV, and an increase in mean cell hemoglobin and mean cell hemoglobin concentration in the blood of rabbits after bifenthrin treatment.

Means weight of liver, heart, gizzard, yolk sac and brain, as a percentage of live body weight for chicken embryos treated with different doses of fenvalerate are presented in Table (3). Data indicated a significant ($P<0.05$) effect of fenvalerate doses on a heart, gizzard, yolk sac and brain relative weight. Fenvalerate caused degenerative changes in the brain and heart of chicken. Changes were more intense in chicken which was injected with fenvalerate by 1/500 LD₅₀ dose. On the other hand, body weight of newly hatched chickens was not different from those of control. However, there was a slight increase in body weight of 1/500 LD₅₀ of fenvalerate treated group. These changes which found may be due to the toxicological effects of fenvalerate on the development of embryos, which caused water retention and non absorption of the yolk sac nutrient.

Results recorded in Table (4) showed that fenvalerate doses (1/500, 1/1000 Of the LD₅₀) caused a significant increase in the activities of both AST and ALT. Percentage of increase in AST with 1/500 and 1/1000 LD₅₀ doses of fenvalerate, compare to control were 13.83 and 19.62 %, respectively. The corresponding percentages for ALT were 17.47 and 37.47 %, respectively. Fenvalerate exposed chicken embryos showed a significant increase in the transaminases activities. One of the most indicators for liver damage and function is the increase of transaminases activities (GOT and GPT) in the serum which has been recorded by Hayes et al. (1989) and Shakoori *et al* (1992). Transaminases (AST and ALT) are important and critical enzymes in biological processes. They play a role in amino acids catabolism and biosynthesis, consequently they are considered as specific indicators for liver damage (Harper, 1979). Several investigators have been showed that the activities of transaminases were increased in human and animals after exposure to pesticides (Abbassy *et al.*, 1989; Shakoori *et al*, 1992; Abbassy *et al*, 1992; Majumder *et al.*, 1997; Gomes *et al*, 1999; Kalaf- Allah, 1999; Youssef *et al*, 1999 and Abbassy *et al*, 2000).

It can be concluded that , the administration of fenvalerate irrespective to the dose- caused a significant decrease in the number of red blood cells and hemoglobin concentration and significant increase in transaminases activities (AST and ALT) which indicate that the pyrethroid had adverse effects on the blood picture and the liver of chicken embryos.

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Table1: Embryonic mortality, pipped chicks and hatchability of chicken embryos exposed to different doses of fenvalerate (X +S.E)

Items	Cont	1/10	1/50	1/100	1/250	1/500	1/1000
No of fertile egg	50	50	50	50	50	50	50
Dead%	30	100	80	80	60	40	-
Pipped%	-	-	20	20	40	20	50
Hatchability%	70	0.0	0.0	0.0	0.0	40.0	60.0

Table 2: Blood hematology of chicken embryos exposed to different doses of fenvalerate (X +S.E)

	Cont	1/10	1/50	1/100	1/250	1/500	1/1000
PCV	41.8+11.8 ^a	0.0	0.0	0.0	0.0	38.2+0.31 ^b	42.7+1.29 ^a
HB	18.2+0.50 ^a	0.0	0.0	0.0	0.0	17.6+0.42 ^a	18.5+0.64 ^a
RBC	2.78+0.22 ^a	0.0	0.0	0.0	0.0	2.14+0.17 ^b	2.78+0.14 ^a
MCV	153.5+11.1 ^b	0.0	0.0	0.0	0.0	182.3+12.5 ^a	154.1+2.93 ^b
MCH	66.8+4.52 ^b	0.0	0.0	0.0	0.0	83.8+5.49 ^a	67.6+4.94 ^b
MCHC	43.8+2.11 ^a	0.0	0.0	0.0	0.0	46.0+0.73 ^a	43.7+2.38 ^a

Means with the same letter in each row are not significantly different

Table 3: Organs weight percentage of chicken embryos exposed to different doses of fenvalerate (X +S.E).

Dose. Para.	cont	1/10	1/50	1/100	1/250	1/500	1/1000
Body wt. at hatch	36.8+1.94	0.0	0.0	0.0	0.0	38.0+0.96	37.3+0.20
Liver %	3.55+0.35	0	0	0	0	4.05+0.39	3.39+0.28
Heart%	0.96+0.09 ^a	0.0	0.0	0.0	0.0	0.74+0.03 ^b	0.85+0.03 ^b
Gazzred%	9.33+0.59 ^a	0.0	0.0	0.0	0.0	4.97+0.47 ^b	9.09+0.40 ^a
Yolk sac	8.90+0.92 ^b	0.0	0.0	0.0	0.0	10.6+2.06 ^a	7.66+0.30 ^b
Brain%	2.35+0.12 ^a	0.0	0.0	0.0	0.0	1.87+0.10 ^b	2.23+0.11 ^b

Means with the same letter in each row are not significantly different

Table 4: Aspartate aminotransferase (AST) and Alanine aminotrasferase (ALT) activities of chicken embryos exposed to different doses of fenvalerate (X +S.E).

Para.	cont	1/10	1/50	1/100	1/250	1/500	1/1000
AST (U/L)	70.0+0.17 ^c	0.0	0.0.	0.0	0.0	82.0+0.02 ^b	86.1+0.70 ^a
ALT (U/L)	11.1+0.27 ^c	0.0	0.0	0.0	0.0	13.0+0.33 ^b	15.2+0.26 ^a

Means with the same letter in each row are not significantly different

الملخص العربى

التأثيرات الضارة للفينفاليرات على بعض مكونات الدم وتطور أجنة الكتا كيت

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تم دراسة التأثيرات الجانبية الضارة لمبيد الفينفاليرات على بعض مكونات الدم ، تم حقن المبيد بالجرعات 10/1، 50/1 ، 100/1 ، 250/1 ، 500/1 ، 1000/1 من الجرعة النصف مميتة خلال الغرفة الهوائية للبيض عند اليوم الثالث من نمو الأجنة.

وتوضح النتائج مايلى :-

المعاملة بمبيد الفينفاليرات تسبب إنخفاض معنوى فى حجم كرات الدم الحمراء، وتركيز الهيموجلوبين وعدد كرات الدم الحمراء. المعاملة بـ 500/1 من الجرعة النصف مميتة من مبيد الفينفاليرات كانت أكثر تأثيرا فى خفض عدد كرات الدم الحمراء وزيادة متوسط كل من حجم الخلية و هيموجلوبين الخلية.

المعاملة بـ 500/1 ، 1000/1 من الجرعة النصف مميتة تسبب زيادة معنوية فى نشاط إنزيمات AST and ALT . زيادة إرتفاع نسبة كل من الأجنة الميتة المبكرة و الكتا كيت المشوهة نتيجة المعاملة بـ 50/10، 100/1 ، 250/1 من الجرعة وكذلك إنخفاض نسبة الكتا كيت الفاقسة.

وبصفة عامة :-

يمكن القول أن حقن الأجنة بمبيد الفينفاليرات تسبب زيادة كل من نسبة الأوضاع الشاذة و نفوق الأجنة. كذلك حدوث تغيرات فى بعض مكونات الدم مثل إنخفاض معدل تخليق كرات الدم الحمراء والهيموجلوبين. تودى المعاملة بالفينفاليرات إلى حدوث زيادة معنوية فى نشاط إنزيمات AST and ALT والتي تعطى دلالة على حدوث تغيرات غير طبيعية فى خلايا الكبد والمخ والقلب.