Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 1045-1050

Received: 27-03-2017
Accepted: 28-04-2017

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Investigation of acute toxicity of Malathion and Cypermethrin on Clinico-hematological parameters in female New Zealand White Rabbits (*Oryctolagus cuniculus*)

Mussarrat Shaheen and Ali Muhammad Yousafzai

Abstract
The aim of the present study was to find the clinical and hematological alterations in female rabbits exposed orally to Malathion and Cypermethrin at a dose of 75mg/kg body weight for seven days through gavage. Animals were divided into three groups i.e. A, B and C comprised of three rabbits each. Group A was considered as control. Group B was intoxicated with Malathion while C was Cypermethrin intoxicated group. All the animals were monitored twice a day for clinical signs. Signs like grinding of teeth, salivation, licking of legs, incoordination, dizziness, increased urinations, and jerky movements persisted for 30 to 90 minutes after intoxication. Malathion intoxication exhibited significant decrease in MCH and PLT and showed decrease in WBCs and MCHC levels. It also produced increase in RBCs, PCV and MCV levels. Cypermethrin administration produced increase in WBCs, RBCs, PCV, MCH, PLT and significant increase in MCHC. It is concluded from the present results that both the insecticides were neurotoxic in nature and have leukopenic and leukemic tendency for female rabbits

Keywords: Pesticides, Malathion, cypermethrin, haematology

1. Introduction
The use of pesticides is expanded many folds i.e. to improve the crop production and human health by controlling or eliminating unwanted insects, animals and plants as well as disease vectors [1]. The excessive use of pesticides for pest control has been widely used in agriculture, however their chaotic use has charged serious harms and problems to human as well as to the biodiversity [2, 3]. Cypermethrin is commonly used pyrethroid which is used extensively in the control of house household and agricultural pests all over the world. The genotoxic effects of cypermethrin is well known [4]. Pyrethroid insecticides affect the permeability of the Na+ voltage dependent channels in the nerve cells. This effect causes the membrane depolarization and synaptic disturbances which are responsible for the hyper excitability in cases of intoxication [5, 6]. Cypermethrin is classified as a possible human carcinogen, because it causes an increase in the frequence of lung tumors in female mice. It has been linked to an increase in bone marrow micronuclei in both mice and humans [7].
Malathion is an organophosphorus insecticide abundantly used in agriculture and houses for the control of diseases vectors. It is a major source of environmental poisoning in developing countries [8]. Malathion itself is of low toxicity; however, absorption or ingestion into the human body results in its metabolism to malaoxon, which is more toxic. In studies the effects of long-term exposure to oral ingestion of malaoxon in rats, malaoxon has been shown to be 61 times more toxic than malathion [9]. Malathion is a lipophilic substance, it may enhance lipid per oxidation by directly interacting with the cellular plasma membrane [10] so it may harm the membranes by inducing lipid per oxidation. Haematological studies are significant because blood is the major transport system of the body, and evaluations of the haematological profile usually provides vital information on the body's response to injury of all forms, including toxic injury [11].
The present investigation was aimed to find out the toxicological effects of malathion and cypermethrin on clinico- hematological parameters in female rabbit´s blood.

2. Materials and Methods

The study was carried out in September 2014 for seven days in the animal house of Islamia college university Peshawar. Nine female New Zealand white rabbits (*Oryctolagus cuniculus*) of about one year age and free from any apparent clinical ailments were procured from the local market. Rabbits were divided into three groups A, B, and C with three rabbits each. Group A was control without any treatment. Group B was Malathion intoxicated while C was cypermethrin treated with a dose of 75mg/kg body weight for 7 days respectively. The rabbits in each group were observed for clinical and behavioral changes twice a day. The marginal ear vein of the rabbits were used to collect the blood samples. At the end of intoxication period the blood samples were collected in the ethylene diamine tetra acetic acid (EDTA) tubes to prevent blood clotting. Blood samples were shifted to ice box and were transported to the laboratory for hematological analysis. The collected blood was used for the estimation of various hematological parameters. Automated Haematology Analyzer Sysmex KX-21N was used for analysis of various hematological indices.

2.1 Statistical Analysis

The data were analyzed by using Analysis of variance (ANOVA) and the mean values were compared by SPSS version (16). The significance level was $p<0.05$.

3. Results

Five to ten minutes after the administration of 75mg/kg body weight of both malathion and cypermethrin doses, the female rabbits started showing clinical signs like grinding of teeth, salivation, sneezing, watery eyes, licking of legs, scratching of skin, incoordination, dizziness, itching, increased urinations, jerky movements and muscular tremors which persisted for 30 to 90 minutes after intoxication.

The studied hematological parameters showed a drastic change in the Malathion and Cypermethrin treated samples as compared to the control samples (Table, I and Fig.1).

Mean± S.E value for WBCs in control samples was 2.47±0.03 $\times10^3/\mu l$, while in malathion intoxicated samples it was 1.83±0.03$\times10^3/\mu l$ and in cypermethrin administered samples it was 4.67±0.12$\times10^3/\mu l$ (Table, 1). The results from ANOVA following t-test showed that malathion in comparison with control samples exhibited a highly significant decrease ($P<0.001$) in WBCs, while the cypermethrin administered samples exhibited a highly significant increase ($P<0.001$) (Fig. 1.1). The value of red blood cells (RBCs) count in control group was 4.73±0.035$\times10^6/\mu l$ while in malathion and cypermethrin treated groups was 5.05±0.085$\times10^6/\mu l$ and 5.29±0.35$\times10^6/\mu l$ respectively. A non-significant increase in both intoxications was observed (Fig 1.2). Haemoglobin value for control sample was 9.73±0.19 g/dl while in Malathion intoxicated sample was 9.53±0.03g/dl and in cypermethrin treated group was 11.17±0.545 g/dl (Table, 1). Malathion administered group displayed a non-significant decrease and cypermethrin treated group showed an increase (Fig. 1.3).

Packed cell volume (PCV) value in control group was 33.7±0.25 % while in malathion and cypermethrin treated group it was 35.63±0.69% and 37.47±1.66% respectively (Table, 1). The results showed an increase in both malathion and cypermethrin intoxicated groups respectively (Fig 1.4). The value of mean corpuscular volume (MCV) in control group was 70.13±0.318fL while in Malathion it was 72±0.305fL and cypermethrin samples was 68.97±1.35fL (Table, 1). Malathion fed group exhibited an increase while Cypermethrin samples showed decrease in MCV level (Fig. 1.5).The value of Mean corpuscular hemoglobin (MCH) for control samples was 20.13±0.04pg/cell while in Malathion administered group was 18.2±0.35pg/cell and cypermethrin treated samples was 20.59±0.31pg/cell (Table, 1). Malathion fed group exhibited a significant decrease while cypermethrin intoxicated group presented an increase (Fig. 1.6).

The value of mean corpuscular hemoglobin concentration (MCHC) of control group was 26.17±0.34g/dl while in Malathion treated samples it was 25.57±0.13g/dl and cypermethrin administered group was 29.97±0.18g/dl respectively (Table, 1). Malathion fed group in comparison with control revealed a decrease in MCHC value while cypermethrin administered group showed a significant increase (Fig 1.7). Platelet count (PLT) in control group was the 328.67±14.25 $\times10^3/\mu l$ while in malathion and Cypermethrin administered samples was 164.33±4.98$\times10^3/\mu l$ and 365±61.73$\times10^3/\mu l$ respectively (Table, 1). Malathion intoxicated group exhibited a more significance decrease while cypermethrin treated group revealed an increase (Fig. 1.8).

4. Discussion

Clinical signs of both malathion and cypermethrin toxicity in female rabbits started 5 to 10 minutes after the administration of both insecticides, and persisted for 30 to 90 minutes. Signs like Itching, scratching of skin, licking of legs and other body parts, grinding of teeth, sneezing, incoordination, dizziness, increased urinations, jerky movements and muscular tremors were observed in both the intoxicated groups. Skin irritation has also been presented in a previous study with cypermethrin treatment in rabbits [12]. The same results such as incoordination, muscular tremors, jerky movements, and dizziness were also observed in cypermethrin intoxicated rabbits. The gating characteristics of voltage-sensitive sodium channels were modified by pyrethroid insecticides in mammalian neuronal membranes [13] to delay their closure which produces a protracted sodium influx. Itching, restlessness and head shaking seem to be nervous signs produced by both malathion and cypermethrin by the mechanism of blockage of sodium channels.

In the present study, among the hematological indices WBCs in malathion treated samples exhibited highly significant decrease while the cypermethrin administered samples showed significant increase. WBCs are the important part of the immune system, because of their main defensive function, they respond immediately to any change in the medium due to xenobiotic transformation. The significant decrease and increase shows that WBCs is the primary infected hematological parameter which reflects the activation of animal defense line against the toxicant or it also shows the blood leukemic tendency during which the number of WBCs increases, while a decrease in number of WBCs may probably be due to the disruption of WBCs synthesis sites in the bone marrow, the disease is commonly known as leukopenia. A highly significant increase in WBCs count after carbofuran and permethrin intoxication in New Zealand white rabbits were observed [14]. Same results in male rabbits after cypermethrin administration were observed [15].

In the current study Red blood cells (RBCs) in both Malathion and Cypermethrin treated samples was increased. Similarly permethrin administration in male rabbits also caused a significant increase in the number of RBCs [14]. A similar significant increase in rabbits after cypermethrin

administration was reported [15]. In another study a significant decrease was observed in RBC count after cypermethrin treatment which is contradictory to the current study [16]. The increased value of RBCs might be due to kidney and pulmonary diseases, dehydration and polycythemia vera, a condition which is characterized by proliferation or hyperplasia of all bone marrow cells, with an increase in red blood cell [17].

The value of hemoglobin in the present study showed a decrease in malathion administered sample while in cypermethrin treated group it exhibited an increase. In another study significant decrease in hemoglobin level is presented in rabbits after bendicarbamate administration [18], while reduction in hemoglobin level was also observed in rabbits after cypermethrin administrations [16, 15]. A highly significant decrease in Hb in another investigation was reported after administration of permethrin in a medium dose to rabbits [19] Low hemoglobin level might weakens the biosynthesis of haeme in bone marrow while at a low and high dose Hb content in rabbits was highly significant increased and unchanged respectively. In another study a highly significant decrease in Hb level in male rabbits after 75mg/kg carbofuran administration for 10 days and also reported a highly significant increase in Hb after Permethrin administration in a dose of 75mg/kg for 10 days [14]. Exposure of pyrethroid damaged the haemopoitic tissues as a result the erythrocyte got damaged [20]. Therefore reduced haemoglobin and total red blood cells count could possibly be due to suppression of erythropoiesis and haeme biosynthesis. Oxidative damage to haemoglobin due to haemolysis led to reduction in total erythrocyte count and haemoglobin. Hypoxia, anemia and hyperthermia are related stresses and decrease the capacity of red blood cells to carry sufficient oxygen unless otherwise compensated by erythropoiesis. The decreased availability of oxygen generally increased the synthesis of hemoglobin, release of blood cells from storage site and enhanced erythropoiesis [21].

In the present investigation, PCV in both malathion and cypermethrin treated groups exhibited an increase. PCV showed highly significant decrease in 75mg/kg and 20mg/kg carbofuran treatment groups in rabbit while in 10mg/kg chronic test in rabbit showed a highly significant increase in rabbit, while Permethrin administered groups in all the three doses showed highly significant increase in rabbit [14]. A significant increase in PCV in male rabbits was reported after cypermethrin administration [15] while a significant decrease in PCV was observed in rabbits after cypermethrin administration [16]. Mean corpuscular volume (MCV) in the present study malathion treated samples exhibited an increase and in cypermethrin treated samples its value is decreased. Hemoglobin content and WBC count remained unaltered, while the RBC and PCV decreased significantly [22]. Levels of hematocrit, hemoglobin, leukocytes and red blood cells and MCHC decreased with increase in cypermethrin concentrations, MCV level was found to be increased and MCH level was not affected [23]. In another study significant increase were found in the TLC, Lymphocytes and MCV in the test group of rabbits [24].

In the present investigation malathion caused significant decrease in both MCH and MCHC levels while cypermethrin caused significant increase in both parameters in female rabbit. Same increase in MCH and MCHC levels in male rabbit after administration of cypermethrin was presented [15]. A similar significant decrease in MCH and MCHC was observed after administration of bendicarbamate in rabbit [18].

In the current study Platelet count (PLT) in malathion administered sample showed a more significance decrease and in cypermethrin intoxicated samples it showed an increase. The significant decrease in hematological indices WBCs, RBCs, Hb and PCV might be caused by the effects of pesticide on liver and bone marrow (blood producing organs) and restriction of many stages of Hb synthesis in rabbits as the results of pesticides administration [25].

The decrease in PCV (Packed cell volume) in medium and high dose treated rabbits was obviously contributed by the decreased cellular counts in blood after cypermethrin treatment. In addition, the reduction in blood parameters (PCV, Hemoglobin and RBCs) may be attributed to hyperactivity of bone marrow [26], leading to the production of red blood cells with impaired integrity which were easily destroyed in circulation by reticuloendothelial system.

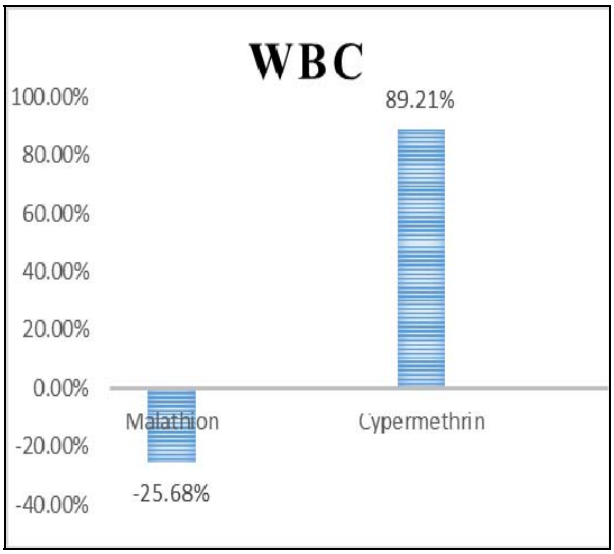
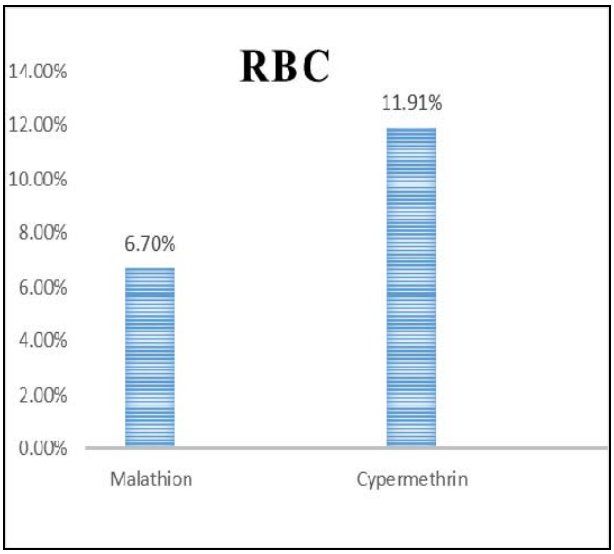


Fig 1.1



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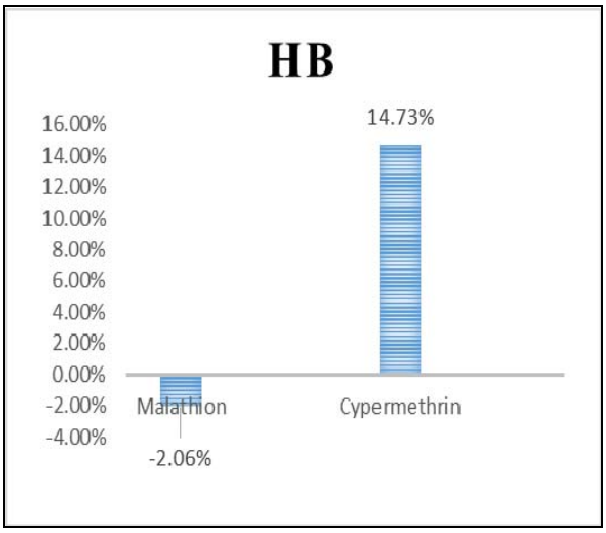

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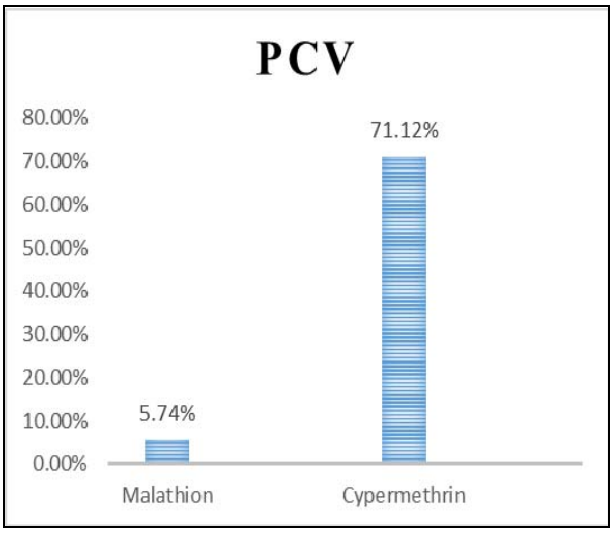
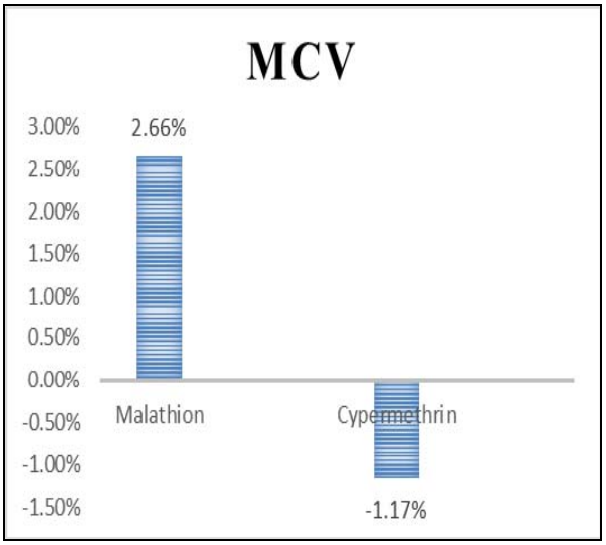


Fig 1.4



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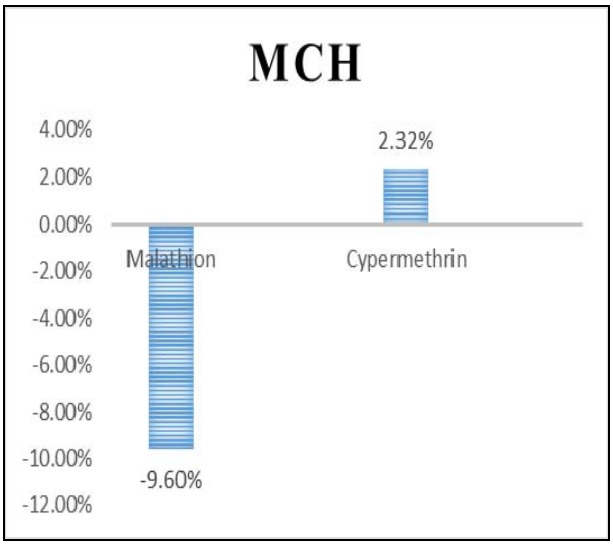

Fig 1.6

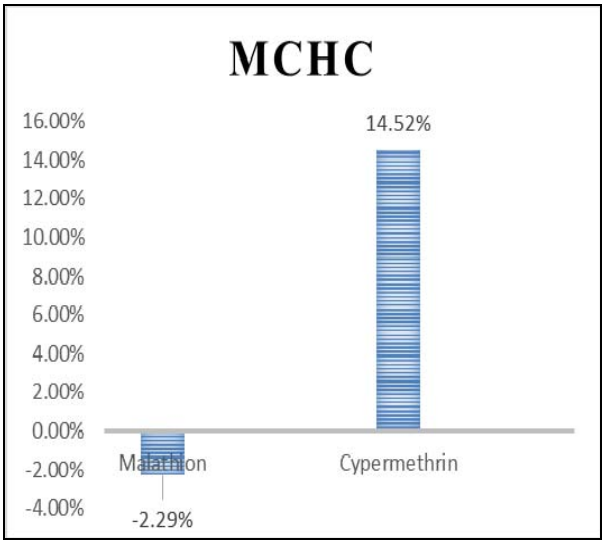

Fig 1.7

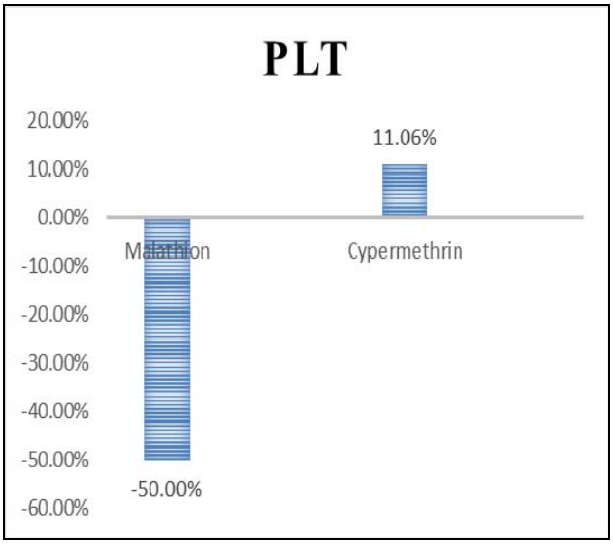

Fig 1.8

Fig 1: Hematological parameters after 7 days of oral administration of Malathion and Cypermethrin in a dose of 75mg/kg to female New Zealand White Rabbit showing % increase (+) or decrease (-).

Abbreviations used: White blood corpuscles (WBCs), red blood corpuscles (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT).

Table 1: Hematological components of blood of *Oryctolagus cuniculus* after treated with 75mg/kg body weight malathion and cypermethrin for 7 days.

S. No	Parameters (Units)	Control	Malathion	Cypermethrin
1	WBC(TLC)×10^3/µl	2.47±0.03	1.83±0.03***	4.67±0.121***
2	RBC×10^6/µl	4.73±0.035	5.047±0.085*	5.29±0.349
3	Hemoglobin g/dl	9.73±0.19	9.53±0.03	11.17±0.55
4	HCT(PCV) %	33.7±0.25	35.63±0.696	37.47±1.66
5	MCV fL	70.13±0.318	72±0.305	68.97±1.35
6	MCH pg	20.13±0.033	18.2±0.35*	20.59±0.31
7	MCHC g/dl	26.17±0.338	25.57±0.13	29.97±0.18*
8	Platelet Count ×10^3/µl	328.67±14.25	164.33±4.98**	365±61.73

The values are expressed as mean of three rabbits Mean± SEM, Student's "t" test, **P*<0.05, ***P*<0.01, ****P*<0.001

Abbreviations used: White blood corpuscles (WBCs), red blood corpuscles (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT).

5. Conclusion

The present study provides the information about the clinical and hematological alterations in rabbits due to oral exposure of malathion and cypermethrin. It also revealed that these insecticides adversely affect the nervous system and showed anaemic, leukopenic and leukemic tendency in female rabbits. Both malathion and cypermethrin are generally toxic to many non-targeted species including man and other desirable forms of life. From the present study it is suggested that insecticides must be examined for their possible adverse effects on animals and human before their application to agricultural fields.

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