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# INTERACTION OF SOME INSECTICIDES AND HEAVY METALS WITH JAPANESE QUAILS ENZYME SYSTEMS

BY

K.S. EL-Gendy, A.A. El-Warraky, N.S. Ahmed, S.A. Soliman and A.H. El-Sebae

*Plant Protection Department, Faculty of Agricultrure, Alexandria University, Alexandria, Egypt.*

## ABSTRACT

The levels of lead ($Pb^{+2}$), copper ($Cu^{+2}$) and cadmium ($Cd^{+2}$) in blood of both domestic and wild quails were monitored. Lead content in domestic quail (4.97 ppm) exceeded that in the wild ones (3.35 ppm). Relatively low levels of copper were detected. Cadmium in the wild, slightly exceeded that in the domestic ones. On the other hand, eight enzyme systems were assayed. They were acetyl cholinesterase (AChE), butyryl cholinesterase (BuChE), adenosine triphosphatase (ATPase), acid phosphatase (APase), glutathione s-transferase (GST), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and delta aminolevulinic acid dehydratase (δ-ALAD). The interaction of heavy metals with quail enzyme systems was studied. Generally, δ-ALAD was affected by most of the heavy metals, although it is highly responsive to lead. Mercury and cadmium showed mild inhibition of AChE and BuChE. The largest interaction of mercury was shown as higher inhibition of APase. On the other hand, thiodicarb showed full inhibition of AChE, while fenvalerate did not show any interaction with AChE. BuChE was moderately inhibited by both insecticides.

## INTRODUCTION

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. The toxic metals are known to cause serious adverse biochemical effects; Zemansky (1974), White and Finley (1978), Eastin et al. (1983), WHO, (1984) and El-Gendy et al. (1988). Pesticides are broad spectrum biocides toxic not only to target pests, but also to nontargets including wildlife; Hill and Mendenhall (1980), Deweese et al. (1983), Wiemeyer et al. (1984) and Littrell (1988).

The objective of this study is to investigate the monitoring of heavy metals (lead, cadmium and copper) pollutants in blood of both domestic and wild quails, and estimating the activity of different enzymes: AChE, BuChE, APase, ATPase, GST, GOT and GPT in liver and δ-ALAD in blood of quails. In addition, the study includes the interaction of lead, cadmium mercury, copper, thiodicarb and fenvalerate with quil enzyme system.

## MATERIALS AND METHODS

**Tested Compounds:-**

Technical grade samples of thiodicarb (larvin), 99.5% was supplied by Union Carbide Company, USA. Fenvalerate (Sumicidin), 99.5% was obtained from Sumitomo Chemical Company, Japan. Copper sulphate, mercuric chloride, cadmium sulfate and lead acetate are pure grade chemical.

**Tested bird:**

Domestic and wild quails were used in this study. The species used is Japanese quail (Coturnix coturnix japonica). Domestic quails locally bred at the Poultry Research farm in Abees, Faculty of Agriculture, Alexandria University. The quails were 45 days old with body weight ranging between 100-120 gm each. Wild quails were trapped in the Alexandria Vicinity, each weighed 60-70 gm.

Methods:-

Domestic and wild quails were sacrificed. Blood samples were collected for determination of metal content of lead, cadmium and copper according to Zinterhofer, et al (1971) method, using atomic absorption spectrophotometer Shimadzu. Also, the blood used for determination of δ-ALAD activity according to Burch and Siegel (1971) procedure. Then, the birds were dissected liver was removed rapidly, weighed and homogenized in 10 volume (w/v) of suitable buffer. Homogenates were centrifuged at 6000 x g for 30 minute. The supernatant obtained were used for determination of different enzymes. In the case of ATPase the supernatant was centrifuged again at 17,000 x g for 30 minutes, and the mitochondrial pellets were resuspended and used for assay ATPase activity using the method of Koch, (1969). AChE and BuChE activities were determined according to Ellman, et al. (1961). GST activity was measured by the simplified procedure of Vessey and Boyer (1984). GOT and GPT were assayed according to the Reitman and Frankel method (1957) using a commercially available Kit from bioMerieux. APase activity was determined according to

Bessey, et al. (1946) . Protein was determined according to Lowry et al. (1951).

The in vitro effect of the tested compounds on the enzymes studied were determined using different concentrations, and the percent inhibition was calculated.

## RESULTS AND DISCUSSSION

### 1. Monitoring of Heavy metal Pollutants in Wild and Domestic Quail:-

Table 1 presents the levels of lead, copper and cadmium in blood of both domestic and wild quail. The level of $Pb^{+2}$ in domestic quail (4.97 ppm) exceeded that in the wild ones (3.35 ppm). This might reflect a local exposure to heavily polluted environment with lead in-put source. The detected levels of lead in blood samples of both domestic and wild quail far exceed the normal levels in wildlife birds abroad; Finley et al. (1976), Eastin, et al. (1983) and Dieter et al. (1976).

Relatively, low levels of copper content were detected in the blood samples of both strains. Again, the domestic quail showed higher levels of copper content, which might indicate higher intake values of copper in the diet, water and the enviornment. However, no acute hazard is expected from such low levels of detected copper.

Cadmium level in the wild quail slightly exceeds that in the domestic one. However, this range of $Cd^{+2}$ is higher than the normal or the permissible levels (WHO, 1984). It is assumed that contaminated feed and water might be the two main sources for in-put of $Cd^{+2}$ as a pollutant, which is capable of being accumulated in the biological tissues. Biomagnification of accumulated levels are expected along the food chain (El-Sebae, 1986).

### II. Activity of Different Enzymes in Both Wild and Domestic Quails:-

Eight enzyme systems were selected to be monitored in the wild and domsestic quails (table 2). The data showed almost similar specific activities for AChE and GST. On the other hand, the three enzymes (GOT, GPT and APase) which are indicative of liver damage showed lower activities in the domestic quail. The values of specific activity were almost 50% of that of the wild quail. Similarly, the activity of the δ-ALAD in blood of domestic quail

was lower than that of the wild ones. This can be easily correlated with heavier level of lead pollutant for the domestic quail blood samples as recorded in table (1).

Table 1
Lead, cadmium and copper levels in blood of domestic and wild quail (Coturnix coturnix Japonica).

| | Concentration (ppm) Mean±SD | |
|---|---|---|
| Metals | Wild quails | Domestic quails |
| Lead | 3.350 ± 0.530 | 4.97 ±1.550 |
| Copper | 0.345 ± 0.162 | 1.04 ± 0.162 |
| Cadmium | 0.490 ± 0.150 | 0.47 ± 0.063 |

ATPase activity was opposite, being higher in domestic quail than wild ones. This may be due to the exhaustion and fatigue of the wild birds, as they reach the Alexandria coast after their long travel. The long consumption of stored energy might leave little room for ATPase additional activity.

The general picture shows how the type of environment and the living habits affect the performance of the biological activities including enzymes which have been adapted to fulfil the requirements of the newly adapted ecological and environmental conditions.

## III. Interaction of Mercury, Cadmium, Lead and Copper with Quail Enzyme Systems:-

The comparative interaction of mercury, cadmium, lead and copper with the selected enzyme systems in liver and blood of both wild and domestic quails is indicated in table (3), where equimolar concentrations ($1X10^{-4}$) of $HgCl_2$, $CdSO_4$, $Pb(CH_3COO)_2$ and $CuSO_4$ were used. Mercury effect was demonstrated in the appreciable inhibition of AChE and BuChE in both wild and domestic quails. The highest interaction of mercury was shown as higher inhibition of acid phosphatase; 95.8 and 87.32% in wild and domestic quails respectively. Interaction of mercury with δ-ALAD in blood was relatively higher than that of cadmium and copper. ATPase, GST, GOT and GPT were not actually affected by mercury. The data also reflects the known fact that mercury is not only a protoplasmic poison, but it is also a neurotoxic agent affecting the cholinergic system with higher affinity towards the acetyl choline

Table 2

Activity of different enzymes of wild and domestic quail.

| Enzymes | Units | S.A. Mean ± SD wild quail | Domestic quail |
|---|---|---|---|
| AChE | μ moles ATCh/ mg protein/ minute | 4.1180 ± 1.060 | 4.246 ± 2.020 |
| BuChE | μ moles BuTCh/ mg protein/ minute | 4.6900 ± 2.490 | 7.200 ± 1.800 |
| GST | OD/ mg protein/ minute | 0.3920 ± 0.085 | 0.329 ± 0.054 |
| GOT | Units/ ml | 14.090 ± 1.620 | 7.500 ± 0.621 |
| GPT | Units/ ml | 15.710 ± 1.480 | 8.410 ± 1.350 |
| ATPase | μ moles of Pi/ mg protein/ hour | 0.0715 ± 0.007 | 0.400 ± 0.012 |
| APase | μ moles of p nitrophenol/ mg protein minute | 6.3500 ± 1.490 | 3.168 ± 0.250 |
| δ-ALAD | Units/ ml erythrocytes/ hour | 204.71 ± 27.56 | 194.0 ± 22.17 |

$$\text{Units of ALAD activity} = \frac{\text{Corrected Absorbance} \times 100 \times 12.5 \times 10}{\text{Hematocrite}}$$

where : 12.5 is diluted factor of the blood.

Table (3)

In Vitro inhibition of some enzymes of wild and domestic quail by 1 x $10^{-4}$ M $HgCl_2$, $CdSO_4$, $CuSO_4$ and Pb $(CH_3COO)_2$.

| Enzymes | % Inhibition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HgCl2 | | CdSO4 | | CuSO4 | | Pb(CH3COO)2 | |
| | Wild | Domestic | Wild | Domestic | Wild | Domestic | Wild | Domestic |
| AChE | 74.37 | 61.08 | 25.00 | 22.38 | 57.5 | 75.07 | 13.04 | 02.87 |
| BuChE | 65.87 | 59.85 | 48.38 | 42.33 | 63.26 | 63.54 | 28.55 | 13.88 |
| ATPase | 04.76 | 20.68 | 31.81 | 17.27 | 05.12 | 32.75 | 27.93 | 10.34 |
| APase | 95.80 | 87.32 | 17.10 | 04.88 | 01.9 | 03.25 | 18.30 | 10.15 |
| GST | 40.25 | 9.06 | 17.02 | 35.19 | 43.43 | 51.78 | 12.50 | 12.60 |
| GOT | 25.88 | 42.07 | 11.09 | 03.64 | 04.53 | 14.3 | 06.58 | 5.41 |
| GTP | 22.26 | 28.22 | 42.67 | 18.13 | 10.62 | 17.66 | 10.20 | 12.30 |
| S-SALAD | 70.27 | 67.59 | 63.88 | 52.31 | 60.19 | 60.40 | 77.39 | 94.90 |

receptor leading to blocking of the receptor function. Dieter and Ludke 1975).

Generally cadmium did not show high specific inhibitory effect to any enzyme of the tested ones except for the δ-ALAD. which is affected by most of the heavy metals. Cadmium was almost inert with these particular enzymes.

Also. most of the classical enzymes were not affected by lead except δ-ALAD in blood. Rozman et al. (1974) , proved similarly that lead shots did not cause inhibition of GOT and GPT enzyme systems in serum of mallard ducks. their results support in general. the present data. Lead was the most potent inhibitor of the δ-ALAD. Dieter. et al. (1976). Dieter and Finley (1979). Franson et al. (1983) and El-Gendy et al. (1988). This agrees with the known high affinity of lead towrads this blood enzyme. which is considered the specific target for lead and which is used for monitoring the impact of lead exposure Stone. et al. (1977), demonstrated that the activity of red blood cells δ-ALAD in Japanese quail is a very sensitive indicator of lead exposure. The highest recorded inhibition of δ-ALAD by lead in the domestic quail more than the wild quail, might be attributed to the already recorded heavier load of lead pollutant in blood of domestic quail (table 1). On the other hand. copper showed mild interaction and inhibition with AChE , BuChE, GST and δ-ALAD. While ATPase. APase. GOT and GPT were not significantly inhibited in liver of both wild and domestic quails.

## IV.Interaction of Thiodicarb and Fenvalerate with Quail Enzyme Systems.

Comparative inhibition percentages of equimolar concentrations of thiodicarb and fenvalerate insecticides are shown in table (4). Thiodicarb showed almost full inhibition of AChE in liver of both wild and domestic quail. while BuChE was moderately inhibited. This reflects the high specificity of thiodicarb as AChE inhibitor more than the BuChE. This study agrees with Rattner. et al. (1982) and Grue (1982). Thiodicarb did not show specific affinity to ATPase. APase. GST. GOT and GPT. On the other hand. fenvalerate did not show any interaction with AChE. while it showed mild inhibition of BuChE inidcating a non specific interaction with this insecticide. Similar mild interaction of fenvalerate with liver ATPase. acid phosphatase and GST were also recorded. However. it is clear that none of these enzymes are the site of action of the synthetic pyrethroids and hence none of these enzymes can be used to assess exposure to synthetic pyrethroids. On the contrary. the acute toxicity exposure of thiodicarb can be detected by monitoring AChE levels.

Table (4): In vitro inhibition of some enzymes of wild and domestic quail by 1 X $10^{-4}$M thiodicarb and fenvalerate.

| Enzymes | % Inhibition | | | |
|---|---|---|---|---|
| | Thiodicarb | | Fenvalerate | |
| | Wild | Domestic | Wild | Domestic |
| AChE | 100.00 | 96.70 | 25.00 | 21.08 |
| BuChE | 59.44 | 68.93 | 50.00 | 48.22 |
| ATPase | 38.31 | 12.50 | 39.70 | 18.75 |
| APase | 12.66 | 06.87 | 51.35 | 52.23 |
| GST | 06.95 | 03.36 | 41.66 | 14.46 |
| GOT | 01.76 | 08.54 | 04.35 | 04.64 |
| GPT | 18.62 | 03.80 | 06.76 | 00.715 |

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## الملخص العربـــى

تم قياس تركيز بعض المعادن الثقيله فى دم كل من السمان البرى والداجن . وقد وجد فى السمان البرى تركيز الرصاص بالجزء فى المليون ٣٫٣٥ ، والنحاس ٣٤٥ر ، والكادميوم ٤٩ر ، أما فى السمان الداجن فكانت كالتالى ٤٫٩ ، ٤٥ر١ ، ٤٧ر على التوالى . كما قدرت عدة أنزيمات فى كبد كل من السمان البرى والداجن وهـى : الاسيتايل كولين استريز ، والبيوتيريل كولين استريز ، والادينوسيـــن ترای فوسفاتيز ، والاسيد فوسفاتيز ، والجلوتاثيون أس ترانس فيـــرايـــز والجلوتاميك أوكسالواسيتات ترانس أمينيز ، والجلوتاميك بيروفيك ترانس أمينيـــــز . كما قدر انزيم الدلتا أمينو ليفيولينيك أسيد ديهيدراتيز فى دم السمان . وقد درس تأثير مبيد الثيودای كارب والغنطاليرات وأربع معادن ثقيله ( الرصاص ـ الكادميـــوم النحاس والزئبق ) على هذه النظم الانزيميه . وأوضحت النتائج ان مبيد الثيـــودای كارب أحدث تثبيطا لانزيمى الاسيتايل كولين استريز والبيوتيريل كولين استريز . أمـا بقية الانزيمات فكان التثبيط فيها منخفض . وأحدثت كل المعادن الثقيله المختبره تثبيط واضح لانزيم الدلتا أمينو ليفيولينيك أسيد ديهيد راتيز وكان أكثرهم تأثيرا هـو الرصاص ( ٩٤٪ ) وأظهر الزئبق والنحاس تأثير واضحا على انزيمى الاسيتايل كولين استريز والبيوتيريل كولين استريز . كما ثبط الزئبق انزيم الاسيد فوسفاتيز بنسبة ٨٧٪ .