

Effect of fungicide (Rizolex-T50%) on hepatorenal functions in *Coturnix coturnix japonica*

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ABSTRACT

We offered male Japanese quail (10 wks age 250-300gm wt) food fortified with fungicide Rizolex-T50% at three concentrations 0.1, 0.25 and 1 of its field rate (FR) (3gm Rizolex added to 1 kg food). Rizolex was mixed well with food. Results were recorded after one, five and ten days. AST, ALT, total protein and bilirubin in serum were determined as immediate indicator for liver function. Furthermore, we detected serum creatinine level as indication to kidney function. We followed food intake for 10 days and recorded the results after one, five and ten days.

Our results revealed that there were decreases in AST in general. There were changes at the 3 tested concentrations after 5 and 10 days in ALT. Total protein appeared to have various differences at all concentrations and at all time intervals. Creatinine level increased significantly at FR and its quarter after feeding for 10 days on contaminated bait. We could not detect any change in food consumption except at 1/4 FR after 5 and 10 days. In addition, a significant change at FR feeding on the treated bait appeared after 10 days.

Keywords: fungicide - hepatorenal functions - *Coturnix coturnix japonica*

INTRODUCTION

In recent years, the use of insecticides has increased intensively as means of crop protection. Intensive use of pesticides in agriculture and veterinary practices has been associated with numerous health problems in human and animals (Benbrook, 1991). One of the major problems associated with pesticides use is the possibility of its biological accumulation in animals and poultry products (Hisashi *et al.*, 2006; Ellis, 1989; Kutches *et al.*, 1970). Moreover avian populations have a role in the ecosystem as seed dispersal, pollination, recolonization and restoration of disturbed ecosystems (Sekercioglu *et al.*, 2004).

Easily, the pesticides can reach the birds directly or/and indirectly through the food chain. Rizolex, that contains phosphorus and carbamate pesticides, Tolclofos-methyl + Thiram, is used as fungicide used as seed treatment. Exposure to organophosphate (OP) and carbamate (CB) pesticides interfere with bird's ability to discriminate contaminated foods. Reductions in food consumption are frequently associated with OP and CB intoxication with symptoms of gastrointestinal stress observed in birds following acute OP or CB exposure (Gure *et al.*, 1991).

The aim of this study is to determine the toxicity of the fungicide Rizolex on Japanese quail. The toxic effect was determined on both liver and kidney functions via the following measurements: AST, ALT, total protein, bilirubin and creatinine. Also, the effect of Rizolex on food consumption was determined.

MATERIAL AND METHODS

Pesticides used:

Rizolex-T50% WP

Active ingredient(s)

[a\) Tolclofos-methyl](#) [b\) Thiram](#)

Chemical class of AI(s)

a) Organophosphate ester b) Dimethyldithiocarbamate

Mode of action (for targeted pest)

Non-Systemic contact with protective & curative

Usage

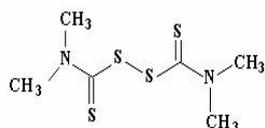
Fungicide

AI concentration(s)

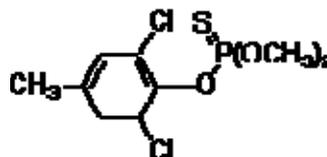
a) 20% b) 30%

Formulation Type

WP



Thiram



[Tolclofos-methyl](#)

Test animals:-

Male Japanese quail 10-week old *Coturnix coturnix japonica* weighing 200-300gm was obtained from a local supplier birds (Ain Shams Farm) and maintained in bird cages under room condition for 15 days before feeding.

Experimental design:

Twenty eight birds weighing 200-300gm each were divided into four groups. The first one was left to eat food mixed with the filed rate (FR) (3gm Rizolex +1kg seeds). The second group ate food mixed with 1/4 FR and the third one fed food mixed with the 1/10 FR. The fourth group served as a control. The birds were left to eat the contaminated food for 24 hrs then the blood samples were taken for the biochemical analysis and also samples were taken after eating for 5 and 10 days. Also bird food consumption was determined after 1, 5 and 10 days.

Biochemical analysis:

- 1- Serum Aspartate Amino Transferase (SAST) or Glutamic Oxaloacetic Transaminase (SGOT) was analyzed according to Bergmeyer and Horder (1980).
- 2- Serum Alanine Aminotransferase (SALT) or Glutamic Pyruvate Transaminase (SGPT) was analyzed according to Bergmeyer and Horder (1980).
- 3- Bilirubin was analyzed according to Walter and Gerade (1970).
- 4- Total protein was analyzed according to Tietz (1994).
- 5- Creatinine was analyzed according to Bartles *et al.* (1972) and Larsen (1972). Also the food consumption was determined.

Statistical analysis:

The significance of the difference among groups was analyzed using ANOVA followed by the least significant difference test for mean separation. A (P) value <0.05 was considered significant. All statistical analysis waves made using the computer software COSTAT. Acute toxicity results were analyzed using the Dose – Reponses software program (Pc Probit) based on Finney analysis (Finney, 1971).

RESULTS

Data presented in Table (1) reflect the extent of harmful effects on male Japanese quail. Regarding liver enzymes AST, ALT, using 0.1gm of Rizolex , there were no differences in their values for three time intervals except ALT after five days

of treatment. This showed lower significant value as it showed 17.67 ± 63 compared to 121.67 ± 10.97 .

When males were treated with 0.25 (of field rate) AST showed lower significant values after one and five days. Whereas after ten days there was no difference from the control value

Table 1: Effect of Rizolex on renohepatic functions after introduced treated bait with field rate or 1/4 or 1/10 field rate to Japanese quail males.

Tests	Field rate(gm)	Time after treatment			L.S.D
		1 st day	5 th day	10 th day	
AST(U/L)	0.10	84.67± 07.51 a	84.57±07.51 a	75.00±24.25 a	30.532
	0.25	52.57±35.38 a	54.67±36.70 b	73.00±05.20 a	62.795
	1.00	84.67 ±07.51 a	10.00±30.00 c	67.33±08.51 b	13.534
Control		84.67 ±7.51 a			
ALT(U/L)	0.10	121.67±18.97 a	017.67±6.35 b	121.67±10.93 a	19.335
	0.25	113.00±25.98 a	113.00±25.93 a	113.00±25.98 a	51.906
	1.00	021.33±6.35 b	121.63±10.97 a	121.67±10.97 a	19.336
control		121.67±10.97 a			
Total protein (mg/dl)	0.10	034.37±5.04 b	129.71±27.99 a	116.86±1.78 a	32.870
	0.25	160.00±5.51 b	147.71±27.99 c	185.14±0.86 b	06.610
	1.00	145.71±1.48 b	116.29±01.31 b	079.71±2.27 c	03.470
control		103.14±0.49 a			
Bilirubin (g/dl)	0.10	05.98±0.29 a	3.83±0.79 b	1.04±1.34 c	1.809
	0.25	23.01±0.09 b	0.19±0.03 c	3.92±0.14 b	1.054
	1.00	06.95±0.21 a	3.31±0.21 c	9.20±0.08 a	0.362
Control		7.47±0.53 a			
Creatinine (mg/dl)	0.10	13.85±0.13 a	04.22± 0.11 b	13.62±0.11 b	0.226
	0.25	14.23±0.22 a	15.36± 1.00 a	15.78±0.22 b	1.213
	1.00	11.45±0.11 c	13.526± 0.16a	19.38±0.06 b	0.223
control		14.37±0.11a			

Moreover AST recorded values in male quail treated with 1gm of Rizolex, after one day there was no difference, whereas it showed highly significant lower value 10 ± 30 and lower significant value after ten days post treatment.

Determining of total protein, there was significant values at 0.1 and 0.25 after one day of treatment, with 0.25 of field rate. There was also significant increase at the 5 periods post treatment.

Apply the field rate, there was a significant increase in total protein after one and 5 days. On the other hand, there was a significant decrease after 10 days post treatment, since the total protein content was 79.71 ± 2.27 comparing with 103.14 ± 0.49 for the control.

Concerning bilirubin values, it appeared that 0.1 FR of field rate caused no difference comparing with the control after one day of treatment since the value was 5.98 ± 0.29 and 1.04 ± 1.34 after one and 10 days respectively. After five days, there was significant decrease, while it showed highly significant decrease after ten days of treatment.

Using 0.25 FR, there was a significant increasing of 23.01 ± 0.09 after one day post treatment comparing with the control 7.47 ± 0.53 . But after five and ten days, there were highly significant decrease values 0.19 ± 0.03 and 3.92 ± 0.14 respectively.

Treatment of male Japanese quail at field rate caused no differences after one and ten days. But it caused highly significant decrease in bilirubin after five days 3.31 ± 0.21 .

Determination of creatinine as indicator for Rizolex effect on kidney function: As creatinine determination, application of 0.1 FR 13.85 ± 0.29 had no variance between the value after one day and the control 14.37 ± 11 . On the other hand, there

was a significant difference at 5th and 10th day post treatment, since the values were 4.22 ± 0.11 and 13.62 ± 0.11 respectively. With 1/4 FR, there was a significant variance only at the 10th day comparing with the control. With the field rate, there was a significant decrease and increase after one and 10 days respectively comparing with the control.

Data in Table (2) refer to the consumption of food of different concentrations by treated birds. After one day feeding on treated food with 0.1, 0.25 or 1, of field rate, the food consumption slightly decreased with no significant variance comparing with the control. After 5 days (18.76gm) treated the food (with 1/4 field rate) food consumption of decreased significantly comparing with the control 32.5. After 10 days, bird feeding on treated foods, with 1/4 and field rate, the food consumption decreased significantly, with a value of 21.53 and 25.368 respectively comparing with 28.01 for a longer period decreased the food consumption. At the same time, bird feeding on treated food with fungicide, a concentration as low as 0.1 field rate did not affect the food consumption even the birds fed for 10 days on treated food.

Table 2: Mean of food consumption by Japanese quail after 1, 5 or 10 days feeding on treated baits of Rizolex.

Treatment (as field rate)	Days after treatment		
	1	5	10
0.10	a 27.803	a 29.400	a 28.284
0.25	a 24.393	b 18.761	b 21.534
1.00	a 27.090	a 33.420	b 25.368
control	a 32.127	a 32.566	a 28.018
L.S.D 5.477	L.S.D 6.034	L.S.D 12.265	

DISCUSSION

Because of the intensive use of pesticides either indoors or outdoors, many deleterious effects may be appeared on the human beings and other animals. Rizolex is a fungicide, a mixture of (OP) and (CB) and used as seed protective agent.

Studies must be done to throw light on Rizolex effect on body systems especially the kidney and liver. As we know, liver is the largest organ in the body that responsible for excretion of the toxic materials and many other vital operations in the body. Also, kidney does an essential role to excrete the liquid toxic materials. We determined the effect of field rate of Rizolex (3gm fungicides + 1kg food) when the *Japanese quail* ate a bait treated with it for one, 5 and 10 days. Also, the 1/10 and 1/4 the field rate was tested in the same manner. The enzymes, AST, ALT, total protein and bilirubin were determined as liver functions and creatinine as a kidney function. Researches in this respect show that with pesticides treatment the enzymes level in the blood increased as a reflection of liver disturbance because of the deleterious effect of the pesticides on the liver cells. AST is a sensitive marker of liver damage, even if the damage is of a sub clinical nature (Kauppinen, 1984; Meyer and Harvey, 1998). Moreover, AST was found to be secreted into the blood during hepatocellular injury (Kalender *et al.*, 2005). ALT is a more specific maker of hepatic injury (Ballantyne, 1988). Both ALT and AST are enzymes produced by hepatocytes and they are involved in the metabolism of amino acids and synthesis of proteins. In damaged cells, these enzymes, leak into the blood stream (Mansour and Mossa, 2010). Changes

in AST level because of pesticides treatment may differ depending on the exposure time and dose. This increase occurs in parallel to time (Seko *et al.*, 2008). Our results, in general, show that the liver enzymes were not affected because Rizolex is one of the pesticides that had no high toxic effects. The concentrations applied (field rate, 1/10 and 1/4 field rate) are low to achieve high activity. Also, the time after treatment where the results were registered is short (1, 5 and 10 days post treatment).

Also, kidney was affected as a result of pesticides treatment and the creatinine level show an elevation in the body.

When the birds ate contaminated baits with Rizolex, and the samples were taken after one, five and 10 days post treatment the fungicide has no obvious bad effects on both liver and kidney functions, perhaps the compound did not represent the extreme toxic material.

Moreover, the bird feeding on the toxic bait with Rizolex is not sufficient to produce deleterious effects. Perhaps the bait was not as good taste as normal for birds to feed on. So, when birds were eating bait mixing with the 1/4 FR they ate more quantity of food and they were more exciting comparing with birds fed untreated food. This result encourages the farmers to mix this fungicide with seeds as seed protective safe agent to gain a high percentages of seed protection and there high germination.

Kidney functions affected significantly with all tests rates after 10 days of the treatment. Perhaps the kidney did its best to go toxic material (fungicides) out of the body.

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ARABIC SUMMARY

تأثير فطريات (Rizolex-T50%) على وظائف الكبد في ذكور طائر السمان

إيمان أ. عبد الهادي و فاطمة م. الجوهري
معهد وقاية النباتات ، الدقى ، القاهرة

تم تقديم عليقة لذكور طائر السمان عمر 10 أسابيع ووزن 250-300 جرام مقسمة إلى ثلاثة أقسام ، مضاف للأول المعدل الحقلى (3جم /1كيلو جرام عليقة ، والثانى مضاف له 10/1 المعدل الحقلى والقسم الثالث مضاف له ربع المعدل الحقلى) . وتم قياس إنزيم AST ، ALT ، البليروبين ، البروتين كدلائل لوظيفة الكبد وقياس الكرياتينين كدليل على وظيفة الكلية بالإضافة إلى كمية المستهلك من الغذاء وذلك بعد يوم ، 5 ، 10 أيام من المعاملة.

أوضحت النتائج وجود نقص فى إنزيم AST بعد 5 ، 10 أيام من المعاملة بينما البروتين أظهر فروقاً مع كل التركيزات سواء زيادة أو نقصاً.

زاد مستوى الكرياتينين عند التركيز الحقلى ، ربعه بعد 10 أيام من التغذية على الغذاء المعامل.

ولم تُلاحظ فروق فى استهلاك الغذاء ما عدا عند تركيز 4/1 المعدل الحقلى بعد 5 و10 أيام من المعاملة. بالإضافة الى وجود اختلاف عند معدل استهلاك الغذاء المعامل بالمعدل الحقلى وذلك بعد التغذية عليه لمدة عشرة أيام .