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INFLUENCE OF TWO PLANT EXTRACTS AND PIRIMIPHOS-METHYL ON SOME BIOCHEMICAL ANALYSIS IN JAPANESE QUAIL BIRDS

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ABSTRACT

The adverse effects of extracts derived from clove, *Eugenia aromatica* and anise, *Pimpinella ansium* compared to an organophosphate insecticide, pirimiphos-methyl, which used commonly as grain protectant, were evaluated by using Japanese quail, *Coturnix coturnix japonica* as test animals.

LC_{90} values of either pirimiphos-methyl or tested plant extracts against the rice weevil, *Sitophilus oryzae* (L.) were used in the biochemical analysis. The birds were fed with either insecticide-or-plant extract-incorporated diet, for 30 days. Pirimiphos-methyl caused 40% mortality of the tested birds while tested plant extracts had no mortality percent. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) assays GOT, GPT, total proteins, albumin, cholesterol and uric acid were determined in serum samples collected from both treated-and control-birds after 30 days of treatment. Pirimiphos-methyl was found to be the most effective on liver functions. The extracts derived from *E. aromatica* showed the lowest effect in this respect. Pirimiphos-methyl and hexane extracts of both *E. aromatica* and *P. ansium* produced alteration in levels of uric acid of treated birds, whereas the ethanolic extracts of both *E. aromatica* and *P. ansium* had the lowest effect in this regard. It is concluded, therefore, that naturally occurring plant substances seem to be generally more safe for human and ecosystem, than the conventional synthetic insecticides commonly recommended for pest control such as pirimiphos-methyl.

INTRODUCTION

The wide spread use of synthetic insecticides possesses serious problems to both man and wild life because of their adverse effects on the environment. One solution of these problems might be to replace sythetic chemicals with compounds which occur naturally in plants. Some plants contain insect repellents (Glob and Webley, 1980) and others act as feeding inhibitors (Ladd *et al.*, 1978 and Warthen *et al.*, 1978). Some plant extracts had reduced adult population (Prakash *et al.*, 1993) and reduced number of egg laid per female and adult emergence (Miah *et al.*, 1993). Several investigators studied the toxic action of different plant extracts (El-Gayar *et al.*, 1975 and 1979; Watson *et al.*, 1979; Abbassy *et al.*, 1981; El-Sayed 1993; Abo Arab *et al.*, 1998; Raja *et al.*, 2001, Salwa, 2001 and El-Kashlan, 2004; Abo-Arab *et al.* 2004 illustrated the efficiency of hexane and ethanol extracts of clove, *Eugenia aromatica* and anise, *Pimpinella ansium* as protectants grains against rice weevil, *Sitophilus oryzae* (L.) and cowpea weevil, *Callosobruchus maculatus*.

Subchronic toxicity tests are designed to examine the adverse effects resulting from repeated exposure over a portion of the average life span of

experimental animals. Properly designed subchronic studies gave valuable information on the cumulative toxicity of a substance on target organs and on physiologic and metabolic tolerance of a compound at low dose of prolonged exposure. The results from such studies can provide information what will aid in selecting doses for chronic, reproductive and carcinogenicity studies. Subchronic studies are also valuable in establishing doses at which no toxicological effects are evident, a critical factor in risk assessment. Therefore, subchronic testing is considered essential for all new chemicals before their specific hazard that can be assessed and legitimate safety assessment made. Subchronic toxicity studies showed always attempt to expose the animals by the same route that man is most likely to be exposed (Mosberg and Hayes, 1989).

Several researchers studied the side effects of some synthetic chemical insecticides on experimental animals e. g., (Abbassy *et al.* 1980, 1988 and 1989); Zidan (1991); El-Harrawie *et al.* (1991); Kandil *et al.* (1991); Abdel-Baki (1993); Zidan *et al.* (1993) and Abd El-All *et al.* (1999).

Aso, the adverse effects of different plant materials on experimental animals have been studied by several researchers e.g,, Meinwald *et al.* (1978); Krause and Adami (1984); Ihsan *et al.* (1987); El-Habbak *et al.* (1988); Mawardi *et al.* (1989); Tennekoon *et al.* (1991); Abrahim *et al.* (1992); Abd El-All (1998); El-Hamady (1999 b); Radwan *et al.* (2001) and Akadugu *et al.* (2001).

Therefore, the objective of our present work was to study some adverse effects of two extracts derived from *E. aromatica* and *P. ansium* compared to an organophosphorus insecticide, pirimiphos-methyl which is commonly recommended for controlling of stored-product insect pests, against the Japanese quail birds.

MATERIALS AND METHODS

Tested materials

LC_{90} concentrations, (which kill 90% of *S. oryzae* adults after five days of feeding on wheat grains treated with either pirimiphos-methyl or ethanol and n-hexane extracts derived from *P. ansium* and *E. aromatica*) were used in the biochemical analysis.

Tested birds

Birds of the Japanese quail, *Coturnix coturnix japonica*, (15 days old) were obtained from the flock breeder by the Poultry Research Farm, Department of Poultry Production, Faculty of Agriculture, Kafr El-Seikh, Tanta University. Birds were kept on Laboratory of Sakha Agriculture Research Station for 15 days in order to adapt with laboratory conditions before treatment. All birds of 30 days old were healthy and have never been subjected to any chemical contamination.

Treatment of birds

Groups of five unsexed Japanese quail birds were used for each tested materials. The tested materials were incorporated in diet (wheat grains) at

LC_{90} concentrations and administrated to the birds for 30 days. Throughout the period of treatment, mortalities in birds treated were daily observed. At the end of experimental period, survival birds were slaughtered. Blood was collected into sterile vials, left until clotting occurred, then the serum (supernatant) was separated and kept frozen till using for biochemical analysis.

Biochemical analysis

Serum samples were analyzed for determining biochemical homeostasis :

1. Liver function assays

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin and cholesterol, were determined in serum sample collected from both treated-and untreated (control)-birds. Procedure followed for analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total protein, albumine and cholesterol were similar to that described by Reitman and Frankel (1957), Henry (1964), Doumas (1971) and Schettler and Nussel (1975), respectively.

2. Kidny function assay

Uric acid was also determined in the same collected samples of serum (Fossatti and Prencipe, 1982).

RESULTS AND DISCUSSION

1. Mortality percent

Data in Table 1 show that 40% of birds were died after 15 days of pirimiphos-methyl treatment. Mortality percentages occurred through duration of treatment are recorded in Table 1. Death might be due to insecticide accumulation, changes in enzyme levels and disruption of physiological and biochemical homeostasis.

2. Effects on liver functions

The activities of AST and ALT enzymes involved in liver function in both treated and untreated (control) birds were estimated (Table 2). Also, serum albumin, cholesterol, and total proteins were determined. Results are presented in Table (2 & 3) and illustrated in Fig. 1. The data indicated that ethanolic extracts from *E. aromatica* and *P. ansium* had no significant alterations in the activities of serum albumin and ALT. However, *P. ansium* resulted in a significant decrease in total protein, while *E. araomatica* had no effect on total proteins. Extracts from *E. aromatica* and *P. ansium* caused a significant increase in cholesterol levels. On the other hand, the organophosphorus insecticide, pirimiphos-methyl significantly elevated albumin, cholesterol, ALT, AST and total protein levels. Hexane extract from *E aromatica* had no effect on all tested parameters. However, the hexane extract from *P. ansium* and also pirimiphos methyl exhibited significant increase for all tested parameters except that of ALT.

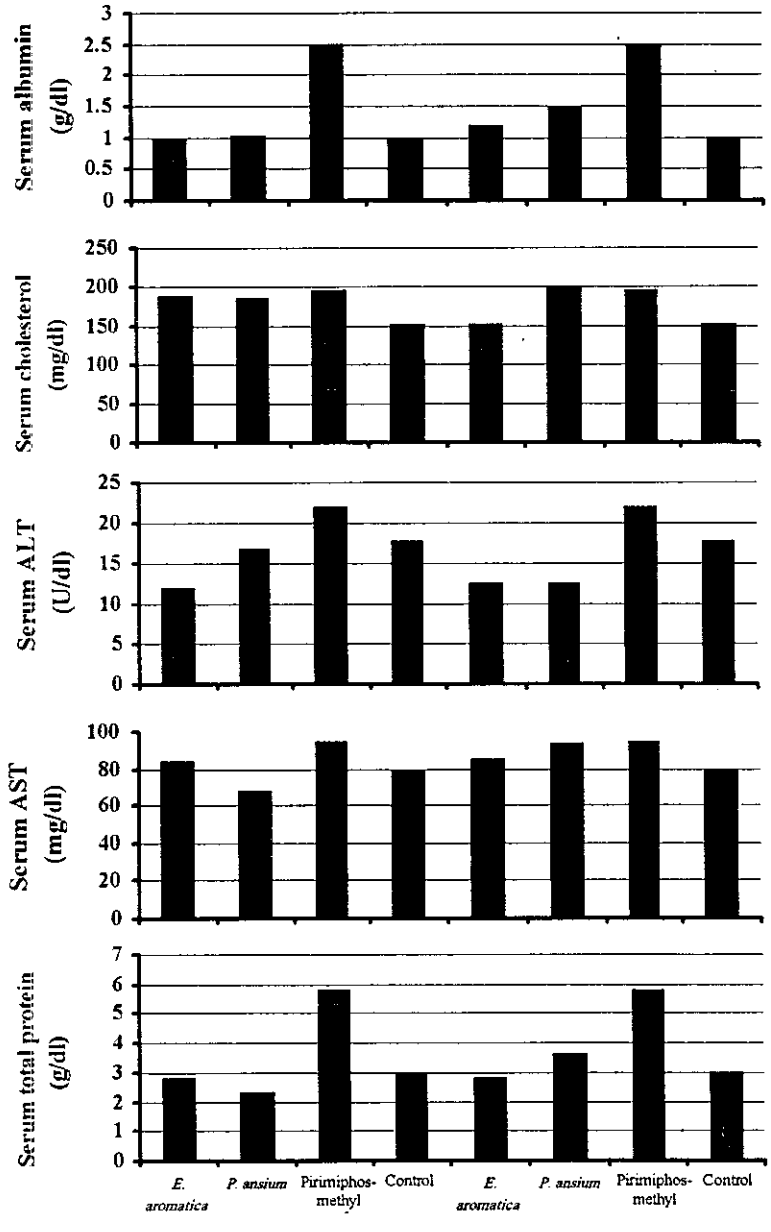

Fig. (1): Effect of ethanol and hexane extracts from *E. aromatica* and *P. ansium* in comparison with pirimiphos-methyl on biochemical analysis of Japanese quail, Coturnix coturnix japonica.

According to Burtis and Edward (1994), tests of hepatic functions could be classified into three categories : 1) Tests of metabolic function which are based on the determination of substances metabolized by the liver (e.g. bilirubin, cholesterol, triglycerrides etc.); 2) Tests of hepatic synthetic function including those based on the determination of substances produced or synthesized by the liver (e.g. albumin); and 3) Tests of hepatic excretories including those based on the determination of substances released from the damaged tissues (e.g. endogenous compounds released by damaged hepatocyte such as the transaminase (ALT and AST) and alkaline phosphatase.

Table 1. Mortality percentage of Japanese quail, *Coturnix Coturnix japonica*, treated with LC_{90} values of tested plant extracts compared with pirimiphos-methyl and control treatments.

Solvent	Tested materials	Number of treated birds	Dead birds	
			Number	% M
Ethanol	*Eugenia aromatica*	5	0	0
	Pimpinella ansium	5	0	0
n-hexane	*Eugenia aromatica*	5	0	0
	Pimpinella ansium	5	0	0
	Pirimiphos-methyl	5	2	40%
	Control	5	0	0

Table 2. The adverse effects of ethanolic extract derived from *E. aromatica*, *P. ansium* and pirimiphos-methyl on biochemical analysis of the Japanese quail birds, *Coturnix Coturnix japonica*.

Treatments	Serum albumin (g/dl)	% of control	Serum cholesterol (mg/dl)	% of control	Serum ALT (µ/dl)	% of control	Serum AST (µ/dl)	% of control	Seum T. protein (g/dl)	% of control
E. aromatica	0.97d	97	188.0a	123.7	12.0b	67.4	84.0b	107.7	2.8c	77.6
P. ansium	1.03cd	103	186.0a	122.4	16.8ab	94.4	67.5a	86.5	2.3b	93.3
Pirimiphos methyl	2.50a	250	197.0a	129.6	22.0a	123.6	94.5a	121.2	5.8a	193.3
control	1.00cd	100	152.0b	100	17.8ab	100	78.0b	100	3.0c	100

Means followed by the same letter are not significantly different at the level of 5% by DMRT (Duncan Multiple Range Test, Duncan, 1955).

The liver is often the primary target for the toxicity of various toxicants. The assessment of liver enzymes in the blood is generally a more sensitive measure of heptotoxicity than histopathologic changes and can be assessed within a short time (Cornelius *et al.*, 1959 and Gradwohl, 1956). The possible mechanism involved in the elevation of transaminase may be due to tissue damage (Korsrud *et al.*, 1972). ALT and alkaline phosphatase released into the blood by the damage liver is one of the most useful tools in the study of hepatotoxicity (Hayes, 1989). Similar findings were obtained by many authors who reported that there were significant alterations in the activities of transaminase in different animal species treated with various pesticides such organophosphates (Ray *et al.*, 1988; Rao *et al.*, 1991; Rajeev *et al.*, 1991 and El-Hamady, 1998. Chicks fed diets containing extract from neem, *Azadirachta indica*, leaves showed increase in lactic dehydrogenase, glutamic oxaloacetic transaminase and alkaline phosphatase (Abrahim *et al.*, 1992). Serum

albumin is formed only in the liver (Talaat, 1955). Hypoalbuminemia (decreased albumine) is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumine (Burtis and Edward, 1994). The hexane extract of *P. ansium* and also pirimiphos-methyl caused a significant increase in albumin (Table 3). These findings are in a good agreement with those reported by Helal (2000) who found that albumine was slightly (but significant) increased by chlorpyrifos, cypermethrin and imidacloprid administered to Japanese quail birds as orally daily doses of 1/50 and 1/100 of LD_{50} for 30 days. It could be concluded that alterations caused for the tested biochemical measurements as a result of animal feeding on either tested plant extracts or pirimiphos-methyl were due to liver dysfunction and tissue injury compared to untreated (control) animals. The safest plant extract in this respect was the ethanolic and hexane extracts of *E. aromatica* had the lowest adverse effects in this respect, whereas pirimiphos-methyl had the highest adverse effects on the liver function assays.

Table 3. The adverse effects of hexane extract derived from *E. aromatica, P. ansium* and pirimiphos-methyl on biochemical analysis of the Japanese quail birds, *Coturnix coturnix japonica*.

Treatments	Serum albumin (g/dl)	% of control	Serum cholesterol (mg/dl)	% of control	Serum ALT (µ/dl)	% of control	Serum AST (µ/dl)	% of control	Seum T. protein (g/dl)	% of control
E. aromatica	1.17c	117	153b	100.7	12.5b	70.2	84.5b	108.3	2.8 c	93.3
P. ansium	1.47b	147	198.5a	130.2	12.5b	70.2	94.0a	120.5	3.6b	120.0
Pirimiphos methyl	2.50a	250	197.0a	129.2	22.0a	123.6	94.5a	121.2	5.8a	193.3
control	1.00cd	100	152.5b	100	17.8ab	100	78.0b	100	3.0c	100

Means followed by the same letter are not significantly different at the level of 5% by DMRT (Duncan Multiple Range Test, Duncan, 1955).

Table 4. Adverse effect of tested materials on uric acid levels of Japanese quail birds *Coturnix coturnix japonica*.

Treatments	Ethanol extracts		n-hexane extracts	
	Serum uric acid (mg/dl)	% of control	Serum uric acid (mg/dl)	% of control
E. aromatica	3.6c	94.7	2.9d	76.3
P. ansium	3.6c	94.7	4.3b	113.2
Pirimiphos methyl	8.3a	218.4	8.3a	218.4
Control	3.8c	100	3.8c	100

Means followed by he same letter are not significantly different at the level 5% by DMRT (Duncan Multiple Range Test, Duncan, 1955).

Effects on kidney function

To study the effect of tested materials on kidney function, the concentration of serum uric acid was determined. Results are presented in Table 4 and illustrated in Fig. 2. The data indicate that the ethanolic extracts from both *E. aromatica* and *P. ansium* had no significant adverse effects on uric acid levels in comparison with that of the control. However, pirimiphos-methyl and hexane extracts of both plant species produced significant alteration in levels of uric acid in treated birds. In general, alterations in uric

acid level may be an indication to the nephrotoxic effect of the tested materials following continuous feeding for 30 days on treated diet. The safest toxicants in this respect were ethanolic extracts of both plant species tested. Uric acid and creatinine concentrations were found to cause significant increase in serum of mice fed on diets containing neem kernesls (El-Fishawi and Sharobeem, 1991). Punia *et al.*, 1987 and El-Hamady 1999a, reported that organophosphate insecticides increased urea and creatinine concentration in serum of various animal species.

Our present results conclude that pirimphos-methyl was the most effective material which caused significant alteration of the upper parameters. These results are in agreement with those reported by Ray *et al.* (1988), Rao *et al.* (1991), Rajeev *et al.* (1991) and El-Hamady (1998). On the other hand, our results demonstrated that the extracts derived from *E. aromatica* had no significant advers effects on liver functions. However, pirimiphos methyl produced alterations of uric acid levels, these results agreed with those found by El-Fishawi and Sharobeem (1991), El-Hamady (1999b).

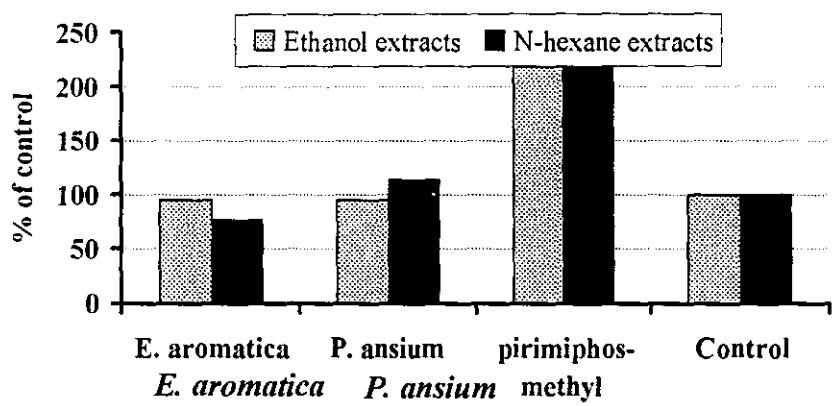

Fig. (2): Effect of ethanol and hexane extracts from *E. aromatica* and *P. ansium* in comparison with pirimiphos-methyl on uric acid of Japanese quail birds, Coturnix coturnix japonica.

They reported that pirimiphos-methyl increased uric acid concentrations. In this respect the ethanolic extracts of *E. aromatica* and *P. ansium* seemed to be the most safe materials tested. Therefore, results of the present study revealed possible hazards occurred by conventional chemical pesticides for human and wild life whereas, naturally occurring substances of plant origin could be more safe in most cases.

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تأثير نوعين من المستخلصات النباتيه مقارنة بمبيد الأكتيليك على بعض التقديرات البيوكيماوية فى طيور السمان

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أظهرت مستخلصات نباتى القرنفل *Eugenia aromatica* والينسون *P. ansium* تأثيرات مبشرة عند تقييمها على بعض آفات المواد المخزونة.

أجريت تجارب على مستخلصات نباتى القرنفل والينسون بالإضافة الى مبيد الأكتيلك (البريميفوس ميثيل) وذلك لدراسة الآثار الجانبية المحتمل حدوثها.

وقد استخدم لإجراء هذه الدراسة طيور السمان لعمل تجارب تحت مزمنة وكانت النتائج كالآتى:

- أدى مبيد الأكتيلك الى موت ٤٠% من الطيور المستخدمة بينما لم تظهر المستخلصات النباتية المختبرة أى نسبة موت على طيور السمان.
- اجريت تحاليل بيوكيماوية لتقدير انزيمى AST, ALT وكذا تقدير البروتين الكلى والألبيومين والكوليسترول وحامض اليوريك.
- كان مبيد البريميفوس ميثيل هو الأشد تأثيرا على وظائف الكبد وكانت المستخلصات الأكثر أمانا فى هذا الخصوص هى المستخلص الأيثانولى ومستخلص الهكسان لنبات القرنفل *E. aromatica*.
- أظهر مبيد البريميفوس ميثيل ومستخلص الهكسان للقرنفل *E. aromatica* والينسون *P. ansium* تغيرا فى مستوى حامض اليوريك للطيور المعاملة بينما مستخلص الإيثانول لـ *E. aromatica* كانت الأكثر أمانا بهذا الخصوص.

ويمكن إيجاز النتائج السابقة فى القول بأن المستخلصات النباتية المختبرة كانت أمنه نسبياً على الإنسان والنظام البيئى من المبيد الكيماوى المستخدم فى هذه الدراسة.