TOXICOLOGICAL EFFECTS OF CERTAIN BIORATIONAL INSECTICIDES ON JAPANESE QUAIL, *Coturnix coturnix Japonica*

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ABSTRACT

The present study was undertaken to make risk assessments of some biorationals i.e., imidacloprid; neem oil; the mineral oil, "Misrona" and the commercial bioinsecticide, "Biofly" (the entomopathogenic fungi, *Beauvraria bassiana* Balsamo) against Japanese quail birds. The study was carried out through acute and subchronic toxicity tests at repeated oral doses each of 1/50 or 1/100 LD50 for 30 days. Chlorpyrifos and Cypermiethrin (evaluated as conventional insecticides for comparison) showed extreme acute toxicities as well as other effects appeared as haernatological lesions, liver and kidney dysfunction, elevated sugar content. Cypermiethrin was less powerful than chlorpyrifos. The damage of liver was ensured by the histopathological examination. A drawback of the advantageous insecticide, imidacloprid is its high acute toxicity to Japanese quail birds and possibly to other avian species. Sublethal doses also exerted hepatic and renal toxicity. Neem oil was acutely non-toxic but was found to pose serious effects appeared as abnormal blood picture, liver damage and biochemical disruption. However, the harmful effects induced by imidacloprid or neem oil were generally less remarkable than those resulted by chlorpyrifos and cypermethrin. Furthermore, detrimental effects caused by imidacloprid, neem oil and possibly cypermethrin were not mostly observed at the lower tested dose and this indicates that the effects are dose dependent. The most conclusive point is the relatively low toxicity of the mineral oil and the bioinsecticide. They were found to be acutely non-toxic. In addition, no haematological, biochemical or histological abnormalities were observed in birds exposed to sublethal doses of these materials even at the higher tested dose. In conclusion, comprehensive toxicological studies are further needed to verify the overall impact of more realistic doses of these materials on other animal and avian species. In this respect, much concern has to be payed to the ecotoxicological risk of the phytochernical constituents of neem seed extracts and fungal mycotoxins.

INTRODUCTION

Increase in the use of chemical pesticides has resulted in widespread environmental problems. As a solution of these problems, a large number of

alternatives known usually as non-conventional pesticides has been produced and applied for crop protection. Of these, mineral oils, botanicals and microbial biocontrol agents are prominent and also referred to as biorationals. On the other hand, synthetic conventional pesticides will continue to play an important role in crop protection for the foreseeable future as there are no practical and realistic alternatives at the moment (Persley, 1996). However, there have been tremendous changes in some of the newer pesticides produced. An example of the newer insecticides, is imidacloprid that belongs to a novel chemical group known as "neonicotinoids".

The compound has a revolutionary mode of action as it is highly specific against insect nicotinic acetylcholine receptors and so it is of low toxicity to vertebrates (Yamamato et al. 1995). Neonicotinoids are generally of low degree of toxicity to mammals and aquatic biota (Okazawa et al. 1998) and also, negative side effects are likely to be much lower with other biorationals (Whitten et al., 1996). However, these materials might pose certain environmental repercussions. Toxicological risk assessments were frequently carried out for the conventional synthetic pesticides but less have been performed for non-conventional ones.

Therefore, the present study aimed to evaluate the relative toxicologici safety of certain non-conventional insecticides i.e. imidacloprid; neem oil; the mineral oil, "misrona" and the commercial product of the entomopathogenic fungi, Beauvaria bassiana Balsamo all compared to chlorpyrifos and cypermethrin. In this respect, acute and subchronic toxicity tests were conducted on Japanese quail.

MATERIALS AND METHODS

1. The tested insecticides :

- **Dursban (chlorpyrifos) 48% EC**: 0, 0-diethyl-0 (3, 5, 6- trichloro-2-pyridyl) phosphorothioate. Dow Chemical Company.
- **Polytreen (cypermethrin) 20% EC**: alpha-eyano-3phenoxybenzyl (±) cis trans 3-(2,2-dichlorovinyl)- 2,2-dimethyl cyclopropane carboxylate. KZ-Cepa Giegy.
- **Confidor (imidacloprid) 20% EC:** 1-(6-ehloro-3-pyridinyl) methyl-4, 5-dihydro-N-nitro-1 H imidazole-2-amine. Nihon Bayer Agrochem.
- **Misrona oil 80% EC:** A product of mineral oil for winter treatment produced by Misr comp. for petroleum oil, Egypt.

- **Neem oil**: Extracted from kernels of *Azadirachta indica* A. Juss. The used sample was provided by Plant Protection Institute. Agric. Res. Center, Dokki, Cairo, Egypt.
- **Blofly:** A commercial liquid preparation of the entomopathogenic fungi, *Beauvaria bassiana* (Balsamo) containing 3 x 10^7 condia/ml. The product has been produced by El-Nasr Comp. for fertilizers and pesticides, Egypt.

2. Test organism:

Males of Japanese quail, *Coturnix coturnix* Japonica (average weight of 120 ± 15 gm) were obtained from the flock bred by the Poultry Research Farm, Dept. of Poultry Production, Fac. of Agric., Kafr El-El-Sheikh, Tanta Univ. Birds were kept in metallic cages provided with water and diet (obtained from the same source of birds) all birds were healthy and have never been subjected to any chemical contamination.

3. Acute toxicity tests:

The acute toxicity (orally or dermally) of the tested materials to Japanese quail birds expressed as LD50 values and their fiducial limits was determined according to the method of Weill , (1952) using groups of birds each of three (n =3). Oral dosing was done by means of a special syringe that has a needle equipped with a ball tip. For the dermal treatment, the method described by Saleh *et al.* (1986) was adopted. Doses were prepared in water except for those of neem and mineral oils which were applied undiluted.

4. Subchronic toxicity studies

Groups of birds each of 15 were daily treated with single oral doses of the tested materials and dosing was repeated for 30 days. Each dose was equivalent to 1/50 or 1/100 LD_{50}. Because oral LD_{50} values were tentatively found to be >20000 & > 10000 mg kg b.w. for the mineral and neem oils, respectively, the corresponding 1/50 and 1/100 LD50 values were supposed to be (400 & 200) and (200 & 100) mg/kg b.w. respectively. At the end of the experimental period, survival birds were clinically examined, sacrificed and blood samples were collected in heparinized or non-heparinized tubes. Livers were eviscerated and subjected to histopathological examination. Heparinized blood was used for hematological examination by means of Coulter Counter Model CBC5. Non-heparinized bloods were centrifuged at 4000 r.p.m for 10 minutes and the obtained sera were kept frozen till used for biochemical analysis. The activities of some enzymes and concentrations

of certain biochemical parameters especially those representing liver and kidney functions were determined. These include alkaline phosphatase (ALP); aspartate aminotransferase, AST (formerly, glutamate oxaloacetate transaminase, GOT), alanine aminotransferase, ALT (formerly, Glutamate pyruvate transaminase, GPT); total protein, total bilirubin, albumin, cholesterol, total lipids, urea, creatinine and blood sugar. All determinations were done using commercial kits produced by Pasteur Lab. Union City, USA. The spectrophotometer, Spekol 11 was used for optical measurements.

Specimens of livers of treated birds were fixed in 10% neutral formalin, embedded in paraffin and sections of about 5 micrometers were prepared and staind with haematoxylin and eosin (Lillie and Fullman, 1976). Statistical analysis of data was carried out according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Acute toxicity studies :

Results of the acute oral and dermal toxicities of the tested materials to Japanese quail birds expressed as LD50 and their fiducial limits are recorded in Table (1).

Table (1): Acute oral and dermal toxicities of the tested materials to Japanese quail

Insecticides	Oral		Dermal	
	LD_{50} (mg/kg b.w.)	Confidence limite	LD_{50} (mg/kg b.w.)	Confidence limite
Chlorpyrifos	8.8	3.3-22.9	155.87	38.9-616.6
Cypermethrin	1247	630.1-2511.9	8957.4	4466.8-17782.8
Imidacloprid	25	12.6-50.0	1757.9	868.3-4623.8
Neem oil	>10000	-	>50000	-
Mineral oil	>20000	-	>50000	-

For oral toxicity and according to Hodgson and Levi (1997), the insecticides could be comparatively classified as follows: "Extremely toxic" for chlorpyrifos and imidacloprid (LD_{50} values: 8.8 and 25 - mg/kg, respectively); "moderately toxic" for cypermethrin (LD_{50}: 1247 mg/kg); 4Crelatively non-toxic" for neem and mineral oils (LD_{50}'s> 10000 and 20000 mg/kg, respectively). Of course the obtained LD_{50} values of the tested formulated insecticides greatly differ from those of technical materials (or active ingredients) currently reported in standard reference books. Formulation of a pesticide may either increase or diminish the,acute

toxicity properties. However, for practical purposes and from the environmental point of view, the pesticide user is more interested in knowing the toxicity of the particular formulation being used. The obtained results concerning the non-conventional insecticides, (i.e. imidacloprid, mineral and neem oils) are somewhat in agreement with those obtained by others. El-Hamady et al. (1999) found that oral LD_{50} of Confidor (imidacloprid) 20% SL against Japanese quail was 38.6 mg a.i/kg b.w. (fiducial limits: 29.7-46.3) Oral LD_{50} of the technical imidacloprid was 31 mg/kg (Grau, 1988a). Thus, the high acute toxicity of imidacloprid to Japanese quail and generally to other birds (Pfluger and Schmuck, 1991) is thought to be a drawback of this advantageous insecticide that belongs to "neo-nicotinoids", a new group of insecticides having a unique mode of action. Nevertheless, proper formulation of this compound (as in Gaucho 70% w.s. for seed treatment) may reduce its acute toxicity and other consequences for the environment.

The present study demonstrates the low acute toxicity of the tested petroleum oil to birds (oral LD50 of Misrona oil is >20000 mg/kg b.w.) and this may be in accordance with previous results. El-Hamedy (1998) found that oral LD_{50} of the commercial petrolium oil, super Royal 9MEC was >15000 mg/kg b.w. of rats. However, at sea, oil slicks are responsible for the death of many birds (Hodgson and Levi, 1997). These deaths might be due to the physical effects caused by the oil coating these birds (Duffus, 1980). Concerning neem oil, the present results show that it might be safe from the view of acute toxicity (oral LDso: > 10000 mg/kg). Isman, (1995) found that extracts of neem-seed preparations (Containing a variety of limonoids and other potentially bioactive compounds) are of low acute oral toxicity to laboratory mammals and generally regarded as safe for use.

For acute dermal toxicity, the tested materials could be classified according to the same ranking scheme as follows: "very toxic for chlorpyrifos (LDso: 155.87 mg/kg); "moderately toxic" for imidacloprid (LD_{50}: 1757.9); "relatively nontoxic" for cypermethrin and both oils (LD_{50}'s : 8957.4 and >50000 mg/kg, respectively). The dermal route is considered to be the most important route of entry into the body during pest control operations (Wilkinson, 1976). Sometimes, the skin exposure may be deliberate as for example the use of pesticides for ectoparasite control. The veterinarian (or household) use of imidacloprid to control fleas, cockroaches, houseflies and others was frequently reported (Jacobs *et al.*, 1997, Wen, *et al.*, 1997, Hopkins, 1998 and Griffin *et al.* 1998). Thus, the moderate dermal toxicity of imidacloprid shown in the present investigation

could be of great interest. Mineral or neem oil exhibited very low acute toxicity to Japanese quail (LD50 >50000 mg/kg)., Deaths of birds (on sea or seashores) exposed to marine spillage of petroleum oils are often due to the physical effects of these oils. This situation is unlikely to occur for mineral or neem oil when applied as crop protectants. Concentrations of spray liquids, initial deposits, or their residues are too minimal to cause death.

Results concerning the acute oral or dermal toxicities of the commercial fungal bioinsecticide are shown in Table (2). The product shows low acute toxicity to quail birds. LD50 values for oral and dermal routes: 6 x 10^8 and >30 x 10^8 conidia/kg b.w. equivalent to 20 and 100 ml of the liquid formulation per kg b.w., respectively. Furthermore, the concentration of the original product (3 x 10^8 conidia/ml) is equivalent to 1000 fold of the field concentration recommended by the producing company (3 x 10^5 conidia/ml). El-Hamady (1998) found that oral LD50 of the same product to white rats was 8.7 x 10^8 conidia/kg The fungi, *B. bassiana* produces mycotoxines as beauvericin, beauverolides and bassianolide (Gillespie and Claydon, 1989). The available literature shows no acute toxicity studies of this toxicant to poultry and any birds. In general, Kadir and Barlow, (1992) reported that, the possibility of mammalian toxicity has limited the development of B. bassiana toxins as mychochemical pesticides.

Table (2): Acute toxicity of the entomopathqgenic fungal product, *B. bassiana* to Japanese quail.

Route of exposure	LD_{50}		Confidence limite (Conidia/kg b.w.)
	Conidia/kg b.w.	Ml*/kg b.w.	
Oral	6 x 10^8	20	3.15x10^8 11.5x10^8
Dermal	> 30 x 10^8	100	-

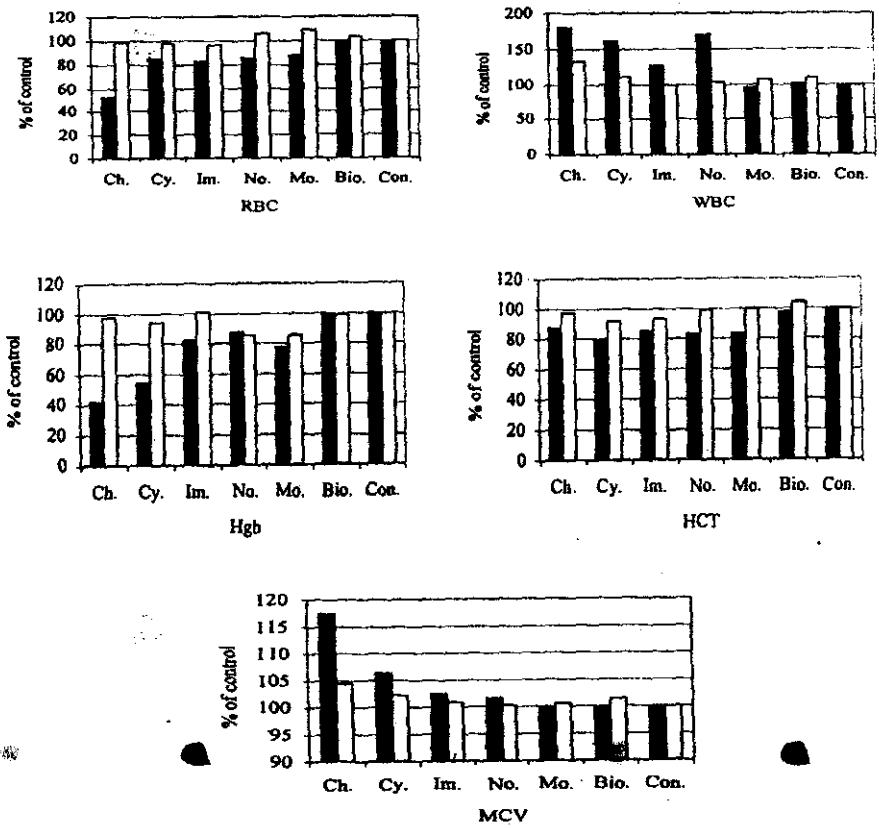
* ml of the liquid commercial formulation

2. Subchronic toxicity :

2.1. Haematological effects

The parameters measured were counts of red blood cells (RBS) & white blood cells (WBC); mean corpuscular volume (MCV); Haematocrit value (HCT) and Hemoglobin content (Hgb). Results are depicted in Fig. (1). It was found that values of blood pictures of control birds were consistent with those observed by Nirmalan and Robinson (1971) in young Japanese quail. . Except for the bioinsecticide, all the tested toxicants at the

dose 1/50 LD50 caused significant reduction in RBC count, Hgb content and HCT values whereas VffiC counts were significantly increased. These effects were less powerful at the dose, 1/100 LD50 where RBC & WBC counts, haematocrit values were not affected by each of the toxicants excluding chlorpyrifos that significantly increased RBC counts (132.14% of control).



1/50 LD_{50} 1/100 LD_{50}

Ch.: Chlorpyrifos, Cy.: Cypermethrin, Im.: Imida-cloprid, No.: Neem oil, Mo.:Mineral oil, Bio.: Bioinsecticide, Con.: Control.

Fig (l): Blood pictures of Japanese quail treated with daily doses of insecticides for 30 days.

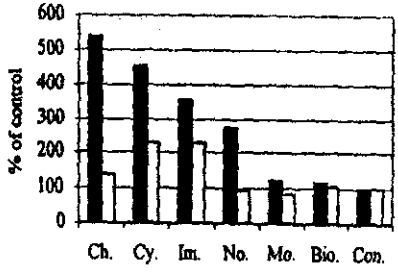
At both doses imidacloprid, the bioinsecticide, neem and mineral oils were of no effect on MCV. It could be concluded generally that repeated daily doses of the bioinsecticide each equal to 1/50 or 1/100 LDso might be of no adverse effects on blood picture of Japanese quail. The rest of the tested

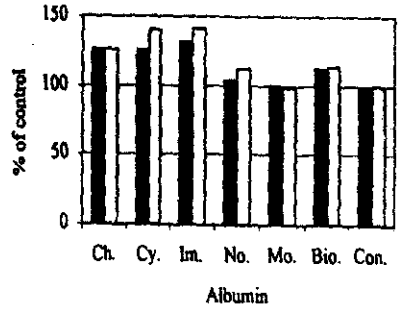
materials (i.e. chlorpyrifos, imidacloprid, neem and mineral oils) were of harmful haematological effects at the higher dose (1/50 LD_{50}) but at 1/100 LD_{50} were of slight effects. Chlorpyrifos was the most dangerous one in this respect. Results are in parallel with those obtained by others. In blood of pharaoh quail, Szubartowska et al. (1992) found that the organophosphate, fenitrothion reduced the RBC, Hgb and HCT values. Similar results were obtained by Zidan (1991 a, b) when treating mice with subchronic doses of organophosphate and pyrethroidal insecticides. The association between the increase of WBC with decrease of RBC counts was also observed by Menz and Luctkemeier (1974). Couillard and Leyhton (1993) reported that crude petroleum oils have caused hemolytic anemia in birds and mammals. In birds, an oxidant damage on circulating red cells has been identified as the primary toxic affect of ingested petroleum oils.

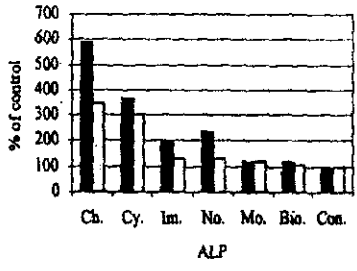
2.2. Biochemical effects

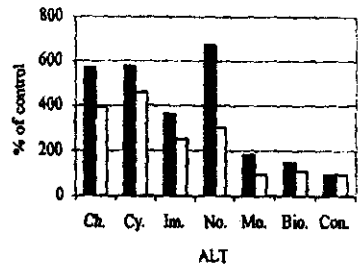
The activities of some enzymes and levels of certain biochemical parameters representing liver function of Japanese quail treated with daily sublethal doses (1/50 or 1/100 LD_{50}) of insecticides for 30 days were determined. Results are illustrated in Figs (2, 3). The mineral oil and the bioinsecticide caused no significant alterations in the activities of alkaline phosphatase (ALP), the transaminase (ALT), and concentrations of total protein, total bilirubin and albumin at the two tested doses.

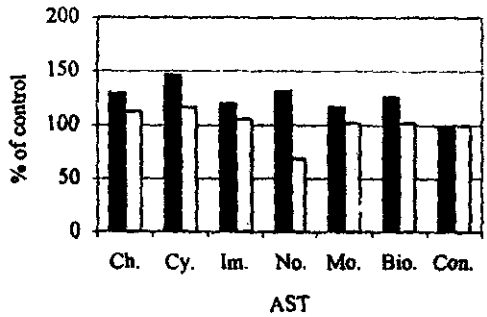
However, these toxicants resulted in a significant increase in AST activity, total lipids and significant reduction in the concentration of cholesterol, particularly at the dose 1/50 LD_{50}. At the dose 1/100 LD_{50}, the mineral oil and the entomopathogenic fungal formulation were of no effect on total cholesterol but the two tested doses were of significant effect on total lipids. Chlorpyrifos, cypermethrin, imidacloprid and neem oil significantly elevated ALP, ALT, AST; total bilirubin, albumin whereas reduced cholesterol and total lipids. The effect was more pronounced for chlorpyrifos and cypennethrin. For chlorpyrifos, the activities of ALP, ALT were (590.19 & 345.45) and (569.95 & 393.78%) of control at the doses 1/50 and 1/100 LD_{50}, respectively. For cyperinethrin, the activities were (369.47 & 300.32) and (576.68 & 455.95 %) of control, respectively.











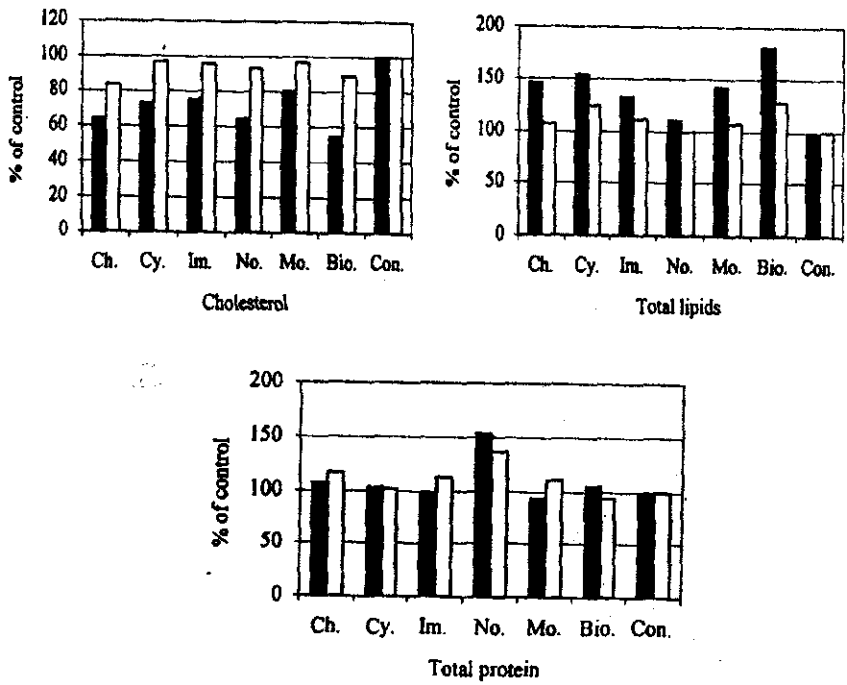
■ 1/50 LD_{50} □ 1/100 LD_{50}

Ch.: Chlorpyrifos, Cy.: Cypermethrin, Im.:Imidacloprid, No.: Neem oil, Mo.:Mineral oil, Bio.: Bioinsecticide, Con.: Control.

Fig. (2): Activities of some liver function enzymes of Japanese quail treated with sublethal doses of the tested materials.

To evaluate the importance of the obtained data, the clinical significance of the tested parameters has to be discussed. Accordingto Burtis and Edward, (1994), tests of hepatic function could be classified into three

categories (1) tests of hepatic synthetic function and these are based on the determination of substances produced or synthesized by the liver (e.g. bilirubin), (2) tests of metabolic function and these are based on the determination of substances metabolized by the liver (e.g. bilirubin, cholesterol, triglycerides etc), (3), tests of hepatic excretory function including those based on the determination of endogenous compounds released by damaged hepatocytes such as transminases, (GOT, GPT) and alkaline phosphatase. The clinical significance of the transaminase, AST is less specific for liver dysfunction (Wilkinson, 1970).



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Ch.: Chlorpyrifos, Cy.: Cypermethrin, Im.:Imidacloprid, No.: Neem oil, Mo.:Mineral oil, Bio.: Bioinsecticide, Con.: Control.

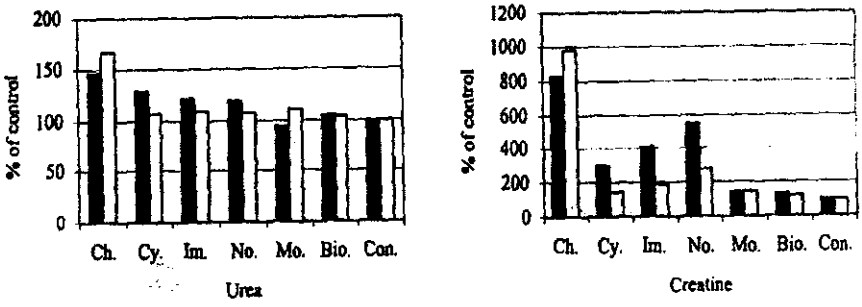
Fig(3): Levels of some biochemical parameters of liver function of Japanese quail treated with sublethal doses of the tested materials.

Thus, the obtained results reveal that chlorpyrifos, cypermethrin, imidacloprid and neem oil at the repeated doses 1/50 or 1/100 LD_{50} may cause liver dysfunction and cliver injury of quail birds. The effect was more pronounced in chlorpyrifos and cypermethrin and generally less powerful at the dose 1/100 LD_{50}. The safest materials in this respect are the mineral oil and the bioinsecticide. The safety of the bioinsecticide should not be generalized because components of the fungal product (i.e. Beauvaria bassiana) were found to induce potential adverse effects, e.g. teratogenecity and other consequences in some fish species (Middaugh and Genther, 1994).

Exposure of birds to sublethal doses of some organophosphate compounds was found to exert hepatotoxicity as shown by increasing some of liver enzymes (Abbassy et al. 1988; Sati 1996 and El-Hamady et al. 1999). Imidacloprid was found also to exert significant alterations in alkaline phosphatase and transaminases in rats and mice treated with sublethal doses (Eweis, 1994 and El-Kashoury, 1999). Chicks fed diets containing leaves of neem trees (Azadirachta indica) showed increase in GOT and alkaline phosphatase. Also, pigs fed on diet containing neem seed kernels showed increase of transaminases and phosphatases (Sastry and Agrawal, 1992).

Concerning the affects on the biomarkers of kidney function, results are shown in Fig (4). It is clear that the mineral oil and the bioinsecticide exhibited no significant effects on urea and creatinine concentrations. Chlorpyrifos, cypennethrin, imidacloprid and neem oil showed significant increase of these parameters especially for chlorpyrifos that elevated urea and creatinine levels to be 146.7 & 166.24 and 831.28 & 976.25% of control at the doses, 1/50 and 1/100 LD_{50}, respectively. At the dose 1/100 LD_{50}, cypermethrin, imidacloprid, neem oil showed no significant effect on urea concentration. Evaluation of blood urea nitrogen and plasma creatinine can be used as index of decreased glomerular filtration in the Kidney (Hayes, 1989). Therefore, the obtained results suggest that chlorpyrifos might be severely nephrotoxic. Cypermethrin, imidacloprid and neem oil are less powerful whereas the mineral oil and the bioinsecticide might be relatively of no renal toxicity. Organophosphate and pyrethroidal insecticides were frequently reported to increase urea and creatinine levels in sera of various animal species especially those treated with sublethal doses (Puina *et al.*, 1987; Dheranetra et al., 1988; El-Hamady et al., 1999) for organophosphates and (Hamza et al. 1981, Saleh et al., 1986, Shakoori et al. 1990) for pyrethroids. Uric acid and creatinine concentrations were found also to increase in serum of mice fed baits containing neem kernels (El-

Fishawi and Sharobeem, 1991). Concerning imidacloprid, results of the present study are also in parallel with those of El-Kashoury (1999) who found that imdiacloprid offered in drinking water to rats at 111 ppm did not change plasma urea level but increased creatinine concentration significantly.

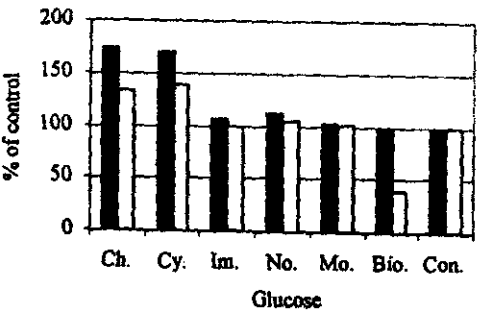


1/50 LD_{50} 1/100 LD_{50}

Ch.: Chlorpyrifos, Cy.: Cypermethrin, Im.:Imidacloprid, No.: Neem oil, Mo.:Mineral oil, Bio.: Bioinsecticide, Con.: Control.

Fig (4) Concentration of urea and creatinine in sera of Japanese quail treated with the tested materials with daily doses for 30 days.

For the effect on blood sugar concentration, results are shown in Fig. (5). Only, the conventional insecticides, chlorpyrifos and cypermethrin caused significant elevation of glucose concentration at the two tested doses 173.85 & 132.69 and 170.05 & 139% of control for chlorpyrifos and cypermethrin at the doses 1/50 and 1/100 LD_{50}, respectively). The nonconventional insecticides (i.e. imidacloprid, neem. oil, mineral oil and the bioinsecticide) were of no significant effects on blood sugar concentration even at the higher dose. Results agreed with those of others. El-Kashoury (1999) found that imidacloporid did not alter plasma, glucose level in rats receiving this insecticide in drinking water at 111 ppm. El-Hamady et al. (1999) found that Japanese quail fed ration pretreated with phosphine + rnalathion/vitavax showed elevated sugar content in blood.



1/50 LD_{50} 1/100 LD_{50}

Ch.: Chlorpyrifos, Cy.: Cypermethrin, Im.:Imidacloprid, No.: Neem oil, Mo.:Mineral oil, Bio.: Bioinsecticide, Con.: Control.

Fig (5):Concentration of urea and creatinine in sera of Japanese quail treated with the tested materials with daily doses for 30 days.

Chattopadhyay et al. (1993) found that leaf extract of Azadirachta indica failed to increase muscle glycogen content and did not influence the effect of exogenous insulin in vitro. Certain organophosphate compounds were found to increase blood sugar in animals (Enan et al., 198 1; Ceron et al. 1996). Blood sugar was also elevated due to chronic or semi-chronic exposure to pyrethroids (Shakoori et al., 1990;Shakoori et al. 1992, El-Hamady et al., 1997 and Sancho et al., 1997). The fasting blood sugar was significantly increased in humans occupationally exposed to pesticides (Berberian and Enan, 1987).

2.3. Histopathological examination

Tissues of livers taken from birds treated with mineral oil or the bioinsecticide at both doses showed no histological changes and seemed to be normal as livers of control birds (Fig. 6). These results are consistent with those of biochemical analysis which showed that, the mineral oil and the bioinsecticide were of no significant effect on liver function. Livers of birds treated with chlorpyrifos, cypennethrhrin, imidacloprid and neem oil at 1/50 or 1/100 LD_{50} showed similar histopathological changes differ in severity. The lesions detected were, in, the form of diffuse vacuolar degeneration of the hepatocytes. The vacoulation was of two types. One is circumscribed vacuoles resembling fat (Fig. 7) and the other was in the form of irregular shaped vacuolation of hepatocytes of diffuse nature representing

hydropic changes (Fig. 8). In addition, focal areas of hepatocytic necrosis were frequently detected especially for liver of birds treated with chloropyrifos at 1/50 or 1/100 LD_{50} in a decreasing order. Focal areas of lytic necrosis of the hepatocytes were observed (Fig. 9). Necrotic foci were heavily infiltrated with mononuclear leukocytes, lymphocytes, macrophage and plasma cells (Figs: 10 & 11). The hepatic sinusoids and viens of hepatic lobules were dilated and engorged with blood (Fig. 12).

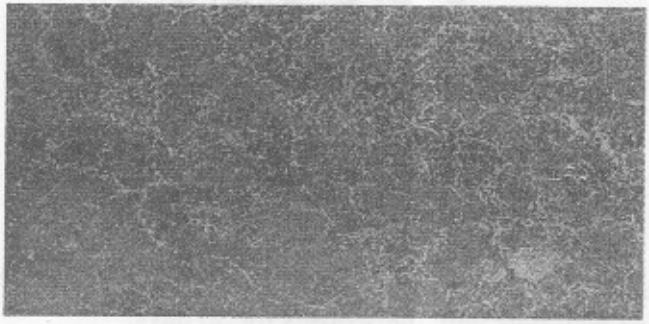


Fig (6): Liver of quail bird treated with repeated doses of mineral oil at 1/50 LD_{50} showing no histological changes and normal liver like birds of control or those treated with the bioinsecticide (H & E, x 200).

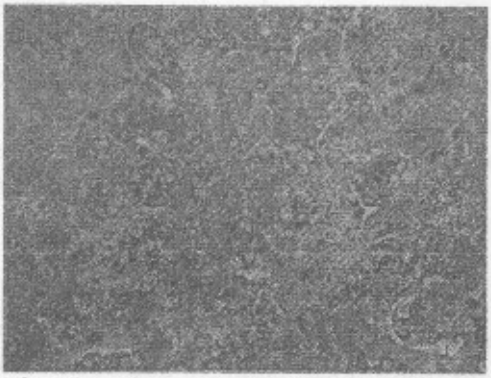


Fig. (7):Liver of birds treated with neem oil at 1/50 LD_{50} shows diffuse circumscribed vacuolation of hepatocytes cytoplasm (H & E, X 400).

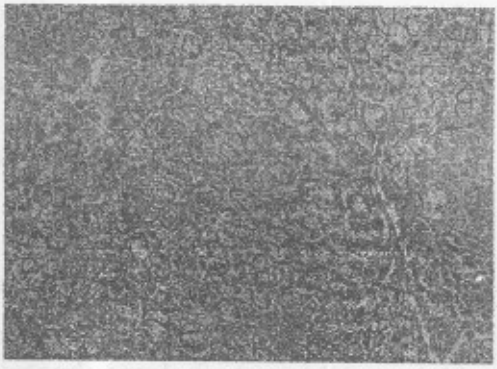


Fig. (8): Liver of birds treated with neem oil at 1/100 LD_{50} showing diffuse irregular shaped vaccoulation of hepatocytes (H & E, X 200).

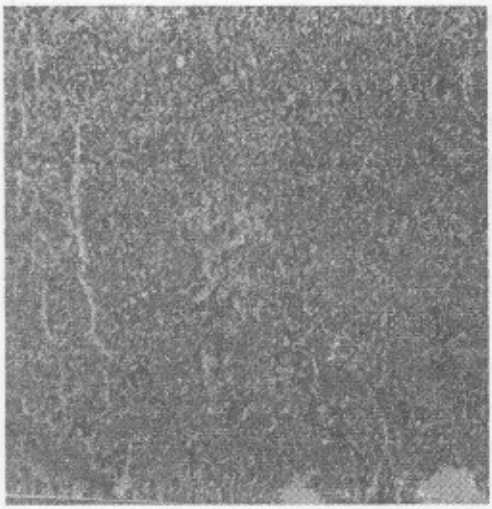


Fig. (9): Liver of quail bird treated with chlorpyrifos at 1/50 LD_{50} showing focal area of lytic necrosis of the hepatocytes and diffuse vaculation (H & E, X 200).

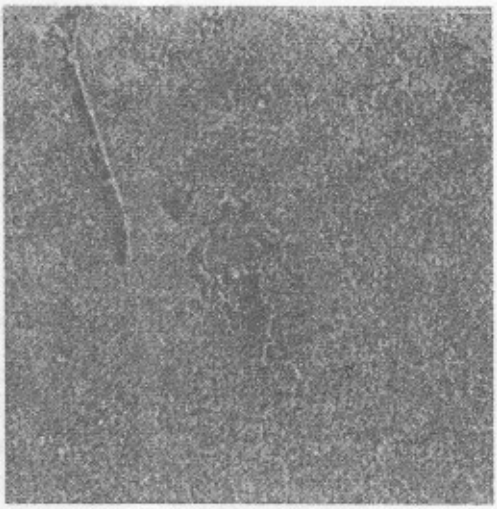


Fig. (10): Liver of quail treated with chlorpyrifos at 1/50 LD_{50} showing focal area of coagulative necrosis and heavy infiltration with mononuclear leukocytes (H & E, X 200).

Fig. (11): Liver of quail treated with cypermethrin at 1/50 LD_{50} showing heavy mononuclear cells infiltration substituting focci of lytic necrosis (H & E, X 200).

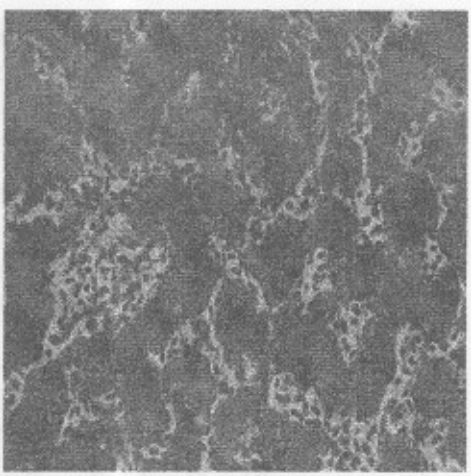


Fig. (١٢): Liver of birds treated with imidacloprid at 1/100 LD_{50} showing moderate degree of sinusoidal congestion (H & E, X 400).

In conclusion, the study suggests that the tested biorational insecticides especially the mineral oil and the fungal bioinsecticide might be toxicologically safer than the conventional synthetic ones. However, comprehensive toxicological studies are needed to be conducted on different animal and avian species to verify the overall impact of these materials on the ecosystem. In this respect, much concern has to be payed to the exotoxicological risk assessment of phytochemical constituents of neem seed extracts and fungal mycotoxins.

REFERENCES

Abbassy, M.A.; Ashry, M.A.; Zein, A.A.; Abdel-Hafez, M.M. and AbdelBaki, M.M. (1988). Side effects of, environmental toxicities. 1. Biochemical and histopathological effects on profenofos and leptophos on hen plasma and liver. J. Agric. Res. Tanta Univ. 14 (1): 414-430.

Berberian, I.G. and Enan, E.E. (1987). Neurotoxic studies in humans occupationally exposed to pesticides. J. Egypt. Soc. Toxicol., 3: 65-75.

Burtis, C. and Edward, A. (1994). Clinical Chemistry. 2 d ed. Vol. 2, Saunders Company. Printed in USA.

Ceron, LL; San'cho, E.; Ferrando, M.D.; Gutierrez, C. and Andreu, E. (1996). Metabolic effects of diazinon on the European eel, Anguilla anguilla. J. of Environmental Science & Health. Pesticides Contaminats & Agricultural Wastes. 31 (5): 10291040.

Chattopadhyay, R.R.; Chattopadhyay, R.N. and Maitra, S.K. (1993). Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Part III. (BIBLIOGRAPHIC CITATION): Fitoterapia. 64: 6, 535-538.

Couillard, C.A. and Leyhton, F.A. (1993). In vitro red blood cell assay for oxidant toxicity of petroleum oil. Journal Environmental Toxicology and Chemistry l2 (5): 839-845.

Dheranetra, W.; Keadtisake, S.; Swatek, F.E. and Fukuto, T.R. (1988). Renal effects in the delayed toxicity of 0, S, S trimethyl phosphorodithioate. Pesticide Biochemistry and Physiology. 30 (l): 95-100.

Duffus, J.H. (1980). Environmental Toxicology. Edward Amold. P. 95.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11: 142.

El-Fishawi, A.A. and Sharobeem, S.F. (199 1). Biochemical studies on the effect of neem kernel, Azadirachta'indica A. Juss on· some metabolic reactions of albino mice. Zagazig. J. Agric. Vol. 18 (3): 849-857.

El-Hamady, Sh. E. (1998). Aphicidal efficiency of certain biorationals with regect to their toxicity to some beneficial insects and mammals 27 Conf. Agric. Der. Res., Fac. Agric., Ain Shams Univ., Cairo. Annals Agric. Sci., Sp. Issue 3, 1013-1027.

El-Hamady, Sh. E.; Abo-Arab, R.B.C.; El-Zeir, F.A.A. and Amer, E.A. (1999). Storage of pesticides-treated maize seeds: Efect on insect infestation, viability of seeds and the safe utility of redundant seeds as poultry feed. Proc. 2 nd Conf. of Pest Control., Mansoura, Egypt Vol. (l): 67-88.

El-Hamady, Sh.E. (1999). Inhibitory effect of imidacloprid on monoamine oxidase in target and non target organisms. J. Agric. Res. Tanta Univ., 25 (l): 100-110.

El-Hamady, Sh.E.; Abo-Sholoa, M.K.A. and Abu-Arab, R.B. (1997). Insecticidal efficiency, applicator exposure and drift hazards of ferivalerate as influenced by two methods of application on cotton fields. J. Agric. Sci. Mansoura Univ., 22 (1l): 3987-4006.

El-Kashoury, A.I. (1999). Subchronic toxicity studies of imidacloprid, profenofas and carbosulfan and their mixtures on albino rats. Ph. D. Thesis, Fac. Agric. Cairo Univ. P. 157.

Enan, E.E.; Enan, O.H. and El-Sebae, A.E. (198 1). Biochemical targets affected by sublethal doses of organophosphorus insecticides. Int. Pest. Control. SeptJOct. 1981.

Eweis, E.E.A. (1994). Subchronic toxicological assessment for some new insecticides on white mice. Ph.D. Thesis in Pesticides. Fac. of Agric. Cairo Univ.

Gillespie, A.T. and Claydin, N. (1989). The use of entomogenous fungi for pest control and role of toxins in pathogenesis. Pestic. Sci. 27, 203-215.

Grau, R. (1988a). Acute oral LD50 of NTN33893 technical against Japanese quail. Intern. Report VW 123. Bayer AG. Institute Ftir bkobiologie. 28, 1, 1988.

Griffin L.; Liege, P.; Kreiger, K.; Kwochka, K.W and Willemse, T. (1998). imidacloprid, a new compound for control of fleas and fleainitiated dermatitis. Advances in Veterinary Dermatology. Vol. 3. Proc. of 3dConf. of Vet. Der., Edinburg, Scotland, 449-450.

Harnza, S.M.; Bakry, H.H.; Abu-Hadeed, A.H. and Kadry, A.M. (198 1). Toxicity of a newly applied synthetic pyrethroid Tenpropathrin", insecticide in dogs. Proc. 4thArab Pesticide Conf Tanta Univ. pp. 19-29.

Hayes, A.W. (1989). Principles and Methods of Toxicology. Raven Press. New York. 2 d ed.

Hodgson, E. and Levi, P.E. (1997). Modem Toxicology. Appleton & Larrge. Printed in USA P. 239.

Hopkins, T. (1998). Imidacloprid and resolution of signs of flea allergy dermatitis in daogs. Canine practice, 23: 2, 18-20.

Isman, M.B. (1995). Leads and prospects for the development of new botanical insecticides. Rev. pestic. Toxicol, 3: 1-20.

Jacobs, D.E.; Hutchinson, M1; Fox, M.T. and Krieger, K.J. (1997). Comparison of flea control strategies using imidacloprid or lufenuron on cats in a controlled simulated home environment. American Journal of veterinary Research, 58: 11, 1260-1262.

Kadir, A.A. and Barlow, H.S. (1992). Pest Management and the Environment in 2000. C.A.B. International, Malaysia, 401 pp.

Lillie, R.D. and Fullman, H.M. (1976). Histopathologic Technique and Practical Histochemistry. McGraw-Hill Book Company. A Blakiston Publication. New York, St. Louis, San Francesco and London.

Menz, M. and Luctkemier, A. (1974). Long term exposure of factory workers. Arch. Environ. Health., 28: 3579.

Middaugh, D.P. and Genther, F.J. (!994). Infectivity and teratogenicity of Beauveria bassiana in Medidia ber Yllina embryous. J. Archives of Environmental Contamination and Toxicology 27 (1): 95-102.

Nirmalan, G.P. and Robinson, G.A. (197 1). Haematology of the Japanese quail (Coturnix coturnixjaponica). Bt. Poult. Sci., 12: 479-481.

Okazawa, A.; Akamatsu, M.; Ohoka, A.; Nishiwaki, W.J.C.; Nakagawa, Y.; Nishimura, K. and Meno, T. (1998). Prediction of the binding mode of imidacloprid and related compounds to housefly head Ach receptors using three-dimensional QSAR analysis. Pestic. Sci., 54: 134-144.

Persley, G.J. (1996). Biotechnology and Integrated Pest Management CAB International. pp. 475.

Pfluger, W. and Schmuck, R. (1991). Ecotoxicological profile of imidacloprid. Pfianzenschutz Nachrichten Bayer, 44/1991, 2 pp: 145-158.

Puina, J.S.; Yadava, S.S.; Jain, S.K. and Garg, B.D. (1987). Haematological and biochemical changes in subacute toxicity of phenthoate. Haryana Veterinarian. 26 (1 & 2): 17-2 1.

Saleh, M.A.A.; Zaher, A.M.; Ahmed. F.A. and Ismail, S.A. (1986). Comparative toxicity of flucythrinate and fenvalerate to albino rats. Egypt J. Food. Sci., 14, No. 1, pp. 31-37. 1

Sancho, E.; Ferrando, M. and Andreu, E. (1997). Sublethal effects of an organophosphate insecticide on the European eel, Anguilla anguilla. Ecotoxicology & Environmental Safety. 36 (1): 57-65.

Sastry, V.R.B. and Agrawal, DX. (1992). Utilization of water washed neem (Azadirachta indica) seed kernel cake as a protein source for growing pigs (BIBLIOGRAPHIC CITATION): Journal of Applied Animal Research, 1992.

Sati, J.M.D. (1996). Liver enzymes as biomarkers of exposure to organophosphorus pesticides. Alex. Sci. Exch., Vol. 17, No. 4, pp. 351-360.

Shakoori, A.R.; Alam, L; Aziz, F. and Ali, S.B. (1990). Biochemical effects of bifenthrin (Talstar) administered orally for one month on the blood and liver of rabbit. Proc. of Pakistan Cong. of Zoology. 10: 61-81.

Shakoori, AK; Aslam, F.; Sabir, M. and Ali, S.S. (1992). Effect of prolonged administration of insecticide (cyhalothrin/Karate). Folia Biologica Warszawa. 40 (12): 91-99.

Szubartowska, E.; Gromysz-Kalkowska, K. and Stepien, M. (1992). Blood morphology in quails after poisoning with Fenitrothion. Comp. Biochem. Physiol., C. Vol. 191 C, no. 2, pp. 263-267.

Weill, C.S. (1952). Tables for convenient calculation of median effective dose (LD50 or ED5o) and instructions in their use. Biometrics, 8: 249-263.

Wen, Z.; Scott, LG. and Wen, Z.M. (1997). Cross resistance to imidacloprid strains of German cockroach (Blatella germanica) and housefly (Musca damestica). Pesticide Science, 49: 4, 367-371.

Whitten, MJ.; Jefferson, R.A. and Daul, D. (1996). Needs and opportunities in: Biotechnology and integrated pest management. Edited by Persley , G1 CAB International p. 17

Wilkinson, C.F. (1976). Insecticide Biochemistry. Press. New York and Physiology. Plenum. p. 704.

Wilkinson, J.11. (1970). Clinical significance of enzyme activity measurements. Clin. Chem. 16: 882.

Yamamoto, L; Yabuta, G.; Tomizawa, M.; Satio, T.; Miyamoto, T. and Kagabu, S. (1995). Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. Nihon Noyaku Gakkaishi (J. Pestic. Sci.), 20: 33-40.

Whitten, MJ.; Jefferson, R.A. and Daul, D. (1996). Needs and opportunities in: Biotechnology and integrated pest management. Edited by Persley , G1 CAB International p. 17

Wilkinson, C.F. (1976). Insecticide Biochemistry. Press. New York and Physiology. Plenum. p. 704.

Wilkinson, J.11. (1970). Clinical significance of enzyme activity measurements. Clin. Chem. 16: 882.

Yamamoto, L; Yabuta, G.; Tomizawa, M.; Satio, T.; Miyamoto, T. and Kagabu, S. (1995). Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. Nihon Noyaku Gakkaishi (J. Pestic. Sci.), 20: 33-40.

Zidan, A.A. (1991a). Biochemical and haematological changes in male white albino mice as affected by sumicidin and sumithion. Fourth Arab Congress of Plant Protection. Cairo 1-5 Dec. 199 1.

Zidan, A.A. (1991b). Biochemical response of male white albino mice to lethal and median lethal doses of sumi-alpha, sumicidin and cyanox. Foruth Arab Congress of Plant Protection, Cairo 1-5 Dec., 199 1.

الملخص العربى

التاثيرات السامة لبعض المبيدات الرشيدة على طيور السمان

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أجريت هذه الدراسة لتقييم اخطار بعض المبيدات الغير تقليدية وهى الإيميداكلوبريد، زيت النيم، الزيت المعدنى "مصرونا" ومستحضر تجارى للفطر الممرض للحشرات *Beauvaria bassiana* على طيور السمان اليابانى وذلك من خلال اختبارات السمية الحادة والتحت مزمنة والأخيرة أجريت عند جرعات تحت مميته متكررة عن طريق الفم تعادل كل منها ١/٥٠ أو ١/١٠٠ من الجرعة النصفية القاتلة وذلك لمدة ٣٠ يوم.

وقد أظهر الكلوربيريفوس والسيبرميثرين (مبيدات تقليدية أشتملت عليها الدراسة للمقارنة) سمية حادة عالية وتأثيرات أخرى ناتجة من التسمم تحت المزمن ظهرت فى صورة تغيرات فى مكونات الدم، اختلال فى وظائف الكلى والكبد بالإضافة الى إرتفاع نسبة سكر الدم. وكان تأثير السيبرميثرين أقل حدة من الكلوربيريفوس. وقد أكد الفحص الهستوباثولوجى الأضرار الواقعة على الكبد. وبالرغم من المميزات المعروفة للأيميداكلوبريد كمركب من مجموعة كيماوية مستحدثة فإنه أظهر سمية حادة عالية ضد طيور السمان مشيرا بذلك الى احتمال ارتفاع سميته الحادة للطيور بصفة عامه كما أن الجرعات التحت مميته من هذا المركب أظهرت سمية على كبد وكلى الطيور المعاملة. أما بالنسبة لزيت النيم فلم يظهر أى سمية حادة ومع ذلك أدى الى حدوث تغيرات بيوكيماوية وتغيرات فى صورة الدم بالإضافة الى تلف أنسجة الكبد وذلك عند المعاملة بجرعاته تحت المميتة. وقد لوحظ أن التأثيرات الضارة الناجمة عن زيت النيم أو الأيمداكلوبريد كانت بصفة عامة أقل حدة من تلك الناتجة عن الكلوربيريفوس أو السيبرميثرين. والأكثر من ذلك أن معظم هذه التأثيرات لم تلاحظ غالبا فى الطيور المعاملة بالجرعة المنخفضة. ومن أبرز النتائج المتحصل عليها فى الدراسة الحالية الإنخفاض النسبى للسمية والذى لوحظ فى الطيور المعاملة بالزيت المعدنى أو المبيد الحيوى فبالإضافة الى انعدام السمية الحادة لهذه المواد تقريبا فإنها لم تحدث تأثيرات معنوية ضارة على صورة الدم أو على النظم البيوكيماوية تحت الدراسة كما أكد الفحص الهستوباثولوجى سلامة أنسجة الكبد فى هذه الطيور ومع ذلك تقترح الدراسة أن الأمر يستلزم إجراء اختبارات توكسكولوجية أخرى شاملة على حيوانات عديدة باستخدام جرعات أخرى من هذه المواد أقرب واقعية وبصفة خاصة لمكونات مستخلص بذور النيم وكذا توكسينات الفطر تحت الدراسة.