

**TOXICOLOGICAL STUDIES OF SOME PESTICIDES ON
CERTAIN VEGETABLE CROPS PESTS
III-SIDE EFFECTS OF SOME COMPOUNDS USED TO
CONTROL CERTAIN VEGETABLE CROPS PESTS ON
WHITE RATS**

**Ahmed, F. A. M. ; A. A. Zein ; M. H. Tag El- Din and J. B. El-naggar
Pesticides Department , Fac. of Agric., Tanta Univ., Egypt .**

ABSTRACT

Side effects of tested compounds i.e., Fenpyroximate, fenarimol ; sulphur and some of their alternatives i.e. mineral oil, Kz-oil and black pepper extract on some enzyme activities in white rats were studied. Results showed that serum ChE activity was significantly decreased in all treatments ,except in fenarimol treatment it increased.Total protein concentration in serum was increased in the case of fenarimol, sulphur, black pepper extract and kz-oil . Albumin concentration in serum was not significantly changed with all tested compounds. Serum alkaline phosphatase activity was significantly increased with all tested compounds.The maximum percent of increasing was found in case of fenpyroximate treatment.Serum AST activity decreased with fenpyroximate and sulphur treatments, while it increased in the black pepper extract treatment.Serum ALT activity decreased with fenpyroximate and sulphur, while it was increased with fenarimol and black pepper extract.Creatinine concentration in serum was not significantly changed with all tested compounds.Urea concentration in serum increased with fenarimol and black pepper extract treatments.With the exception of sulphur treatment all tested compounds caused liver damage as pyknosis of karyolysis of the hepatic nuclei, congestion of the protoliver with infiltration of the protal area with lymphocytes, coagulation necrosis of the hepatocytes . Fenpyroximate and fenarimol caused increase in the marginal zone of white pulp, but Kz-oil caused decrease in the size of white pulp.All tested compounds caused kidney damage as mild cloudy swelling of the renal epithelium, congestion of glomerular tuft inter tubular, necrosis of glomerular renal epithelium.Brain tissue was not affected in any treatment.

INTRODUCTION

The use of chemical pesticides during the last few years has rapidly increased because of their fast action and prolonged protection; this has created a pollution problem to the environment . Pesticide residues after application on vegetable crops should be followed by many processes to avoid side effects of pesticide residues .Many authors studied side effects of pesticide residues on the albino white rats El-Harrawie *et al.* (1991); Kandil *et al.* (1991); Abd-Elbaki *et al.* (1993); Tag El-Din *et al.* (1996) and Abd-Allah (1998).This study was directed to investigate the effect of the tested compounds fenarimol, fenpyroximate (1/3 LD₅₀), sulphur,kz-oil (1/3 recommended dose) and black pepper extract at (5000 ppm) where combined with the food on some important enzymes which affect ed some biological functions.

MATERIALS AND METHODS

1-Tested Compounds :

Five compounds were used in this study. All tested compounds were in the formulated form and dosage were calculated on the basis of ppm. of active ingredient. The chemical names of the tested compounds are as follows.

1-a. Acaricide:

Ortus : The common name is – fenpyroximate (5% S.C.)

The IUPAC name is: tert-butyl (E)- α - (1, 3-dimethyl -5- phenoxy pyrazol-4-yl methylene-amino-oxy) - P-toluate.

It was supplied by Nihon Nohyaku Company-Tokyo-Japan.

1-b.Fungicides:

Rubigan: The common name is – fenarimol (12 % E-C.)

The IUPAC name is: (+) -2,4'-dichloro- α -(pyrimidin-5-yl) benzhydryl alcohol.

It was supplied by Eli Lilly Company Dow Elanco Germany

1-c. Flowable sulphur: (52% S.C.) provided by stoller chemical Company – U.S.A

1-d. Mineral oil: Kz-oil: formulated mineral oil supplied by Kafr El-Zayat pesticides and chemicals Company as 95% E.C. This oil is recommended to control the scale insects in Egypt.

1-e.Plant extract of black pepper (*Piper nigrum* fruits):The plant extract was prepared according to Abbassy *et al.* (1993)

2-Rearing of animals

A pure strain of healthy white albino male rats; *Rattus norvegicus* were purchased from Medicin Faculty, Tanta Univ., and reared under laboratory conditions of $25 \pm 5^\circ\text{C}$ and $65\% \pm 5\%$ R.H. The rats were housed in metallic cages 45 x 35 x 20 cm, fed daily with balanced ratio consisted of bread, lettuce, carrot, cucumber and tomato. Male rats of 3-4 months old with the mean average weight of 120-150 gm were used in the current study.

3-Biochemical experiments:

3-a.Treatments of white rats:

Six groups of male rats (4 rats/group) equal in age and weight as described before were used in the experiment for each compound. Additional four rats were reserved as control check, Rats were given contaminated diet (tomato, cucumber and pepper) in daily doses of $1/3$ LD₅₀ (as described in the pesticides Manual (1994) for fenpyroximate and fenarimol and $1/3$ recommended dose for sulphur and KZ-oil, but for black pepper extract 5000 ppm. was used to contaminate the diet. Throughout the experimental period (28 days), rats were observed for any mortalities or any clinical symptoms.

3-b.Determination of enzymes activity and histopathological studies:

Four rats from each group were weighed and slaughtered after 28 days of treatments and blood samples were collected in clean sterile vials.

The blood was centrifugated for 15 minutes at 4000 r.p.m. Serum was used in this experiments to determine the different enzymes activities of cholinesterase (ChE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total protein, albumin, creatinine and urea. Brains, kidneys, spleens and livers were obtained and kept in formalin 10% for histopathological studies.

The activity of cholinesterase (ChE) was determined according to the method of Ellman *et al.* (1961), Transaminases activity were determined by colorimetrically method according to Reitman and Frankel (1957), the method of Rec (1972) was used for determination of alkaline phosphatase, determination of total protein was carried out by the method of Henry (1964), determination of albumin was carried out by the method of Doumas and Watson (1971), the determination of creatinine was carried out according to Schirmeister, J. (1964) and the determination of urea was done as follows: The enzymatic colorimetric test was carried out by the method of Patton and Crouch (1977).

4- Histopathological studies

This experiment was carried out to study the histopathological lesions of brain, liver, spleen and kidney tissues of rats treated with 1/3 LD₅₀ of tested compounds as daily dose in food for 28 days. The brain, liver, spleen and kidney of slaughtered rats were removed and fixed in formalin 10% and processed to paraffin sections according to the method of Lillie and Fullman (1976). The sections were stained with Hematoxyline and Eosin. All histopathological study was done in the Dep. of Histology, Fac. of Vet. Medicine, Mansoura Univ. sections were examined and the data were recorded.

5-Statistical analysis

statistical analysis of the enzyme activity data was carried out according to Duncan's multiple range test (Duncan's, 1955).

RESULTS AND DISCUSSION

1-a Effects of the tested compounds on cholinesterase activity:

The effects of daily administration of treated fruits (tomato, cucumber and pepper) of tested compounds (1/3 LD₅₀ of fenpyroximate and fenarimol, 1/3 recommended doses of sulphur and Kz-oil and at 5000 ppm black pepper extract) on serum ChE activity in treated male rats were summarized in table (1). The results showed that, serum ChE activity was significantly decreased with all treatments with the exception in fenarimol treatment it increased (unexpected data). These results are in agreement with those found by Tag-El-Din *et al.* (1996); Abd-Allah (1998) Abd-Elbaki *et al.* (1999) and Saleh (1990), who concluded that ChE activity was significantly decreased after exposure to organophosphorus or carbamate insecticides in white rats.

Table (1): Effect of daily administration on untreated and treated fruits (tomato, cucumber and pepper) for 28 days on serum AchE^{*}, total protein and albumin of white rats.

Treatments	Activity of AchE* \pm sE	Total protein conc (gm/100ml)	Albumin conc. (gm/100ml)
		Mean \pm sE	Mean \pm sE
Control	0.289 \pm 0.009 b	5.827 \pm 0.183 d	2.87 \pm 0.48 ab
Fenpyroximate	0.259 \pm 0.005c	6.24 \pm 0.15 cd	3.38 \pm 0.57 a
Fenarimol	0.314 \pm 0.019a	6.57 \pm 0.29 bc	2.56 \pm 0.042 b
Sulphur	0.191 \pm 0.005 d	7.21 \pm 0.07 a	2.46 \pm 0.09 b
Black pepper extract	0.284 \pm 0.011 bc	6.937 \pm 0.31 ab	3.22 \pm 0.41 ab
Kz-oil	0.262 \pm 0.006 c	6.523 \pm 0.29 bc	2.59 \pm 0.33 b
L.S.D _{0.95}	0.01875	0.14419	0.7007

* activity = μ moles Asch/min./mg protein.

1-b. Effects of the tested compounds on total protein:

Results presented in table (1) indicated that, total serum protein concentrations were significantly increased in rats treated with fenarimol, sulphur, black pepper extract and Kz-oil. Many authors reported that total serum protein concentrations were increased after exposure to different pesticides (El-Harrawie *et al.*, 1991 and El-Hamady 1997). On the other hand Eweis *et al.* (1995) reported that the amount of total protein was significantly decreased after exposure to Ivermectin in white rats.

1-c. Effects of the tested compounds on albumin:

Results concerning the effects of the tested compounds on albumin concentration are illustrated in table (1). The data showed that albumin concentration was at the normal level, hence there is no significant difference in serum albumin concentration of all treated or untreated rats. Reduction in albumin concentrations was observed by many authors in animals exposed to various insecticides (Saleh, 1990 and El-Hamady, 1997). On the other hand Abd-Allah (1998) found that albumin concentration was increased in treated white rats with chloropyrifos methyl and pirimicarb after 60 days from treatment with daily oral dose.

1-d. Effects of the tested compounds on alkaline phosphatase:

Results in table (2) showed that, alkaline phosphatase activity was significantly increased in serum of the treated male rats for all tested compounds. Similar effects were obtained by many authors, El-Harrawie *et al.* (1991); Kandil *et al.* (1991); Abd-Elbaki *et al.* (1993); Tag El-Din *et al.* (1996) and Abd-Allah (1998).

1-e. Effects of the tested compounds on transaminases activity (AST and ALT):

Results recorded in table (2) showed that, serum AST activity was decreased with fenpyroximate and sulphur, while it was increased with black pepper extract compared with untreated rats ALT activity was decreased with fenpyroximate and sulphur. On the other hand, ALT activity was increased

with fenarimol and black pepper extract. These results were in agreement with those of many authors, Saleh. (1990); El-Harrawie *et al.* (1991); Abd-Elbaki *et al.* (1993); Tag El-Din and Salama (1996); El-Kassabany (1995); Tag-El-Din *et al.* (1996) and El-Hamady (1997) who reported that, transaminases activity was increased after exposure to different pesticides in white rats. On the other hand, other investigators observed decreasing in levels of transaminases activity after exposure to different pesticides in white rats, Zidan *et al.* (1991); Bayoumi *et al.* (1995) and Eweis *et al.* (1995).

Table (2): Effect of daily administration of untreated and treated fruits (tomato, cucumber and pepper) for 28 days on serum alkaline phosphatase (ALP), AST and ALT of white rats:

Treatments	ALP (u/L)*	AST (u/L)*	ALT (*u/L)
	Mean ± SE	Mean ± SE	Mean ± SE
Control	223.61 ± 5.46 f	45.36 ± 3.72 ab	50.59 ± 2.47 a
Fenpyroximate	333.03 ± 5.49 a	37.84 ± 2.63 c	44.74 ± 2.98 b
Fenarimol	299.06 ± 4.16 d	47.33 ± 5.4 ab	56.28 ± 2.96 a
Sulphur	261.79 ± 5.45 e	41.42 ± 3.76 bc	41.27 ± 3.63 b
Black pepper extract	324.51 ± 2.73 b	52.63 ± 3.42 a	56.16 ± 3.6 a
Kz-oil	309.97 ± 4.17 c	46.84 ± 2.03 ab	53.12 ± 2.86 a
L.S.D _{0.95}	7.725	6.795	5.742

* Activity = U/L

1-f. Effects of the tested compounds on Creatinine and urea:

Results of the effects of the tested compounds on Creatinine and urea are recorded in table (3). Data showed that no significant effect on creatinine concentration of all tested compounds in rats. However, fenarimol and black pepper extract caused significant increase in urea concentration.

Table (3): Effect of daily administration of treated and untreated fruits (tomato, cucumber and pepper) for 28 days on serum enzymes representing kidney function of rats.

Treatments	Creatinine (mg/100ml) Mean ± SE	Urea (mg/100 ml) Mean ± SE
Control	1.43 ± 0.05 a	2.32 ± 0.24 b
Fenpyroximate	1.6 ± 0.1 a	2.46 ± 0.05 ab
Fenarimol	1.75 ± 0.062 a	2.6 ± 0.11 a
Sulphur	1.62 ± 0.025 a	2.367 ± 0.08 ab
Black pepper extract	1.92 ± 0.021 a	2.62 ± 0.16 a
Kz-oil	1.34 ± 0.02 a	2.483 ± 0.13 ab
L.S.D _{0.95}	-	0.2329

2-Histopathological examinations:

2-a Histopathological effects on liver of treated rats:

Figure(1) shows the normal structure of liver. Microscopical examination showed that liver of animals treated with fenpyroximate at dose of 1/3 LD₅₀ showed pyknosis of karyolysis of the hepatic nuclei beside hydrobic degeneration (Fig. 2). Fig. (3) shows the liver of animals treated with fenarimol at dose 1/3 LD₅₀, and indicate congestion of the protoliver with infiltration of the protal area with lymphocytes. In case of iver of rats treated with Kz-oil (1/3 recommended dose), the examination showed coagulation necrosis of the hepatocytes with sever congestion of the hepatic sinusoids (Fig. 4). Fig. (5) shows the liver of animals treated with black pepper extract at 5000 p.p.m , was severely damaged as Necrosis of the hepatocytes beside infiltration of the protal area with round cells (inflammatory cells).

2-b. Histopathological effects on spleen of treated rats:

Figure (6) shows the normal structure of spleen. Rats treated with fenpyroximate showed increased marginal zone of white pulp (Fig.7). Fig. (8) shows the spleens of rats treated with fenarimol which caused chromatin clumping of white pulp. Spleen of rats treated with Kz-oil showed decrease in the size of white pulp (Fig. 9). These results are in agreement with that of Abassy *et al.* (1988) , Abassy *et al.* (1989) , Hanafy *et al.*(1991) , Abd-Elbaki *et al.* (1993) .

2-c. Histopathological effects on kidney of treated rats:

Fig. (10) shows the normal structure of kidney. Kideny of rats treated with fenpyroximate showed mild cloudy swelling of the renal epithelium (Fig. 11). Fig. (12) shows the kidney of rats treated with fenarimol which caused congestion of glomerular tuft with moderate cloudy swelling of the renal epithelium. Kideny of rats treated with Kz-oil showed congestion of the glomerular tuft inter tubuler blood vessels beside cloudy swelling of the renal epithelium (Fig. 13). For rats treated with black pepper extract tissues of kidneys showed necrosis of the glomerular renal epithelium (Fig. 14).

2-d.Histopathological effects on brain of treated rats:

Histopathological examination of brain of rats treated with 1/3 LD₅₀ as daily administration of tested compounds showed no histological alterations in its tissues.

fig

REFERENCES

- Abbassy, M. A.; M. A. Ashry; A. A. Zein; M. M. Abdel-Hafez and M. M. Abd-Elbaki (1988). Side effects of environmental toxicants 11-Biochemical and histopathological effects of profenofos and leptophos on hens plasma and liver. *J. Agri Res. Tanta Univ.*, 14 (1): 414-430.
- Abbassy, M. A.; A. A. El-Swak; Y. A. Hussein and M. Tag. El-Din (1989). Side effects of environmental toxicants VII. Effect of the organophosphorus insecticides sulprofos on serum enzymes and liver of laying hens. *Egypt. J. Appl. Sci.*, 4 (3): 595-600.
- Abbassy, M. A.; Sh. E. E. El-Hamady and M. A. Abd-Elbaki (1993). Efficiency of certain natural and synthetic pesticides in controlling whiteflies and mites on cucumber grown under plastic tunnels. *J. Agric. Res. Tant Univ.*, 19 (2) : 474-479.
- Abd-Allah, S. A. A. (1998). Toxicological studies of some pesticides in relation to their side effects. M. Sc. Thesis, Fac. Agric Kafr El-Sheikh, Tanta Univ.
- Abd-Elbaki, M. A.; S. Dora; Sh. E. E. El-Hamady; A. A. El-Sawak and M. A. Abbassy (1993). Toxicological, histopathological and mutagenicity studies of some fungicides on rats and microorganisms. *J. Agric. Res. Tanta Univ.*, 19 (2) : 480-494
- Abd-Elbaki, M. A.; Sh. E. E. El-Hamady; L. A. Reda and M. M. F. El-Naggar (1999). Assessment of safety period for consumption of some vegetables containing pirimiphos-methyl residues in rats. *Egypt. J. Zool.*, 32 : 441-458.
- Bayoumi, O. C.; A. Abdel-Azeez; Kh. Khilo; A. A. El-Fikey and Sh .A.Hemeda (1995). Hepato-renal and cytogenetic studies on the effect of the insecticide chlorpyrifos methyl (Reldan), the foliar fertilizer, (ff multicri I) and or their mixture on rats. *Vet. Med. J. Giza*, 43 (1): 115 -122.
- Doumas, B. and W. Watson (1971). (In :Colorimetric method for determination of albumin serum, Diamond DiagnosticCo, .Germany .Clin. ,Chim. Biocon. Acta. 31:87-89.
- Duncan, B. D. (1955) .Multiple range and multiple F test. *Biometrics* ,11:1-42.
- El-Hamady, Sh. E. E. (1997).Pesticidal efficiency and mammalian toxicity of Abamectin applied at different field concentrations to control certain pests on some vegetables. 7th Nat. Conf. of Pest and Dis. of vegetables and fruits in Egypt, 664-681.
- El-Harrawie, M. A.; F. M. El-Nattar; M. M. Farid and H. M. Salem(1991). Acute and subchronic toxicity of methamidophos for albino rats. 4th Arab Conf. Plant Protec., 270-278.
- El-Kassabany, S. A. (1995).Haematological and biochemical changes induced by Folpet and Captan in rats. *Com. in Sci. and Dev. Res.*, 52 (781) :15-22 .
- Ellman, G. L.; K. D. Courteney; V. Andres and R. M. Featherstone (1961). Anew and rapid colormetric determination of acetylcholinesterase activity. *Bioch. Pharmac.*, 1 : 88-95.

- Eweis, E. A.; N. Eihwashy; H. K. Said and M. A. Kandil (1995). Acute toxicity of Ivermectin in laboratory animals. 1st Int. conf. of Pest control, Mansoura, Egypt, 89-96.
- Hanafy, M. S. M.; M. S. Arbid and M. M. H. Afify (1991). Biochemical and histopathological effect of the organophosphorus insecticides tamaron in rats-Indian Journal of Animal Sciences, 61 (1) : 43-47.
- Henry, R. J. (1964). Clinical chemistry, Harper and row publishers, New york: 181(In colorimetric method for determination of total protein in serum. Diamond Diagnostics Comp., Egypt).
- Kandil, K. A.; F. M. El-Nattar; M. U. Mohamed and M. H. E. Gad (1991) . Effect of cyanophos on certain enzymes activities and thyroid function of mice. 4th Arab Conf. Plant Protec. Cairo 1-5: 279-285.
- Lillie, R. D. and H. M. Fullman, (1976). Histopathologic technique and paractical histochemistry. Mceraw-Hill Book coppany. A. Blakiston publication. New York, St. louis, Sanfraneisco and London.
- Patton, C. J. and S. R. Crouch (1977). Anal. Chem. 49 : 464-469. (In colorimetric method for determination of urea in serum. Diamond diagnostics Co., Egypt).
- Rec, G.S.C.C (1972). J. Clin Chem. Clin Biochem. 10 : 182 (In colorimetric method for determination of alkaline phosphatase in serum Diamond Diagnostics Co., Egypt).
- Reitman, S. M. D. and S. Frankel (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Amer. J. Clin. Path., 28 : 56-63.
- Saleh, F. (1990). Metabolic effects of the carbamate insecticide methomyl on rats : II Changes in serum cholinesterase and transaminases following treatment of the insecticide. J. Phys. Sci., 14 (1-2) : 55-64.
- Schirmeister, J. (1964). Dtsch, Med. Wschr 89 : 1940 Colorimetric method for determination of creatinine, C.F. (Commercial Kits of Pasteur lab. U.S.A).
- Tag El-Din, M. H. and M. A. Salama (1996). Residual and toxicological studies on Rubigan treated potatoes. 6th International Conf. Environmental Protection is a Must" 21-23 May , Alexandria , Egypt, PP:642-657.
- Tag El-Din, M. H.; O. C. Bayoumi and Sh. E. El-Hamady (1996). Efficiency of chlorpyrifos, carbosulfan and cypermethrin against the adult of rice weevil *Sitophilus oryzae* with respect to their deterimenal side effects on white rats. J. Agric. Sci. Mansoura Univ., 21 (6) : 2335-2342.
- Zidan, A. A.; M. I. Hussein and S. M. Dahroug (1991) .Effect of certain insecticides on some biochemical aspects of male mice blood by oral administration. 4th Arab Conf. plant Protec. Cairo : 293-300.

دراسات توكسيكولوجية لبعض المبيدات على بعض آفات محاصيل الخضر
٣-التأثيرات الجانبية لبعض المركبات المستخدمة في مكافحة بعض آفات
الخضر على الفئران البيضاء

فرحات عبد المولى محمد أحمد – أمين عبد الباقي زين – محمود حسن تاج الدين –
جيهان بدوى النجار
قسم المبيدات- كلية الزراعة بكفر الشيخ – جامعة طنطا

تم دراسة تأثير المركبات المختبرة على بعض الاثريمات فى الفئران وذلك عن طريق تغذية هذه الفئران على غذاء (طماطم – خيار – فلفل) ملوث بتركيز يمثل LD_{50} 3/1 لكل من مركبى الفيناريمول والفينبيروكسميت و 3/1 الجرعة الموصى بها حقليا لكل من مركبى الكبريت والزيت المعدنى وتركيز 5000 جزء فى المليون من المستخلص النباتى لبذور الفلفل الأسود لمدة 28 يوم وتم ذبح الفئران. وأوضحت النتائج حدوث انخفاض فى نشاط أنزيم الكولين استريز cholinesterase (ChE) مع كل المعاملات و حدوث زيادة فى تركيز البروتين الكلى total protein مع كل من الفيناريمول والكبريتوالمستخلص النباتى والزيت المعدنى ك – زد ولم تحدث تغيرات معنوية فى تركيز الالبومين Albumin فى كل المعاملات بينما: حدث ارتفاع فى نشاط الفوسفاتير القلوى Alkaline phosphatase مع كل المركبات المختبرة وحدثت أقصى نسبة ارتفاع مع مبيد الفينبيروكسميت و حدث انخفاض فى نشاط Aspartate aminotranferase (AST): حدث مع كل من مركب الفينبيروكسميت والكبريت بينما حدث ارتفاع فى نشاطه مع المستخلص النباتى وحدث أيضاً انخفاض فى نشاط Alanine aminotransferase (ALT) مع كل من الفينبيروكسميت والكبريت بينما حدث ارتفاع فى نشاطه مع كل من الفيناريمول والمستخلص النباتى. لم يحدث تغير معنوى فى تركيز الكرياتنين فى الفئران المعاملة أما بالنسبة لتركيز اليوريا فقد حدث زيادة فى الفئران المعاملة بمركب الفيناريمول والمستخلص النباتى لبذور الفلفل الأسود وأظهرت الدراسات الهستوباثولوجية صغر فى حجم الخلايا الكبدية وتحلل أنويتها وتضخم فى الأورده وارتشاح فى الخلايا الدموية فى جميع المركبات عدا مركب الكبريت حيث لم يكن له تأثير كما أحدث كل من مركبى الفينبيروكسميت والفيناريمول زيادة فى حجم الكتلة البيضاء للطحال بينما أحدث الزيت المعدنى صغر فى حجمهاو أظهرت جميع المركبات المختبرة تضخم فى الأوعية الدموية فى الكلية مع تلف فى الغشاء الطلائى المبطن للكلية عدا مركب الكبريت لم يكن له تأثير. و لم يظهر أى تأثيرات للمركبات المختبره على أنسجة المخ فى الفئران المعاملة .