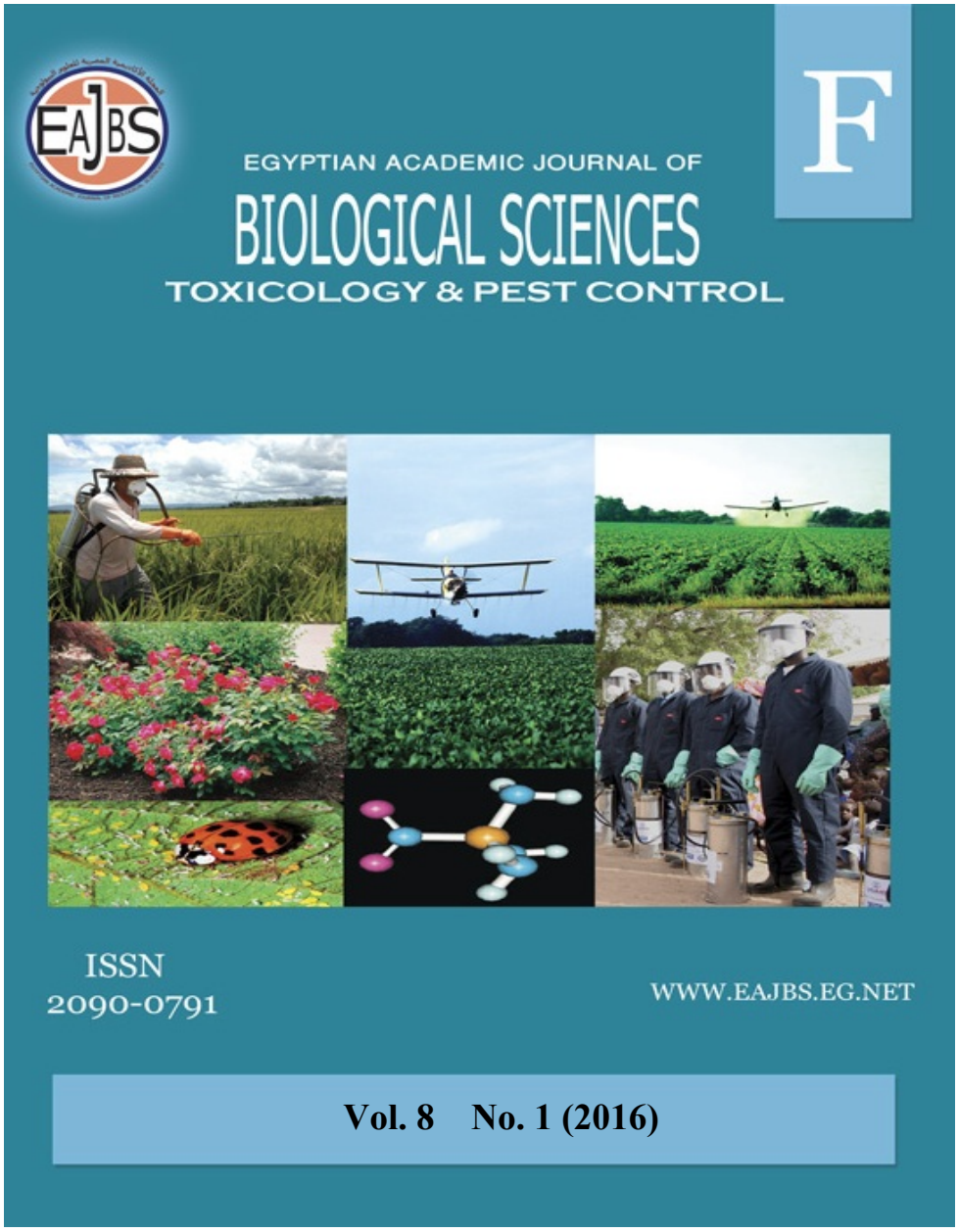

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Reproductive Toxicity of Dermally Applied Mixture of Organophosphate and Pyrethroid Insecticides in A commercial Formulation to Male Albino Rats.

Naser A. Al-Ansary, Fouad A. F. Ali, Mohamed F. El-Tawil and Emad M. Marzouk

Plant Protection Department, Division of Pesticides, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.
E.Mail; alansarynaser@ymail.com

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ABSTRACT

Mixing organophosphate (OP) and pyrethroid (PYR) insecticides becomes common in Egypt since mid-1980s and result in an impairment of male reproductive function. The present investigation was conducted to study certain criteria used to evaluate the reproductive toxicity of treated male rats with the commercial preparation of OP/PYR mixture, namely Runsave, which contains 30% chlorpyrifos (CPF) and 3% lambda-cyhalothrin (LTC) compared to its individual commercial components of 48% E.C (CPF) and 5% E.C (LTC). Adult male rats were treated dermally with sublethal dose of each toxicant (6 rats each) on alternate day for 8 weeks, then they were sacrificed except few rats that treated with Runsave mixture. The relative testes weights, semen picture, activities of plasma and brain cholinesterases as well as histopathological changes in testes were determined. The remaining rats that treated with Runsave were allowed to mate with untreated adult females. Results showed that the candidate toxicants brought about marked reduction in testes relative weights, sperm counts and motilities, activities of plasma and brain cholinesterases (ChEs) and caused histopathological alterations in testes of treated rats, all in comparison with those of corresponding control group. Generally, the commercial preparation Runsave has potentially greater toxic impact for rats than its components alone. Male rats treated with Runsave and allowed to mate with receptive untreated females, their reproductive capabilities were greatly diminished.

INTRODUCTION

In developing countries, the exposure to mixtures of pesticides and the extensive use of their commercial formulations are additional factors that increase health risks.

In Egypt, organophosphorus (OP) and pyrethroid (PYR) insecticides have commonly been mixed since mid-1980s to manage pest complex of cotton and other crops. These mixtures are available as premixes from pesticide companies or they are tank-mixed by farmers.

Ideally, the insecticides having different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pest (Abd El-Mageed and Shalaby, 2011).

The majority of previous investigations reported that (OP/PYR) mixtures had higher toxicity to insects and mammals compared with the toxicity of their individual alone (Ahmad, 2004, 2007; Latuszynska *et al*., 1999, 2001, 2003; Abdallah *et al*., 2010; Noaishi *et al*., 2013). These authors believed that OP compounds inhibit the metabolism of pyrethroids by decreasing the activities of metabolizing esterases (mainly carboxylesterases).

Chlorpyrifos, a phosphorothioate ester, was first registered in U. S. A in 1965. It is one of the most widely used insecticides in agriculture worldwide to control wide range of insect and arthropod pests. It represents more than 50% of global insecticide use (Cassida and Quistad, 2004). It inhibits acetylcholinesterase (AChE), a key enzyme that hydrolyzes the neurotransmitter acetylcholine to choline and acetic acid. Inhibition of ACh results in the accumulation of acetylcholine and the overstimulation of cholinergic receptors, which in turn overstimulates neurological activity in the organism (Grue *et al*., 1997).

Pyrethroid insecticides have been used since the 1970s and represent an increasing proportion of world pesticide sales. Lambda-cyhalothrin is a pyrethroid type Π which contains cyano group in the alpha position of phenoxy benzyl moiety. Pyrethroids are potent lipophilic insecticides recognized as neurotoxicants. They are known to act directly on the axon through interference with the sodium channel-gating mechanism that underlies the generation and conduction of nerve impulses. This effect is responsible for repetitive activity in sense organs, membrane depolarization, and block of excitation (Soderlund *et al*., 2002).

Most occupational exposures to pesticides are through inhalation or dermal exposure (Sullivan and Krieger, 1992). However, Fenske and Elkner (1990) reported that dermal exposure was greater than inhalation and accounted for about 73% of the total exposure. Because there is no established simple experimental models for studying different multiple mixtures of insecticides, methods have been established with rats and mice to study this problem (Ortiz *et al*., 1995; Joshi and Sharma, 2011).

In the literature available, no data were found concerning the adverse effects of dermally-applied Runsave mixture to mammals.

In Egypt, as the whole bulk of spray workers and pest control exterminators are men and their occupational poisoning is mainly through dermal exposure, the present investigation was conducted to study, certain criteria used to evaluate the reproductive toxicity of treated male rats with Runsave in relation to its individual insecticides. Thus, the side-by-side comparison would be accurate since it had occurred under identical test conditions.

MATERIALS AND METHODS

Insecticides used: Chlorpyrifos (48% E.C): *O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, Lambda-cyhalothrin (5% E.C): is a mixture of two isomers:[1α (S*),3α(Z)](±)-cyano(3-phenoxy phenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl) -2,2 dimethyl cyclopropane carboxylate and the commercial preparation of OP/PYR mixture (Runsave, 33% E.C). These insecticides were purchased from Dow Agro Sciences Company, Al-Rwad

Chemicals and Pharmacutica Chemicals Company, Egypt, respectively.

Test Animals: Thirty six adult male Sprague-Dawley rats (190-210g), *Rattus norvegicus albinus*, were purchased from the Biological Products and Vaccines Holding Company, Helwan farm, Cairo. Rats were maintained under the laboratory conditions of 25±5°C and 65±5% R.H for two weeks before starting the experiments for acclimatization. They were housed in metal cages (65 x 25 x 20 cm) with a 12:12 h- light/dark cycle and maintained on *ad Libitum* diet and water. Twelve adult female rats were obtained from the same place to be used later on.

Experimental design: Experiments were carried out after the Ethics Committee of the Faculty of Agri., Al-Azhar Univ. approved the protocol of the experiments and procedures used in the study.

For chlorpyrifos and lambda-cyhalothrin the selected sublethal doses were 1/30 of their dermal LD_{50} values as reported in Pesticides Manual (Anonymous, 2003). The estimated sublethal doses used as formulated products for chlorpyrifos and lambda-cyhalothrin were 139 and 442 mg/kg, respectively. For Runsave mixture, the chosen sublethal dose of its formulated product was 121.2 mg/kg. The selection of that sublethal dose was based on both preliminary experiments (which didn't cause any rat mortality after two months) and in the neighborhood of those reported previously with OP/PYR mixtures (Latuszynska *et al.*, 1999; Shalaby and Abd El-Mageed, 2010; Noaishi *et al*., 2013).

Rats were divided randomly to six groups, each group had six male rats, and the experiments were divided into two parts. Part I: included five groups: two groups treated with Runsave, one group with chlorpyrifos and one group with lambda-cyhalothrin all in comparison with control group. Part Π: included a group that were treated with Runsave then allowed to mate with untreated females in comparison with the sixth control group. Male rats of Part I were treated dermally with sublethal doses of candidate toxicants on alternate day for 8 weeks. The fur on the later abdominal area of approximately 4×4 cm of all animals were clipped off as described by Dikshith *et al*. (1976) using animal grooming clipper (WAHL model 305 G) and these areas were kept depilated during the exposure period. Rats were treated dermally using 0.5 ml Hamilton syringe with blunt-pointed needle at a rate of 0.1 ml of toxicant solution per 100g rat body weight. Before treatment, the shaved area was cleaned by a piece of cotton soaked with the diluted solution (acetone: ethyl alcohol 1:3) to remove any oily dirt. After administering the toxicant, rats were immobilized for at least 2 hrs (Bartek *et al*., 1972). Rats were observed daily for overt signs of toxicity and their weights were determined to estimate body weight gain or loss from the initial body weights. At the end of the experimentation period (8 weeks), rats were sacrificed by decapitation, blood samples were collected, left till clotting occurred and centrifuged at 4000 rpm for10 mins. The obtained sera were used for measuring the activities of ChE (pseudo cholinesterases or BuChE). Brain isolated from each rat, was homogenized in a 1% triton x-100 in 0.1M phosphate buffer solution (pH 8.0) to give a 10% (W/V) brain homogenate. The crude homogenate was centrifuged at 3000rpm for 10mins in cold centrifuge. The supernatant was used for measuring the activities of AChEs (true cholinesterases). Both activities of serum ChE and brain AChE were determined spectrophotometrically using the method described by Ellman *et al.* (1961).

Testes from each sacrificed rat were dissected out, trimmed of excess fat and weighted.

The relative testes weight = Absolute testes weight/ Whole body weight ×100.

Sperm suspension was obtained from each sacrificed rat by the modified method of Lubicz-Naworcki and Change (1974). For histopathological examination, the isolated testes were fixed in Bouin's solution and processed by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks, then sectioned at 4 µ thicknesses. The paraffin sections were stained by haematoxylin and eosin (Pearse, 1968) and then histopathological examination was carried out microscopically.

For part Π: After 8 weeks of repeated dermal exposure with sublethal dose of Runsave mixture, each male rat was paired overnight with pro-oestrous untreated females in the ratio (1 male:2 females) in a separate cage. Vaginal smears of the females were taken on the following morning. The presence of spermatozoa indicates a successful mating and was considered day one of pregnancy. If spermatozoa were absent, daily vaginal smearing was done until a successful mating was achieved and the number of nights were counted. Pregnant females were divided into two groups. The first group was giving the chance to complete their gestation periods till delivery. Both the longevity of gestation period (in days) and number of deposited viable fetuses were counted. In the second group, pregnant females were anesthetized and cesarean were performed (at 20th day of gestation) in which their gravid uteri were removed photographed and examined for the number of formed viable fetuses.

Statistical analysis:

The significance of differences was calculated by using one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

General observation:

Rats treated dermally with sublethal doses of Runsave mixture and its individual insecticides, did not show any signs of intoxication or death except those treated with lambda-cyhalothrin that showed skin sensations in their shaved areas. These skin sensations "Paresthesia" was previously observed by Le Quense *et al*. (1980) and Lawrence and Cassida (1999). They reported that the intensity of these sensations was more pronounced with pyrethroids type Π than type I and these burning spots were most likely to be due to repetitive firing of sensory nerve endings. The only conspicuous feature of intoxication with the candidate toxicants, was the reduction of body weight gain of all treated rats compared to control as presented in (Table 1) and Fig. (1).

Table 1: Average weekly body weight gain (g) from initial body weights for control and treated male rats.

Treatments / Times (weeks)	Control	Runsave Mixture	Chlorpyrifos	Lambda-cyhalothrin	Mean
1st	10.72	6.28	1.22	(-)3.33	3.72
2nd	24.72	14.67	13.00	2.89	13.82
3th	39.04	17.75	24.42	(-)0.33	20.22
4th	45.78	22.67	36.33	(-)0.89	25.97
5th	52.56	25.89	43.44	3.78	31.42
6th	63.00	32.33	46.42	7.58	37.33
7th	75.00	40.92	46.33	8.50	42.69
8th	84.56	43.89	50.22	14.44	48.28
9th	90.11	46.44	57.33	22.33	54.05
Mean	53.94	27.87 (48.33%)	35.41 (34.81%)	6.11 (88.75%)	
L.S.D	at 1%		at 5%		
Times	0.58		0.44		
Treatments	0.47		0.36		
Times×Treatments	1.42		1.08		

- Each number presented in the table represent the average of at least 6 replicates (rats).
- (-) sign indicate loss weight from initial weight.
- Parenthetical numbers show the percent reduction of weight of each treatment in relation to the control.

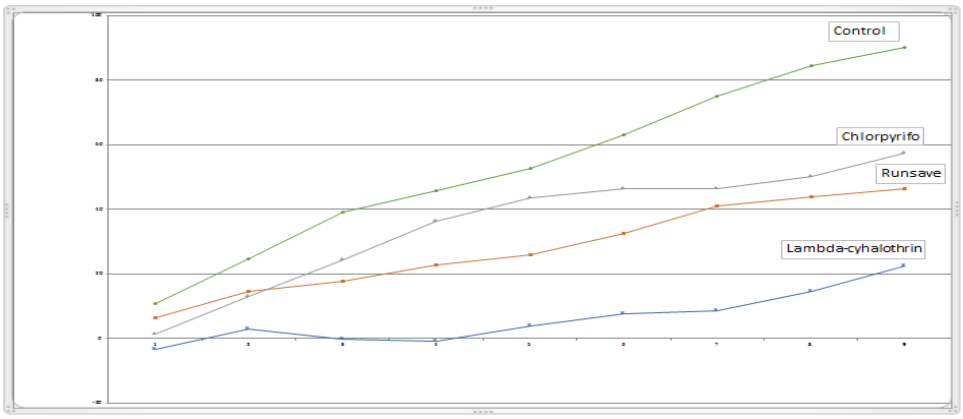


Fig. 1: Average weekly body weight gain (g) from initial body weights for control and treated male rats.

The percent reduction of body weight gain of treated rats compared to control could be arranged descendingly as: lambda-cyhalothrin (88.75%) > Runsave mixture (48.33%) > chlorpyrifos (34.81%). Thus, all rats treated with the examined toxicants suffered reduction in body weight gain compared to control. This piece of result is in accordance with those obtained previously. For OP/PYR mixtures (Wang *et al*., 2012), for chlorpyrifos (Ambali *et al*., 2010, 2011), for lambda-cyhalothrin (Ratnasooriya *et al*., 2002; Abd EL-Kawy *et al*., 2013). These authors suggested that the reduction of body weight gain might be due to anorexia which accounted for the reduction of food intake. However, Ambali *et al*. (2011) believed that the reduction of body weight gain might be a combination of oxidative, toxic and cholinergic stress.

Effect on the activities of serum and brain ChEs

Data shown in Table (2) revealed that the impact of prolonged dermal application of tested toxicants for 8 weeks, resulted in significant inhibition of brain and serum ChE activities. Regardless, the type of ChEs, the maximum percent of inhibition occurred with Runsave mixture followed by chlorpyrifos and lambda-cyhalothrin. The present result corroborated the previous reports (Latuszynska *et al*., 2001, 2003; Wielgomas and Krechnik, 2007; Shalaby and Abd El-Mageed, 2010) who concluded that a mixture of OP/PYR caused a significant inhibition of both brain AChE and plasma BuChE.

Table 2: Activity of brain AChE and plasma BuChE of male rats treated dermally with sublethal doses of examined toxicants for 8 weeks.

Treatment	Brain AChE			Plasma BuChE		
	Activity µ mole/mg protein/min	(%) Activity of control	(%) Inhibition	Activity µ mole/mg protein/min	(%) Activity of control	(%) Inhibition
Control	88.48	100	0.00	4.48	100	0.00
Runsave mixture	9.86	11.1	88.9	0.31	6.96	93.04
Chlorpyrifos	29.76	33.6	66.4	0.67	14.91	85.09
Lambda cyhalothrin	76.15	86..1	13.9	3.59	80.80	19.20
L.S.D at 5%	5.76			0.90		

Data presented in Table (2) showed that, compared to chlorpyrifos, lambda-cyhalothrin produced the least inhibition effect to both enzymes. It has believed that pyrethroid insecticides do not inhibit cholinesterases, which is the target of OP insecticides. Data of that table showed that serum BuChE was more sensitive to candidate toxicants than brain AChE. This piece of result is in agreement with that obtained by Moser (1995) and Amitai *et al*. (1998). Therefore, serum ChE (BuChE) is commonly used to monitor occupational exposure to OP compounds (Khan *et al*., 2008).

There are many environmental toxicants inducing alteration of reproductive functions concurrently with impaction on the central nervous system and behavior, which are so called neuroendocrine disrupters operating through hypothalamo-pituitary-gonadal axis (Sarkar *et al*., 2000; Gore, 2001).

The examined toxicants may act as neuroendocrine disruptors via inhibition of AChE activity and increase of acetylcholine level in brain which may be linked to the suppression of the brain's release of hormones that stimulate the gonado- trophic hormones (LH and FSH) (Zidan, 2009).

Effect on semen picture:

All treatments affected sperm count and motility (Table 3). The descending order of their effects is: Runsave > chlorpyrifos > lambda-cyhalothrin. This result is in agreement with that reported previously in which all treatments had spermicidal effects on experimental animals or humans.

Table 3: Sperm count and sperm motility of male rats treated dermally with sublethal doses of examined toxicants for 8 weeks.

Exposure time	8 weeks	
Treatment	Sperm count ($N\times10^6$/ml)	% Motility
Control	57.86	87.30
Runsave mixture	49.97	72.67
Chlorpyrifos	55.33	78.67
Lambda cyhalothrin	56.80	83.00
L.S.D at 5%	4.32	4.61

For OP/PYR mixtures (Shivaraj *et al*., 2011; Wang *et al*., 2012), for chlorpyrifos (Zidan, 2009), for lambda-cyhalothrin (Kumar *et al*., 2004). Holdcraft and Braun (2004) suggested that the reduction of sperm count might be due to the effect of toxicant on spermatogenesis which is controlled by two main regulatory processes, i.e., endocrine regulation via the gonadotropin hormones and local regulation via inter-cellular communications. Uzun *et al*. (2009) mentioned that the toxicant had the ability to cross the blood-testis barrier and induced oxidative stress and lipid peroxidation that damaged the biological membranes in the testes, which in turn might cause the degeneration of spermatogenic and Leydig cells, which disrupted spermatogenesis and reduced sperm count. They added that the oxidative stress affected the activities of mitochondrial enzymes and the structure of the microtubules in the sperms which in turn reduced their motilities. Lasram *et al*. (2008) found that malathion caused an inhibition of sperm glycolysis which prevented normal ATP production leading to insufficient energy supply for spermmotility. Kenfack *et al*. (2015) suggested that the weak sperm motility could be due to the fact that a great majority of sperm was liberated prematurely from germinal epithelium of treated rats, or to cytotoxic effects of toxicant.

Relative testes weights:

Genital organ weight was among of the criteria used to evaluate the reproductive toxicity of the tested toxicants to rats. Data pertaining to the impact of the tested insecticides on relative testes weights are shown in Table (4), which indicated that all treatments caused reduction of relative testes weights.

Table 4: Relative testes weights of male rats treated dermally with sublethal doses of examined toxicants for 8 weeks.

Treatment	Control	Runsave Mixture	Chlorpyrifos	Lambda cyhalothrin	L.S.D at 5%
	1.21	0.81	0.88	1.12	0.27

This piece of result is in agreement with that obtained previously (for OP/PYR mixture, Wang *et al*., (2012); for OPs, Zidan, 2009; for PYRs, Sandhia and Kumaran, 2013). The reduction of testes weight might be due to the reduction of the bioavailability of androgen, reduce the number of germ cells, inhibition of spermatogenesis and steroid genic enzyme activity (Sandhia and Kumaran, 2013). Several pesticides have reduced the organ weights by affecting either hypothalamus or pituitary or both (Okazaki *et al*., 2001).

Histopathological evaluation:

The present study was focused on studying the testis alterations as it is the essential organ for reproduction. Figure (2) showed photomicrographs of control and intoxicated testes with the examined toxicants after 8 weeks exposure period. Sections of testes of control rats showed normal seminiferous tubules with their complex stratified epithelium and sertoli cells were bounded by basal lamina.

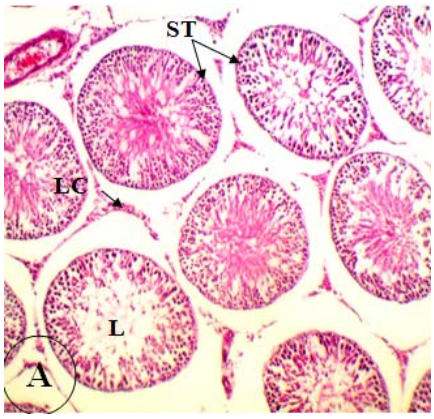
The newly formed sperms were mainly found facing the lumen of the tubules in between the spermatids and attached to the apical parts of sertoli cells. However, some of them were dispersed in between the spermatogenic cells. Some seminiferous tubules were found containing highly active spermatogenic cells with different phases of mitosis. The testicular interstitium was found external to the basal lamina and in between the seminiferous tubules containing blood vessels and compact groups of Leydig cells.

In spite of the great number of testes specimens collected from male rats, only one or two photomicrographs were selected to represent the testicular alterations occurred for each toxicant.

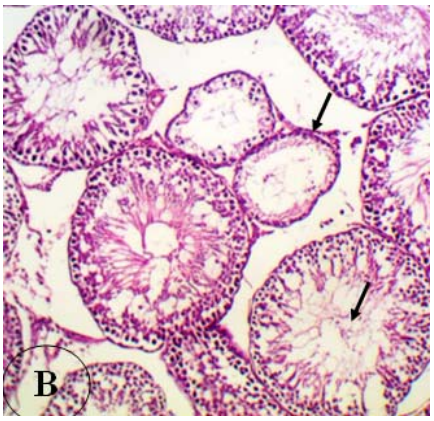
The majority of the seminiferous tubules of treated rats appeared collapsed and deformed with protruded or irregular parts of their lamina. Some of the tubules showed congested blood vessels, while others showed well circumscribed vacuoles replacing some spermatogenic cells. Runsave mixture showed more pronounced impairments than its individual insecticides. The above testicular alterations, observed from the present work, are in harmony with those obtained previously. For OP/PYR mixture, Wang *et al*., (2012), for OPs, Uzun *et al*., (2009), and for PYRs, Assayed *et al*. (2010).

Part Π: Reproductive capacity of treated male rats with Runsave mixture.

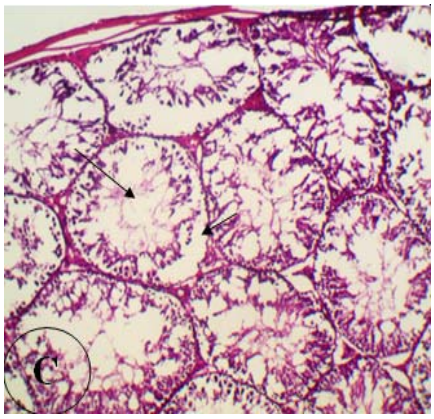
Following exposure of male rats to repeated sublethal dermal dose of Runsave for 8 weeks, they allowed to mate with virgin untreated females. It was observed that, the number of viable pups deposited after delivery (1st group of pregnant females) was nearly equal to the number of viable fetuses found in the gravid uteri of the 2nd group.



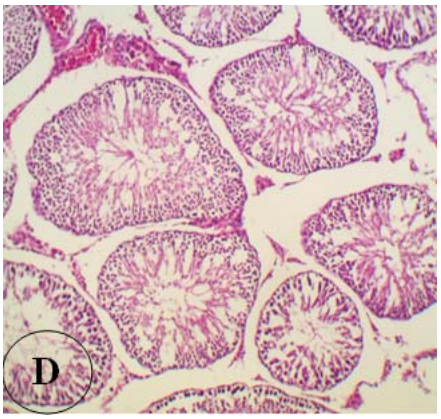
A: Section of control testis showing testicular capsule which contains many seminiferous tubules (S.T) which are uniform in size and shape and are lined by regularly arranged rows of spermatogenic cells at different stages of maturation. Leydig cells (L. C) are found in the interstitial connective tissue between S.T



B: Section of intoxicated testis with Runsave showing irregular shape of S.T. which adversely affect the structure and function of L.C, severe damage of all spermatogenic cells and enlargement of the interstitium



C: Section of intoxicated testis with Chlorpyrifos showing deformed S.T, necrosis of many S.T. and atrophy of all spermatogenic cells



D: Section of intoxicated testis with Lambda-cyhalothrin showing an improvement in the general architecture and all spermatogenic cells are noticed

Fig. 2: Photomicrographs of testes of control and treated rats after 8 weeks (E/H X 100).

Fig. (3) Shows the shape of uteri isolated from pregnant females of each serial mating after 20 days of gestation (i.e. before the normal delivery). In control group, the uterine horns were equal in shape and length with regular distribution of implanted embryos. In females impregnated with treated males their uterine horns appeared to be unequal with irregular distribution of implanted embryos and resorption sites. As shown in Table (5) couples of healthy untreated males with adult untreated females (control) took an average of 1.67 nights for successful mating and the pregnant females gave an average of 8.33 viable fetuses after 22 days gestation period.

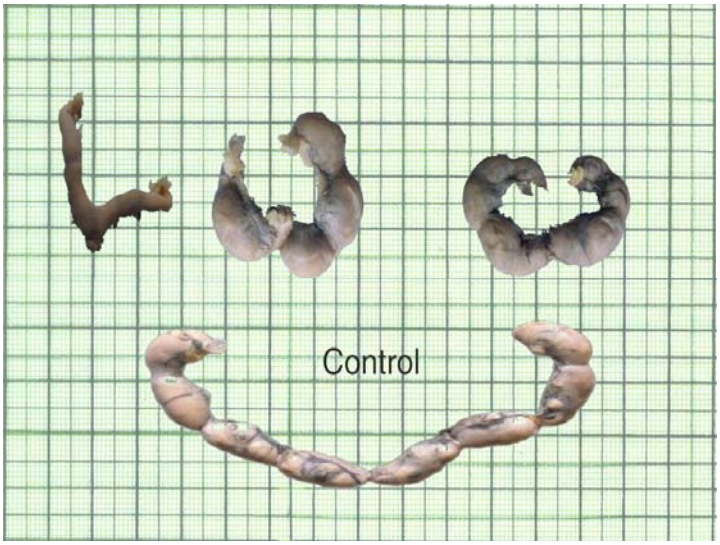


Fig. 3: Isolated uteri of control pregnant females and females impregnated with treated males with Runsave.

Table 5: Effect of serial matings between treated males (♂) and untreated female rats (♀) on some reproductive parameters.

Couples	Mean number of nights required for successful mating	Mean gestation period (days)	Mean number Of viable fetuses
Control (♂)×(♀)	1.67	22.00	8.33
Males treated with Runsave Mixture (♂) × (♀)	8.33	25.33	5.33
L.S.D at 5%	5.24	2.45	1.30

Couples of untreated females impregnated with treated male with Runsave mixture required more nights for successful mating, their pregnant females took longer gestation period until delivery and gave fewer number of viable fetuses. Thus, treated male rats with sublethal dose of Runsave were sufficient to reduce their reproductive capacities compared with those of controls. Same results were obtained previously by Morgan and Abd El-Aty (2008) and Kenfack *et al.* (2015), who used chlorpyrifos as examined insecticide.

The impairment of reproductive capacity due to toxicants could be attributed to :(1) significant suppression of testosterone biosynthesis (SaiLinlin *et al.*, 2014). (2) increased degenerate germ cells in seminiferous tubules (Wang *et al.*, 2012). (3) increased all parameters of sperm toxicity (Pina-Guzman *et al.*, 2009). Morgan and Abd El-Aty (2008) cited that the decreased reproductive performance in animals exposed to OP insecticides could be attributed to antiandrogenic activity, altered androgen metabolism, pathological changes in the testes and suppressive effect on the functional activity of accessory sex glands. The more acceptable explanation was reported by Ratnasooriya *et al.* (2002) who found that treatment of male rats with lambda-cyhalothrin (trade name, ICON) affected their reproductive behavior when they mated with receptive

females. Treatment had no effect on fertility, but sexual competence was seriously impaired: Libido (assessed in terms of pre-coital sexual behavior, and numbers of mounting, intromission and ejaculation), sexual arousability/motivation (in terms of latencies for mounting, intromission and ejaculation and sexual vigour (judged by frequencies of mounting and intromission or copulatory efficiency). In addition, ICON suppressed intromission ratio, indicating erectile dysfunction. ICON-induced sexual dysfunction was mediated by multiple mechanisms, mainly toxicity, stress, sedation and possibly via dopaminergic system.

CONCLUSION

All the obtained results indicate, without any doubt, that the commercial preparation Runsave has potentially greater reproductive toxicity to rats than its components alone. Accordingly, it would be advisable to study further the interactions between insecticides forming OP/PYR mixtures.

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ARABIC SUMMARY

سمية مستحضر تجارى لمخلوط من مبيد فوسفورى وبيريثرويد على القدرة الإنجابية لذكور الفئران البيضاء المعاملة عن طريق الجلد

ناصر عبد الهادي عبد المولى الأنصاري- فؤاد أحمد فهمي على - محمد فوزي محمد الطويل
عماد الدين محمد أحمد مرزوق
قسم وقاية النبات – كلية الزراعة بالقاهرة – جامعة الأزهر

أجرى هذا البحث لدراسة بعض القياسات المستخدمة لتقييم فاعلية المستحضر التجارى رانسيف [الذى يحتوى على 30% من المبيد الفوسفورى (كلوربيريفوس) + 3% من مبيد البيريثرويد (لمباداثيهالوثرين)] على القدرة الانجابية لذكور الفئران البيضاء البالغة مقارنة بفاعلية كل مبيد على حده فى صورته التجارية (الكلوربيريفوس 48% مركز قابل للاستحلاب، لمباداثيهالوثرين 5% مركز قابل للاستحلاب). قسمت ذكور الفئران إلى أربعة مجموعات ، ثلاثة منها عوملت بالمركبات الثلاث السابقة عن طريق الجلد بعد حلاقة فروتها فى المنطقة البطنية السفلية بجرعات غير مميتة يوم بعد يوم لمدة ثمانية أسابيع تلاها ذبح هذه الذكور ما عدا بعض الذكور المعاملة بمخلوط الرانسيف كما استخدمت المجموعة الرابعة كمجموعة ضابطة (كنترول). خلال مدة المعاملة تم ملاحظة أعراض السمية وتدوين أوزان الفئران. الذكور التى تم ذبحها تم تقدير الوزن النسبى للخصى، تحليل السائل المنوى، قياس نشاط إنزيمات الكولين إستريز فى كل من المخ والبلازما كذلك ملاحظة التغير الحادث فى التركيب التشريحى للخصى مجهريا وذلك مقارنة بالمجموعة الضابطة. بالنسبة للذكور المعاملة بمخلوط الرانسيف والتى لم تذبح تم تزاوجها مع إناث ناضجة غير معاملة.

أوضحت النتائج أن المعاملة بالمركبات السابقة أدى إلى قلة معدل الزيادة فى أوزان الفئران مقارنة بالكنترول كما لم يحدث أى موت لأى منها. بالنسبة للذكور التى ذبحت لوحظ حدوث نقص فى كل من الوزن النسبى للخصى، عدد الحيونات المنوية وحركتها ، نشاط إنزيمات الكولين إستريز ، بجانب حدوث تغيرات مرضية فى نسيج الخصى وذلك مقارنة بنظيراتها فى المجموعة الضابطة. عموما كانت جميع التغيرات السابقة أوضح فى الفئران المعاملة بمخلوط الرانسيف عن المعاملة بكل مبيد منفرد.

لوحظ عند تزاوج الذكور المعاملة بمخلوط الرانسيف مع إناث غير معاملة أن زادت فترة حملها مع قله فى عدد المواليد مقارنة بالكنترول.