J. Pest Cont. & Environ. Sci. 14 (2): 1-18 (2006).

Reproductive toxicity of some biorational pesticides in albino rats (*Rattus norvegicus* var. *albus*)

El-Hamady, Sh. E.; A.E. Salama; I.I. El-Fakharany and N.A. Zidan
Pesticides Dept. Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt

ABSTRACT

The toxicity of abamectin and bioinsecticide *B.t.* (*Bacillus thuringiensis*) was evaluated against white albino rats. Rats received dietary doses each approximately equivalent to 1 / 10 or 1 / 100 LD_{50}'s of the tested pesticides for consecutive 60 days. The reproductive toxicity was dosage dependant and the symptoms appeared as alternations of genital organs weights and sex hormonal levels, e.g. reduced testis and seminal vesicles and reduced testosterone levels. In females, ovarian and uterine weights and progesterone levels were abnormal. Fertility characters of spermatogenic cells were severely affected as well. In all cases, lesions onset was ensured by the histopathological manifestation. The forementioned effects were poorly exhibited by abamectin, especially at the lowest dose. However, abamectin might affect mating capability of male rats. Furthermore, pregnancy and neonatal indices e.g. pregnancy rate, litter size/pregnant female, sex ratio and pub weight) were not significantly affected by each of the tested pesticides.

Keywords: Reproductive toxicity, abamectin, *Bacillus thuringiensis* which albino rats.

INTRODUCTION

Colborn *et al.* (1996) recorded that very low concentrations of environmental contaminants could potentially resulted in adverse effects on reproduction and development in humans and wildlife (Servos *et al.,* 2001), such contaminants are frequently referred to as endocrine disrupters (EDS). Experimental, and epidemiological studies have recently focused on various aspects of adverse effects in male reproductive function, such as rising incidence of testicular cancer, reduced and probably low semen quality, high and possibly increasing frequencies of undescended testis and hypospadias, and growing demand for assisted reproduction (Skakkebaek *et al.,* 2001). Potentially adverse effects occur in some wildlife at the individual and

population levels caused by disruption of one or more endocrine systems (Ankley and Giesy, 1998).

In the current study, the reproductive toxicity of certain biorational pesticides i.e. vertimec and a formulation of the microbial bioinsecticide, *Bacillus thuringiensis* was evaluated on white albino rats.

MATERIALS AND METHODS

1. Pesticides used:

1.1. Vertimec (Abamectin) 1.8 % EC: A mixture containing a minimum of 80 % avermectin B_{1a} (5-0-demethyl avermectin A_{1a}) and a maximum of 20 % avermectin B_{1b} (5-0-demethyl-25-de-(1-methyl-propyl) 25-(1-methylethyl) avermectin A_{1a}). The commercial product was supplied by MSD AGVET; MSD sharp & Dohme GmBH, Germany.

1.2. Xentari®: 13.3 % *Bacillus thuringiensis* subspec: *aizawai*. The product was produced by Abbott Laboratories. Chemical and Agricultural Products Division, North Chicago, USA, and Provided by Bayer Company,

2. Test animals: Males and females of albino rats (*Rattus norvegicus* var. *albus*) each of 3 weeks age and 60 $\pm$ 10 gm weight were obtained from the Organization of Serum and Vaccine (Helwan Farm), Cairo, Egypt. Rats were kept under healthy normal laboratory conditions, provided *ad libitum* with a diet containing yellow corn (57 %), soybean meal (28.5 %), layer concentrate (10 %), fish meal (1.5 %), corn oil (1.9 %), wheat bran (1 %) and methionine (0.1 %).

3. Acute toxicity tests: The acute toxicity of the tested pesticides to albino rats (each of 2 months, age and 100 $\pm$ 10 gm, weight) was determined. Oral LD_{50} values and their fiducial limits were assayed according to Weill (1952).

4. Reproductive toxicity tests:

4.1. Preparation of the toxic diet: To calculate the quantities of pesticides required to be mixed with diet, preliminary experiments were performed to determine the quantity of the diet normally consumed by each rat per day. The desired quantity of diet was diluted in a proper volume of water and mixed thoroughly with diet. The mixture was allowed to dry at room temperature and then kept in a deep freezer till being used. This

mixture was considered a fresh treated diet for three days, afterward, new mixtures were periodically prepared by the same manner. The rate of pesticide in the mixture was calculated on the basis that toxic diet ingested by an animal/24 hr, should carry the required quantity of a pesticide that is nearly equivalent to the desired doses (i.e. 1 / 10 or 1 / 100 LD_{50}).

4.2. Experimental design: Male and female albino rats (each of 2 months old and 100 $\pm$ 5 gm, weight) were used. Six groups each of 16 rats (8 male + 8 female) were specific for six pesticidal treatments, where animals of each group were fed diet containing the tested pesticides at quantities equivalent to 1 / 10 LD_{50} or 1 / 100 LD_{50} of the tested pesticide. Rats of an additional group of the same numbers of rats were offered diet free from pesticides and considered as control. Feeding on the pesticide-treated-diet continued for 30 days and through which females were completely isolated from males. At the end of this period, rats of each group were coupled (1 male + 1 female), caged for mating and fed on the toxic diet for additional 30 days.

4.3. Tissue specimens and genital organs examination: At the end of the first 30 days of treatment, blood samples were collected for hormonal assay. Blood samples were collected from the orbital sinus by the method described by UFAW (1976). At the end of the experimental period (60 days), male rats were sacrificed by decapitation; genital organs were taken and weighed. Epididymal spermatozoa were examined. Tests and epididymis were prepared and kept for histopathological examination. Females fed the toxic diet for 60 days were allowed to survive additional 21 days on non-toxic diet. Fertility test was performed, then the animals were sacrificed. Genital organs were obtained, weighed and prepared for histopathological examination.

4.4. Fertility testing: Examination of epididymal spermatozoa was carried out as described by Ali (1992). Epididymis of rats fed pesticide-treated diet (for 60 days) was obtained. Epididymal contents were prepared according to Hancock (1951) to determine the number of alive as well as the morphologically abnormal spermatozoa. The percentages of dead and morphologically abnormal spermatozoa were determined by recording their number among 200 spermatozoa examined randomly, 100 in each of the two smears. The progressive, individual motility of sperms was examined according to the method reported by Bearden and Fugnay (1980) as adopted by Farag (1998). A small droplet of semen was added to one drop of sodium

citrate 2.9 % on warm slide. Several fields were microscopically examined and the percentages of individual motility of sperms were recorded. Concerning females, tests were carried out on virgin females fed toxic diet for 30 days followed by additional 30 days of the same treatment. Pregnant females and their neonates were counted. According to Harlod and Eric (1989) and Farag (1998), the following fertility parameters were calculated:

Pregnancy rate = (No. of pregnant females/total No. of females cohabited) x 100
Litter size/pregnant female = (total No. of pups/total No. of pregnant females) x 100.

Other parameters e.g. weight of pups, length of pups, sex ratio and survival of neonates up to 21 days of birth, were calculated.

4.5. Hormonal assay: Levels of testosterone, progesterone and LH were determined in serum of rats adopting Elecsys system and using kits of Roche Diagnostics GmBH, D-68298, Mannheim, Germany. Assays were carried out at the Diagnostic Laboratory of Dr. Saleh El-Sharkawy, Prof. of Biotechnology, Fac. of Pharmacy, Mansoura Univ., Egypt using Elecsys Analyzer, D-Vi-S.

4.6. Reproductive behaviour: Vertimec was evaluated for its possible effect on reproductive behaviour of male rats. In this respect, the serial mating technique was described by Lee and Dixon (1972).

4.7. Histopathological examination: Tissue specimens fixed in 15 % formalin saline were dehydrated, embedded in paraffin blocks, sectioned and stained by haematoxylin and eosin (Harris, 1998). Microscopic examination was carried out by Dr. Ragia A. Fahmy, Assistant Professor of Pathology, Early Cancer Detection Unit, Obstetrics of Gynecology Hospital, Ain Shams Univ., Cairo, Egypt.

RESULTS AND DISCUSSION

1. Acute toxicity: Results of the acute oral toxicity of the tested pesticides are recorded in Table (1): LD_{50} values: 16.4 and > 3000 mg / kg, for vertimec and B.T. bait, respectively. According to Hodgson and Levi (1997), the pesticides could be classified as follows: vertimec is extremely toxic to rats whereas *B. t.* is moderately toxic. The obtained results are in agreement with those of others. The oral LD_{50} for abamectin (vertimec) on

rats is 10 mg / kg and on mice ranged from 14 to greater than 80 mg / kg (Kamrin, 1997). The LD_{50} is greater than 5000 mg/kg for the *B. t.* product Javelin in rats and greater than 13000 mg / kg in rats exposed to the product, thuricide (Kamrin, 1997). All acute studies showed that *B. t.* formulations might be safe for rats, rabbits and humans (Joung and Cote, 2000).

Table (1): Acute oral toxicity of pesticides to white albino rats.

Pesticides	LD_{50} (mg/kg)	Confidence limits
Vertimec	16.4	5.9-42.7
Xentari (*B. t.*)*	> 3000	-

* *B. t. Bacillus thuringiensis*

2. Genital organs weights: Weights of testes, epididymis, seminal vesicles and prostate gland of rats are shown in Table (2). Weights of epididymis in vertimec or *B. t.*-treated rats were not significantly affected whereas; those of testes and seminal vesicles were significantly elevated in B. t.-treated rats at the two tested doses. This effect was not fully observed in the case of vertimec. Weights of prostate glands were not significantly affected in rats treated with vertimec or *B. t.* Many pesticides of various chemical groups were found to reduce weights of male genital organs, testes, epididymis, seminal vesicles and prostate glands. the effects are always accompanied by a reduction in plasma testosterone level and a decrease in sperm count and motility (El-Ashmawy *et al.,* 1993; Hassan *et al.,* 1993; Abd El-Aziz *et al.,* 1997; Farrag, 1998 and Abd El-Khalek *et al.,* 1999).

Table (2): Genital organs weights of male rats treated with dietary daily doses of pesticides for 60 days.

Pesticide	Approximate dose (mg/kg)	Dosage** in diet (ppm)	Mean relative weight (gm organ/100 gm b.w)							
			Testes		Epididymis		Seminal vesicles		Prostate Gland	
			Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control
Vertimec	1/10 LD_{50}	6.4	1.09^{a}	72.19	0.48^{a}	84.21	0.24^{b}	64.86	0.54^{a}	78.26
	1/100 LD_{50}	0.64	1.06^{a}	72.19	0.48^{a}	84.21	0.66^{a}	178.38	0.62^{a}	89.86
Xentari (*B. t.*)*	1/10 LD_{50}	1200	0.75^{b}	49.67	0.59^{a}	103.50	0.77^{a}	208.11	0.66^{a}	95.65
	1/100 LD_{50}	120	1.26^{a}	83.45	0.58^{a}	101.75	0.72^{a}	194.59	0.59^{a}	85.51
Control			1.51^{a}	100	0.57^{a}	100	0.37^{b}	100	0.69^{a}	100

In the same column, values followed by the same letters are not significantly different at 5 % level according to DMRT. **B. t. Bacillus thuringiensis,* doses considered to be 300 and 30 mg / kg for 1 / 10 LD_{50} and 1 / 100 LD_{50}, respectively. **Calculated approximately.

Relative weights of (ovary + uterus) were not affected by vermectin or *B. t.* in female rats (Table 3). Brake *et al.* (2004) found that *B. t.* prepared in corn as diet had no measurable or observable effect on fetal postnatal, pubertal or adult testicular development.

Table (3): Weights of some genital organs of female rats treated with dietary daily doses of pesticides for 60 days.

Pesticide	Approximate dose (mg/kg)	Dosage ** in diet (ppm)	Relative weight of (ovary + uterus)	
			Mean (gm/100 gm b.w)	% of control
Vertimec	1/10 LD_{50}	6.4	0.19 [b]	111.77
	1/100 LD_{50}	0.64	0.18 [b]	105.88
Xentari (*B. t.*)*	1/10 LD_{50}	1200	0.20 [b]	117.65
	1/100 LD_{50}	120	0.18 [b]	105.88
Control			0.17 [b]	100

Values followed by the same letters are not significantly different according to DMRT.
**B. t. Bacillus thuringiensis,* doses considered to be 300 and 30 mg / kg for 1 / 10 LD_{50} and 1 / 100 LD_{50}, respectively. **Calculated approximately.

3. Hormonal status: Sera of rats (treated with daily dosages each equal to 1/10 or 1/100 LD_{50} of the tested toxicants for 30 days) were subjected to hormonal assay. Results are recorded in Table (4). Rats treated with *B. t.* showed levels of testosterone, 80.03 and 77.12 % of control at the doses of 1/10 and 1/100 $LD_{50,}$ respectively. Vertimec showed normal level of this hormone even at the highest dose. *B. t.* showed values of progesterone concentrations, 36.05 and 31.29 % of control for the doses of 1/10 and 1/100 LD_{50}, respectively, and normal values of LH level, whereas, vertimec caused no significant alterations in the levels of LH and progesterone. It is clear that vertimec showed no effects on testosterone, LH and progesterone in serum of treated rats. However, this compound exerted abnormal levels of testosterone, estradiol and progesterone in Japanese quail birds especially those treated with 1/10 LD_{50} (Zidan 2005).

Table (4): Concentration of certain serum hormones in male and females of rats exposed to the pesticides for 30 days.

Pesticide	Approximate dose (mg/kg)	Dosage** in diet (ppm)	Serum hormone concentration					
			Males		Females			
			Testosterone (ng/ml)		LH (m IU/ml)		Progesterone (ng/ml)	
			Mean	% of control	Mean	%of control	Mean	% of control
Vertimec	1/10 LD_{50}	6.4	2.86^{abc}	83.23	0.1^{a}	100	8.63^{b}	89.34
	1/100 LD_{50}	0.64	2.95^{ab}	85.85	0.1^{a}	100	10.11^{b}	104.66
Xentari (*B. t.*)*	1/10 LD_{50}	1200	2.75^{bc}	80.03	0.1^{a}	100	3.48^{c}	36.05
	1/100 LD_{50}	120	2.65^{bc}	77.12	0.1^{a}	100	3.02^{c}	31.29
Control			3.44^{a}	100	0.1^{a}	100	9.66^{b}	100

In the same column, values followed by the same letters are not significantly different at 5 % level according to DMRT. **B. t. Bacillus thuringiensis,* doses considered to be 300 and 30 mg / kg for 1 / 10 LD_{50} and 1 / 100 LD_{50}, respectively. **Calculated approximately.

The contradiction might be explained on the basis that vertimec undergoes metabolism by different pathways leading to less toxic metabolites in rats. The histopathological examination ensured the results, where tissue of ovaries and testes of rats showed no histological changes. However, El-Betieha and Da (2003) found that adult male rats exposed to tap water containing abamectin at 1.19-2.13 mg / kg b.w /day, showed decreased serum level of testosterone. Testosterone is the principal male hormone produced by the interstitial (Leyding cells) of the male testes and in smaller amount by the adrenals and the female ovaries. Thus, testes are responsible for the synthesis of the male sex hormones, or androgens, and for the production of spermatozoa. The most important androgen, both in terms of potency and the amount secreted in testes is the steroidal compound, testosterone, a powerful anabolic hormone. It is a vital to the development of secondary sexual characteristics in males and is essential for spermatogenesis (Guyton, 1991 and Mycek *et al.,* 1997). Testosterone is secreted by the Leyding cells of the testis under the influence of LH. Krause (1977) reported that decreased testosterone levels might be due to a direct damage of Leyding cells or to a lowered stimulation of these cells by LH. Spermatogenesis is not only depending upon LH but also upon sortoli cells of seminiferous tubules in the testes which are simulated by follicle stimulating hormone (FSH). Disorders of male genital function (Hypogonadism) are manifested by a decrease in plasma testosterone level.

Hypogenadism may occur with defective seminiferous tubules function or defective Leyding cell function and this leads to infertility through decreased production of spermatozoa (Mycek *et al.,* 1997).

4. Effects on spermatozoal morphology and viability: Results recorded in Table (5) showed that percentages of living sperms decreased significantly in rats treated with the tested pesticides at both tested doses (percentages ranged 51-65 % versus 92 % in control rats). The least incidence of dead sperms was noticed in rats treated with vertiemc at 1/100 LD_{50} (living sperms: 65 %).

Table (5): Effect of pesticides on epididymal sperm characters in male rats.

Pesticide	Approximate dose (mg/kg)	Dosage ** in diet (ppm)	Living sperms (%)	Individual motility (%)	Abnormality (%)			
					Bent tail	Coiled tail	Protoplasmic droplet	Total
Vertimec	1/10 LD_{50}	6.4	51.67^{d}	40.00^{d}	8.0^{a}	4.0^{ab}	2.67^{ab}	14.67
	1/100 LD_{50}	0.64	65.00^{b}	60.00^{b}	4.33^{b}	3.0^{bc}	2.67^{ab}	10.0
Xentari	1/10 LD_{50}	1200	55.00^{cd}	43.33^{d}	7.33^{a}	4.33^{a}	2.67^{ab}	14.33
(*B. t.*)*	1/100 LD_{50}	120	63.33^{b}	58.33^{b}	3.67^{b}	1.0^{de}	0.33^{c}	5.0
Control			92.33^{a}	85.00^{a}	1.0^{c}	0.33^{e}	0.33^{c}	1.66

In the same column, values followed by the same letters are not significantly different at 5% level according to DMRT.**B. t. Bacillus thuringiensis,* doses considered to be 300 and 30 mg/kg for 1/10 LD_{50} and 1/100 LD_{50}, respectively. **Calculated approximately.

Percentages of individual sperm motility also significantly decreased for all pesticidal treatments (43-60 % versus 85 % in control rats). The least effect was observed for vertimec at 1/100 LD_{50} (60 %). Sperm abnormalities were observed for each of the tested pesticides. Total sperm abnormalities ranged 5-14.67 % versus 1.66 % in control rats. Abnormalities appeared as sperms of bent or coiled tails or those having protoplasmic droplets (Figs. 1-4). The present study reveals that vertimec or *B. t.* might have adverse effect on male rat reproduction. The least toxic compound in this respect might be Vertiemc, especially at the lowest dose. This result is in consistent with that previously obtained in the present study, where the lowest dose of vertimec showed no effects on testis weights and testosterone concentration of male rats. This is supported by the histopathological examination of testis that shows normal testicular structure of tissues and partial atrophic changes. In contrast, El-Betieha and Da (2003) found that abamectin (vertimec) given to rats via drinking water at 571-1714 ppm for 6 weeks showed a reduction of

serum testosterone and decreased epidiymal and testicular sperm counts. In general the adverse effects exerted by *B. t.* and possibly by vertimec on male reproductive organs, testosterone level and semen picture might be attributed to the direct cytotoxic effect on the testis, that is related to the testicular degeneration accompanied with destruction of sertoli and Leyding cells as well as disturbed maturation of the spermatozoa. The pronouncing histopathological alterations observed in testis tissues as will discussed later on supports this opinion.

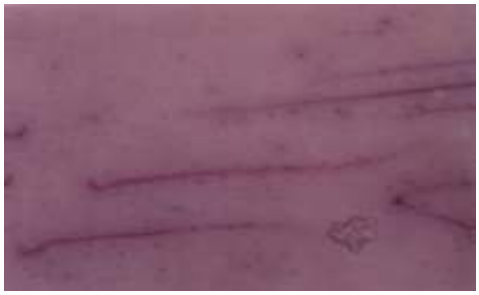

Fig. (1): Shows normal spermatozoa (control).

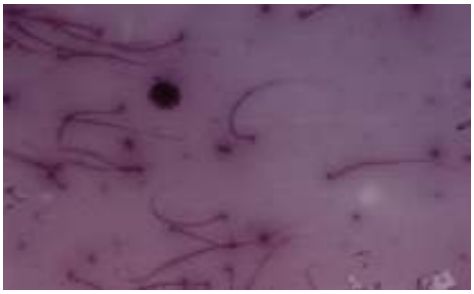

Fig. (2): Sperms of rats treated with 1/10 LD_{50} of Vertimec, showing curved middle piece.

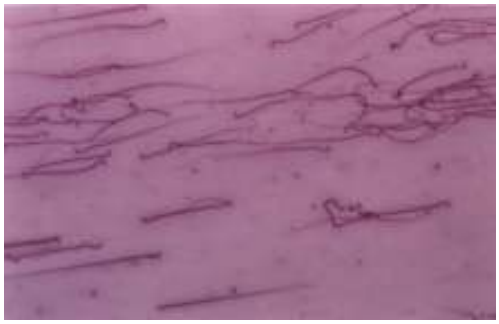

Fig. (3): Spermatozoa of rats treated with 1/10 *B. t.,* showing, bent tail.

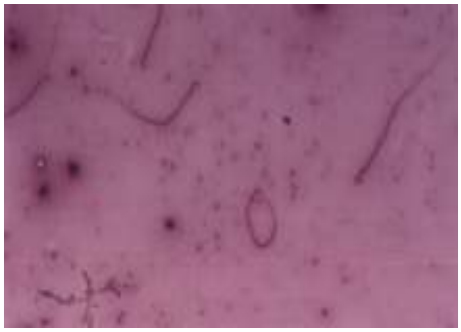

Fig. (4): Spermatozoa of rats treated with 1/10 LD_{50} of *B. t.* showing coiled tail.

5. Effect on pregnancy and neonatal indices: Results recorded in Table (6) revealed that the vertiemc or *B. t.* exhibited no significant effects on pregnancy rate, litter size/pregnant female, sex ratio, and pup weight. However, significant effects on pup length and percents of survival pups were observed. It is evident that, although parent females were mated with males whose fertility characters of spermatogenic cells was significantly

affected as mentioned earlier their neonatal outcome have not been affected by either of test materials. Many pesticides were found to exert detectable effects on parturition and neonatal indices. Ivermectin (a dihydrosynthetic form of abamectin) administered orally to rats at 0.05-3.6 mg / kg b.w/day for 3 consecutive generations showed increase in postnatal pup mortality and a decrease in pup weight and surviving offspring (Lankas *et al.,* 1989). However, the same compound injected S.C. at 200 µg / kg b.w. to rabbits, 2 weeks before mating exhibited no significant effects on gestation period, parturition rate, live birth rate and mean little weight (Ferreria *et al.,* 1989).

Table (6): Effect of pesticides on pregnancy rate, litter size and other neonatal properties when dietary administered to rats for 60 consecutive days.

Pesticide	Approximate dose (mg/kg)	Dosage** in diet (ppm)	Pregnancy rate (%)	Litter size/ pregnant female		Pup weight (gm)		Pup length (cm)		% survival pups	Male Sex ratio (%)
				Mean	% of control	Mean	% of control	Mean	% of control		
Vertimec	1/10 LD_{50}	6.4	77.5^{a}	7.75^{a}	96.88	5.91^{a}	121.57	6.66^{ab}	109.56	22.58^{b}	87.5^{ab}
	1/100 LD_{50}	0.64	93.75^{a}	9.5^{a}	118.75	5.81^{a}	119.51	6.46^{bc}	106.27	89.47^{a}	97.5^{a}
Xentari (*B. t.*)*	1/10 LD_{50}	1200	100.0^{a}	8.75^{a}	109.38	4.89^{a}	100.59	6.61^{ab}	108.73	94.29^{a}	97.5^{a}
	1/100 LD_{50}	120	100.0^{a}	8.0^{a}	100	5.91^{a}	121.57	6.9^{a}	113.51	90.63^{a}	73.5^{b}
	Control		100.0^{a}	8.0^{a}	100	4.86^{a}	100	65.08^{c}	100	71.88^{a}	97.0^{a}

In the same column, values followed by the same letters are not significantly different at 5% level according to DMRT.

**B. t. Bacillus thuringiensis,* doses considered to be 300 and 30 mg/kg for 1/10 LD_{50} and 1/100 LD_{50}, respectively.

**Calculated approximately.

6. Effects on reproductive behaviour: Results recorded in Table (7) showed that out of twenty cases of housing a pair of vertimec-treated rats (1 male + 1 female), only 11 cases of mating have occurred, compared to 19 cases of control treatments. Thus, percentage of mating is 55 % versus 95 % in control. These values might be good indicators to the number of mountings, thrusts and ejaculation. Hence, vertimec might affect mating capability of male rats. This suggestion is supported by the continuous observations of treated male rats that showed slow motion and tendency to sleep. The number of mountings, thrusts and ejaculation can each be quantitated as indicators of reproductive behaviour. Failure of mating might be associated with decreasing of testosterone, and or due to behavioural and neuromuscular defects.

7. Histopathological examination: Normal tissues of control rats are shown in Figs. (5-7) for vertiemc at 1 / 10 LD_{50}, tissues of testes revealed partial spermatogenic arrest within 20 % of acini with thickened membrane denoting granual onset of early atrophic changes (Fig. 8). At the dose 1 / 100 LD_{50}, the tissues revealed normal testicular structures with interstitial inflammatory cell infiltrate. Tissues of epididymis of Vertiemc-treated rats (at 1 / 10 LD_{50}) showed partially affected epididymal function, whereas those dosed with 1/100 LD_{50} revealed normal epididymis (Fig. 9). In *B. t.*-treated rats (at 1 / 10 LD_{50}) tissues of epididymis revealed severely marked inflammatory reaction with completely deneeded epithelium, but at 1 / 100

Table (7): Effect of vertimec on reproductive behaviour and libido of rats, expressed as mating capability, using serial mating technique.

Treatments	Existing of vaginal plug				Mating times (%)
	1st female	2nd female	3rd female	4th female	
Vertiemc-treated rats*					
1st male	+	+	-	+	
2nd male	-	+	-	+	
3rd male	+	+	+	-	55
4th male	-	-	-	+	
5th male	-	-	+	+	
Control rats					
1st male	+	+	+	+	
2nd male	+	+	+	+	95
3rd male	+	+	+	-	
4th male	+	+	+	+	
5th male	+	+	+	+	

* Rats previously given dietary daily doses each equivalent to 1/10 LD_{50} for 30 days.
(+): Mating occurred. (-): Mating did not occur.

Fig. (5): Testes of control rats, showing normal secretory activity (H & E, x 40).

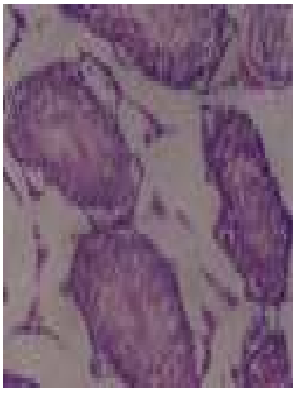

Fig.(6): Testes of control rats, showing normal spermatogonia (H & E, x 40).

Fig.(7): Testes of control rats,showing all spermatogenic series (H & E, x 10).

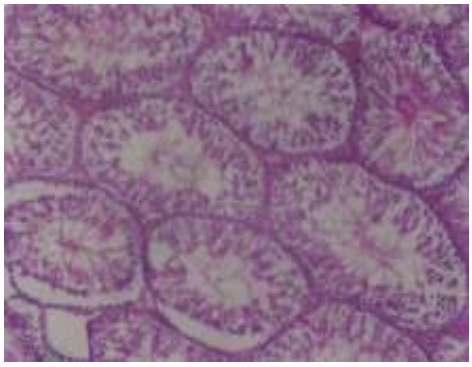

Fig. (8): Testes of vertiemc-treated rats at 1/10 LD_{50} level, showing partial spermatogenic arrest (H & E, x 40).

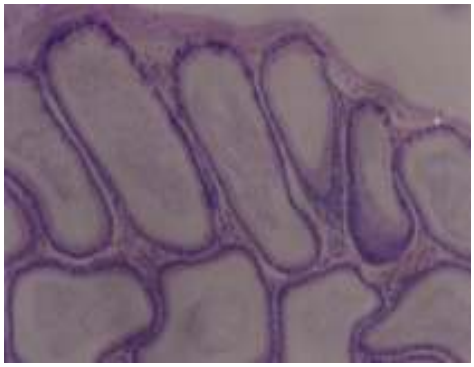

Fig. (9): Tissues of epididymis of vertiemc-treated rats at 1/100 LD_{50} level, showing normal epididymis (H & E, x 10).

LD_{50}, the tissue shows completely deneeded epithelium with extensive hyalinosis and heavy non-specific inflammatory infiltrate (Fig. 10). Ovaries of Vertimec-treated rats at 1 /10 or 1/100 LD_{50} showed normal ovulatory activity like those of control rats (Fig. 11). Ovaries of *B. t.*-treated rats at 1/10 LD_{50} showed normal ovulatory activity with marked oedema and congestion (Fig. 12). For the lower dose, the normal ovulatory activity is associated with oophritis (non-specific inflammatory lymphocytic

aggregates) (Fig. 13). Vertimec-treated rats at 1/10 LD_{50} showed uterus with normal endometrium infiltrated by hemosiderin-laden macrophages with oedema (Fig. 14). For the lower dose, sections revealed myometrial tissue infiltrated by hemosiderin-laden macrophages and hiseocytes. Endometrium showed normal excretory activity (Fig. 15). Uterus of *B. t.*-treated rats at 1/10 LD_{50} shows endometrium with normal excretory activity and with normal myometerium (Fig. 16). For the lower dose, the myometrium showed foamy histeocytic cells (Fig. 17).

In conclusion, the bioinsecticide *B. t.* showed dose-related reproductive toxicity to rats and that was poorly manifested by vertimec especially at lower doses. However, for both insecticides, productivity (expressed as pregnancy rate, number of neonates and sex ratio) was not affected.

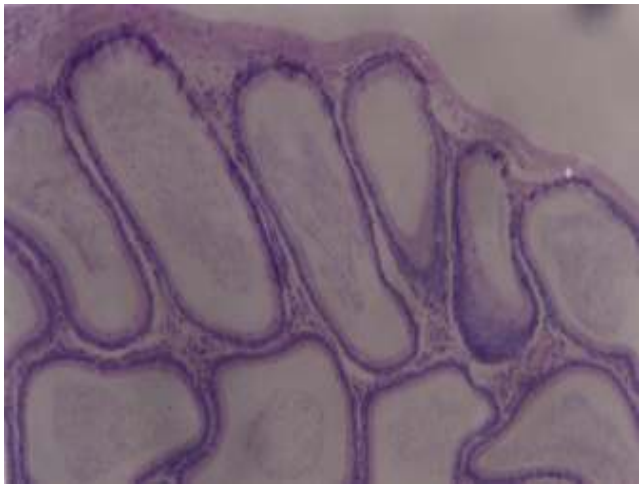

Fig. (10): Tissues of epididymis of *B. t.*-treated rats (at 1/100 LD_{50} level), showing completely deneeded secretory activity and flattened epithelium (H & E, x 10).

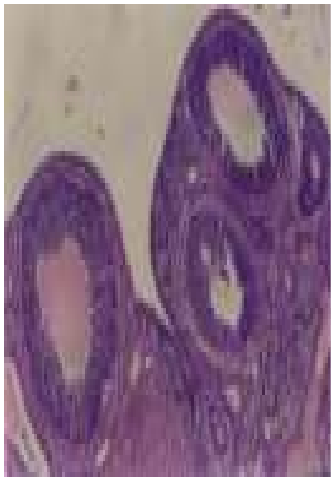

Fig. (11): Ovaries of control female rats, showing normal ovulatory activity and growing follicles. This is similar to those of Vertiemc-treated rats (at 1/10 or 1/100 LD_{50}), (H & E, x 40).

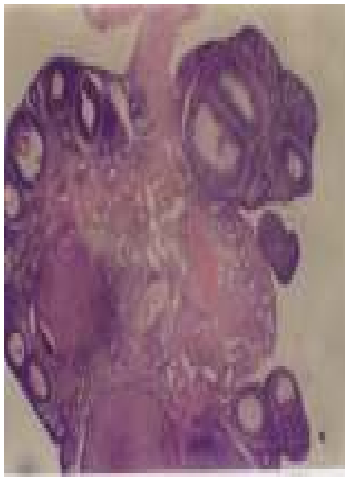

Fig. (12): Ovaries of *B. t.*-treated rats (at 1/10 LD_{50}) showing marked congested ovarian stroma (H & E, x 40).

Fig. (13): Ovaries of *B. t.*-treated rats (at 1/100 LD_{50}) showing lymphoid aggregate (H & E, x 4).

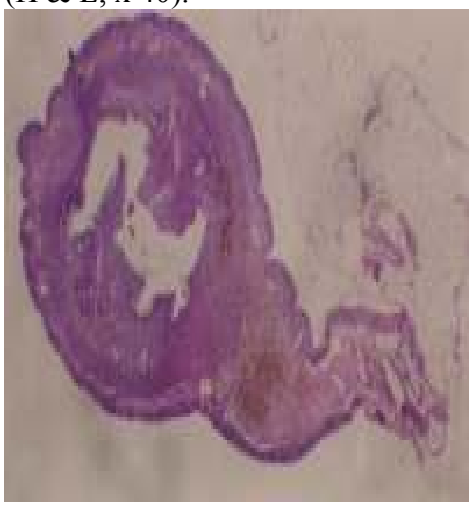

Fig. (14): Uteri of vertiemc-treated rats at 1/10 LD_{50} showing hemosiderin laden macrophage and hemorrhage (H & E, x 10).

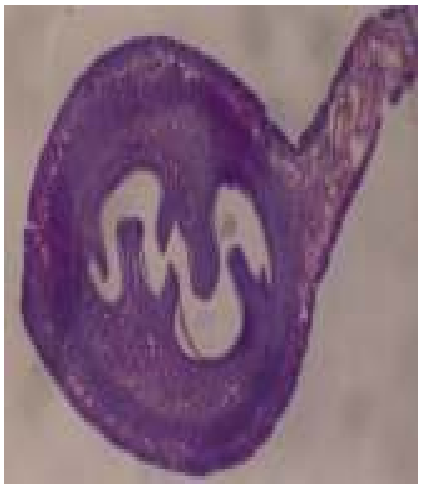

Fig. (15): Uteri of vertimec-treated rats at 1/100 LD_{50}, showing marked congestion(H & E, x 10).

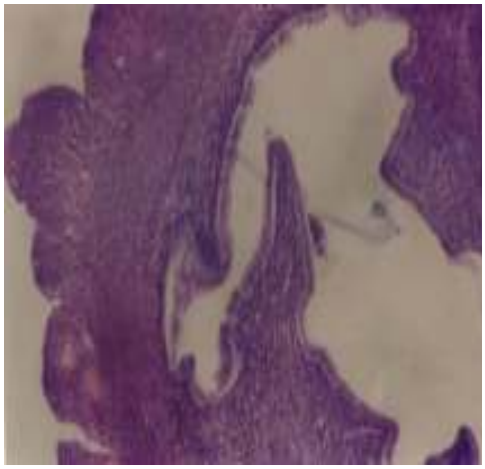

Fig. (16): Uterus of rat treated with 1/100 LD_{50} of *B. t.,* showing normal endometrial secretory changes (H & E, x 4).

Fig. (17): Uterus of *B. t.*-treated rat at 1/100 LD_{50}, showing hemosiderin and laden of foamy histeocytic cells (H & E, x 40).

REFERENCES

Abd El-Aziz, M. I.; A. M. Sahalab and M. Abd El-Khalek (1997). Influence of diazinon and deltamethrin on reproductive organs and fertility of male rats. Deutsche-Tieraraztliche-Wochenschrift,101(6): 230-232.

Abd El-Khalek, M. M.; N. A. Rahmy and H. H. Haleem (1999). Effect of the pyrethroid insecticide, cypermethrin on fertility in male rats. Vet. Med. J., Giza. 47 (3): 295-305.

Ali, U. A. (1992). Reproductive toxicity of the pesticides, hostathion, sumithion and dithane M-45 in rodents. Ph.D. Thesis Fac. of Veterinary Medicine, Cairo Univ.

Ankley, G. T. and J. P. Giesy (1998). Endocrine disruptors in wildlife. A weight of evidence perspective. In Kendall, R., Dickersson, R. Suk W. Giesy J. Principles and Processes for Assessing Endocrine Disruption in Wildlife. SETAC, Pensacola Fl, USA.

Bearden, H. J. and J. Fugnay (1995). Applied Animal Reproduction. Restor Published Co., Inc., Reston, Virgina, p. 158-160.

Brake, D. G.; R. Thaler and D. P. Evenson (2004). Evaluation of *B. t.* (*Bacillus thuringiensis*) corn on mouse testicular development by dual parameter flow cytometry. J. Agric. Food Chem. 52 (7): 2097-2102.

Colborn, T.; D. Dumanosk and J. P. Myers (1996). Our stolen future. Dutton, New York, pp. 186-188.

El-Ashmawy, I. M.; A. O. Zakaria, S. H. A Hemed; S. El-Fikey and Y. A. Hussein (1993). Cytotoxic effects of the pyrethroid insecticide (Matox) with reference to its influence on the reproductive hormone. Vet. Med. J. Giza, 41(3): 125-130.

El-Betieha, A. and S. I. Da (2003). Assessment of antifertility activities of abamectin pesticide in male rats. Ecotoxicology and Environmental Safety, Environmental Research, 55 (3): 307-313.

Farag, A. G. (1998). Toxicological studies of some pesticides on white albino rats. M.Sc. Thesis, Fac. of Agric. Zagazig Univ.

Ferreira, S. R. A.; J. D. Lima; W. M. Ferreira and L. S. Viana (1989). Use of ivermectin in pregnant rabbits. Arquivo-Brasileiro-de Medicina-Veterinaria-Zootcnia. 41(6): 527-533.

Guyton, M. (1991). Textbook of Medical Physiology. 8th Ed. pp. 334-481.

Hanock, J. A. (1951). A staining technique for the study of temperature shock in semen Nature: 107-323.

Harlod, Z. and C. D. Eric (1989). Assessment of male reproductive toxicity. A risk assessment approach. In principles and methods of toxicology (2nd Ed, Hayses. A.W. editor. Raven Press, LTD, New York.

Harris, H. E. (1998). Cited by Carleton, M.A.; Drury, R.A.B.; Wallington, E.A. and Cameren, H., 1967 4th ed., Oxford Univ. Press. New York.

Hassan, A. B.; G. A. Soliman; A. A. Farag and H. M. Sobhy (1993). Effect of the synthetic prethroids. Sumicidin and S-3206 on male rat fertility. Vet. Med. J., Giza 41 (2): 33-38.

Hodgson, E. and P. E. Levi (1997). Modern toxicology Printed in USA. p. 239.

Joung, K. P. and J. C. Cote (2000). A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*. Technical Bulletin No. 29 Horticultural Research and Development Center.

Kamrin, M. A. (1997). Pesticides profiles. Lewis Publishers, New York. p. 531-535.

Krause, W. (1977). Influence of DDT, DDVP and malathion on FSH, LH and testosterone concentration in testis. Bull. Environ. Contam. Toxicol. 18: 231-242.

Lankas, G. R.; D. H. Minsker and T. R. Robertson (1989). Effects of ivermectin on reproduction and neonatal toxicity in rats. Food and Chemical Toxicol., 27(8): 523-529.

Lee, L. P. and R. L. Dixon (1972). Effects of procarbozin on spermatogenesis studied by velocity sedimentation cell separation and serial mating. J. Pharmacol. Exp. Ther., 181: 219.

Mycek, M. I.; R. A. Harvey and P. C. Champe (1997). Lippincotts illustrated reviews: Pharmacology. 2nd ed. Liprincott-Raven Philadelphia, New York.

Servos, M.; Van Der Kraak; J. Glen and M. Wade (2001). Scientific assessment of endocrine disrupting substances in the Canadian environmental. Water Quality Research Journal of Canada, 36(2): 171-173.

Skakkebaek, N. E.; E. Rajpert and K. M. Main (2001). Testicular dysgenesis syndrome: an increasingly common development disorder with environmental aspect. Human Reproduction. 16 (5): 972-978.

UFAW (1976). The UFAW handbook on the care and management of laboratory animals. 5th ed. p. 207. Churchill Livenstone, Edinburgh London and New York.

Weill, C. S. (1952). Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics, 8: 249-263.

Zidan, N. A. (2005). Toxicological effects of selected agrochemicals on some test animals. Ph.D. Thesis Pesticides Dept. Fac. of Agric., Tanta Univ.