Cytogenetic Effects Induced by the Natural Pyrethrins and the Synthetic Lambda Cyhalothrin in Mice *in Vivo*

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Summary The potential genotoxicity of the natural pyrethrins and the synthetic lambda (λ) cyhalothrin was evaluated in mice *in vivo*. Single oral treatment with the doses 45, 90, 180 mg kg⁻¹ b.wt. of pyrethrins and 1.25, 2.5, 5 mg kg⁻¹ b.wt. of λ cyhalothrin (1/8, 1/4, 1/2 LD₅₀) induced modest but statistically significant increase in the frequency of sister chromatid exchanges (SCE's) in bone-marrow cells. Such frequency reached 8.20 ± 0.41 and 8.67 ± 0.24 per cell after treatment with the highest tested dose of both insecticides respectively compared with 5.32 ± 0.28 for the control. Marked induction of SCE frequency (12.40 ± 0.90) per cell was observed after treatment with mitomycin C which is used as a positive control. Single oral treatment with the doses 90, 180 mg kg⁻¹ b.wt. of pyrethrins and 1.25, 2.5, 5 mg kg⁻¹ b.wt. of λ cyhalothrin, induced significant increase in the percentage of chromosomal aberrations in mouse bone-marrow. With respect to germ cells only the highest tested dose of both insecticides (1/2 LD₅₀) induced a significant percentage of chromosomal aberrations in mouse spermatocytes after single oral treatment. Chromosomal aberrations were also recorded after repeated treatment. For studying sperm abnormalities mice were orally treated for 5 consecutive days with 22.5, 45, 90 mg kg⁻¹ b.wt. of pyrethrins and 0.63, 1.25 and 2.5 mg kg⁻¹ b.wt. of λ cyhalothrin. Significant percentage of sperm abnormalities was observed after treatment with the 2 higher doses of both insecticides. In conclusion, both insecticides pyrethrins and λ cyhalothrin induced some clastogenic effects with the high tested doses.

Key words Pyrethrins, Lambda cyhalothrin, Sister chromatid exchanges, Chromosomal aberrations, Sperm abnormalities, Genotoxicity (mouse).

The concept of utilizing certain plants possessing pesticidal activity against different species of insect, has been confirmed. The extract of pyrethrins from *Chrysanthemum cinerariaefolium* flowers, is a typical example as botanical insecticide. Synthetic pyrethroids are analogs of a natural chemical moiety, pyrethrins.

Synthetic pyrethroids are a group of potent insecticides that are environmentally compatible by virtue of their moderate persistence, low volatility, and poor aqueous mobility in soil (Agarwal *et al.* 1994). Further a wide margin of safety to mammals (Casida *et al.* 1983, Casida and Quistad 1995) makes them ideal insecticides for widespread use.

The natural pyrethrins and the synthetic pyrethroids are extensively used allover the world, including Egypt in order to control mosquitoes (Mutinga *et al.* 1992), mites (Collison *et al.* 1981), human head lice (Dodd 2000) and many agricultural pests (Stadler 1996) as well as veterinary and public health programmes (Leahey 1985).

The genetic toxicity/mutagenicity studies on pyrethroids are rather controversial (Leahey 1985, Agarwal *et al.* 1994). With the use of pyrethroids steadily rising, it may be an urgent need to identify the possible adverse effects that may be associated with their use. The aim of the present work is to evaluate the possible genotoxic effects of the natural pyrethrins, and one of the synthetic pyrethroids lambda (λ) cyhalothrin using different cytogenetic parameters in male mice *in vivo*.

Materials and methods

Animals

Mature male white Swiss mice (9–12 weeks old, weighing 25–30 g) were used in all experiments. The animals were obtained from a closed random-bred colony at the National Research Centre, maintained under controlled conditions of temperature and humidity and receiving food and water *ad libitum*.

Chemicals

Two insecticides were used in the present study.

I. Pyrethrins: Natural extract of *pyrethrum* flowers, produced by Scientific Chemical Industries. Firm Scien Co. Egypt, 60% active ingredients. This crude extract is fully identified as esters of 3 alcohols with chrysanthemic and pyrethic acids as shown in Fig. 1.

II. Lambda cyhalothrin: Synthetic pyrethroid insecticide, trade name "Karate" (Fig. 2). CAS chemical name: (R+S)- α -cyano-3-(phenoxyphenyl)methyl-(1S+1R)-*cis*-3-(z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane-carboxylate, CASRN 91465-08-06 was from Zeneca Agrochemicals. England (2.5%).

Treatment and cytological preparations

Sister chromatid exchange: Three different doses were tested 45, 90, 180 mg kg⁻¹ b.wt. for pyrethrins (dissolved in corn oil) and 1.25, 2.5, 5 mg kg⁻¹ b.wt. for λ cyhalothrin (dissolved in distilled water). Such doses are correspondent to 1/8, 1/4, 1/2 of the experimental LD₅₀ of both insecticides respectively. The method described by Allen (1982) for conducting *in vivo* sister chromatid exchange was applied with modifications. 5-Bromodeoxyuridin (BrdU, Sigma) tablets weighing 55 $\pm$ 5 mg were placed subcutaneously for 21 h. The mice were orally treated by gavage with the insecticides 8 h after BrdU implantation, and were injected with colchicine (0.6 mg/kg) 2 h prior to killing. Bone-marrow cells were obtained in KCl 0.075 M at 37°C, keeping the cells 20 min at the same temperature then fixed in methanol-acetic acid solution 3:1. Smears were prepared and stained with fluorescence plus Giemsa method of Goto *et al.* (1978). Five animals were taken for each treatment and at least 30 well spread metaphases per animal were examined microscopically for SCE's.

Chromosomal aberration: For bone-marrow or spermatocytes, groups of 5 animals were orally treated by gavage with a single dose of 22.5, 45, 90, 180 mg kg⁻¹ b.wt. of pyrethrins or with 0.63, 1.25, 2.5, 5 mg kg⁻¹ b.wt. of λ cyhalothrin. Samples were taken 24 h post treatment. For subacute treatment, mice were orally treated by gavage for 5 consecutive days at the dose levels of 22.5, 45, 90 mg kg⁻¹ b.wt. for pyrethrins and 0.63, 1.25, 2.5 mg kg⁻¹ b.wt. for

	R ₁	R ₂
Pyrethrin I	CH ₃	CH ₂ CH=CHCH=CH ₂
Jasmolin I	CH ₃	CH ₂ CH=CHCH ₂ CH ₃
Cinerin I	CH ₃	CH ₂ CH=CHCH ₃
Pyrethrin II	COOCH ₃	CH ₂ CH=CHCH=CH ₂
Jasmolin II	COOCH ₃	CH ₂ CH=CHCH ₂ CH ₃
Cinerin II	COOCH ₃	CH ₂ CH=CHCH ₃

Fig. 1. Crude extract of pyrethrins.

Fig. 2. Structure of the pyrethroid insecticide lambda cyhalothrin C₂₃H₁₉ClF₃NO₃.

λ cyhalothrin. Samples were taken 24 h after the last treatment. Bone-marrow chromosomes were prepared according to the method of Yosida and Amano (1965). Chromosomes of diakinesis-MI spermatocytes were prepared according to the air drying technique of Evans *et al.* (1964). Slides were stained with 7% Giemsa in phosphate buffer (pH 6.8). At least 75–100 well spread metaphases were analyzed per each animal to score different types of aberrations.

Sperm-shape abnormalities: Groups of animals (5/group) were orally treated by gavage for 5 consecutive days at the dose levels of 22.5, 45, 90 mg kg⁻¹ b.wt./day for pyrethrins and 0.63, 1.25, 2.5 mg kg⁻¹ b.wt./day for λ cyhalothrin. Animals were killed on the 35th day after administering the first dose. Sperms were prepared according to the recommended method of Wyrobek and Bruce (1978). Mice were killed by cervical dislocation. The epididymides were excised and minced in 2 ml physiological saline and filtered to exclude large tissue fragments. Smears were prepared and stained with 1% Eosin Y. Thousand sperms were counted for each animal to score different types of sperm abnormalities (head-tail).

For all the above experiments, control groups (non-treated and corn oil) were run alongside the test material in addition to the positive control group treated intraperitoneally with 1 mg kg⁻¹ b.wt. mitomycin C as recommended by Preston *et al.* (1987). It is acceptable that a positive control may be administered by a route different from the test agent and sampled at only a single time (Hayashi *et al.* 1994).

Statistical analysis

In all experiments the resulting data between groups were statistically compared using Fisher's least significant differences and Duncan's multiple range test. The significance of the incidence of abnormalities in experimental *versus* control data was calculated using the t-test.

Results and discussion

The results of sister chromatid exchange (SCE) analysis in bone-marrow chromosomes after oral treatment with pyrethrins and λ cyhalothrin are summarized in Table 1 and Fig. 3a, b. The results reveal modest induction with statistical significance at all the tested concentration levels (1/8, 1/4, 1/2 LD₅₀) as compared to the solvent control group. The frequency of the induced SCE's increased as the dose was raised. Further the SCE's induced by λ cyhalothrin were slightly higher in

Table 1. Frequency of sister chromatid exchanges (SCE's) in mouse bone-marrow cells after oral administration of pyrethrins and λ cyhalothrin

Treatment and doses	Mice No.	No. of examined metaphases with differentiation	SCE's No.	SCE's/cell mean $\pm$ S.E.
I Control (corn-oil)	5	150	798	5.32 $\pm$ 0.28
II Pyrethrins				
45 mg kg ⁻¹ b.wt.	5	150	957	6.38 $\pm$ 0.30*
90 mg kg ⁻¹ b.wt.	5	150	1065	7.10 $\pm$ 0.23*
180 mg kg ⁻¹ b.wt.	5	150	1230	8.20 $\pm$ 0.41**
III Control (non-treated)	5	150	768	5.12 $\pm$ 0.28
IV λ Cyhalothrin				
1.25 mg kg ⁻¹ b.wt.	5	150	1125	7.50 $\pm$ 0.26*
2.5 mg kg ⁻¹ b.wt.	5	150	1207	8.05 $\pm$ 0.40*
5 mg kg ⁻¹ b.wt.	5	150	1301	8.67 $\pm$ 0.24**
V Mitomycin C (positive control)				
1 mg kg ⁻¹ b.wt.	5	150	1860	12.40 $\pm$ 0.90***

* Significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level (t-test).

Fig. 3. Metaphases from mouse bone-marrow showing: a-b) SCE's, c) chromatid gap, d) chromatid gap and chromatid break, e) fragments, f) fragment and robertsonian translocation. Metaphases from mouse spermatocytes showing: g) x-y univalent, h) autosomal univalent and x-y univalent, i) fragment induced after oral treatment with pyrethrins and λ cyhalothrin.

frequency than that induced by pyrethrins. Such frequency of SCE's reached 8.20 ± 0.41 and 8.67 ± 0.24 per cell ($p < 0.01$) after treatment with the highest tested dose of both insecticides respectively compared with 5.32 ± 0.28 for the control. Marked increase in the frequency of SCE's (12.40 ± 0.90) per cell was observed after intraperitoneal treatment with 1 mg kg^{-1} b.wt. mitomycin C which is an alkylating mutagen used as a positive control. This result is in agreement with the previous reports concerning the induction of SCE's by pyrethroids in mouse bone-marrow cells (Amer *et al.* 1993, Chauhan *et al.* 1997), in human lymphocyte cultures (Puig *et al.* 1989, Dolara *et al.* 1992), in CHO cells (Caballo *et al.* 1992). Other pyrethroids such as supermethrin and deltamethrin give negative results (Dianovsky and Sivikova 1995, Villarini *et al.* 1998).

With respect to chromosomal aberrations in bone-marrow cells. Table 2 shows that single oral treatment with the doses 22.5 , 45 mg kg^{-1} b.wt. of pyrethrins and 0.63 mg kg^{-1} b.wt. λ cyhalothrin had no significant effect compared with the control groups. Significant increase in the incidence of chromosomal aberrations was observed at the doses 90 , 180 mg kg^{-1} b.wt. of pyrethrins and 1.25 , 2.5 , 5 mg kg^{-1} b.wt. λ cyhalothrin. The percentage of the induced aberrations reached 7.3 ± 0.6 and 8.3 ± 0.7 ($p < 0.01$) after treatment with the highest dose ($1/2 \text{ LD}_{50}$) of pyrethrins and λ cyhalothrin respectively compared with 2.3 ± 0.5 for control. Such values are greatly lower than that induced by the positive control mitomycin C (21.3 ± 0.9), which may indicate a weak clastogenic effect of both insecticides. Moreover repeated treatment (5 consecutive days) concerning different doses of both insecticides show slight increase in the percentage of chromosomal aberrations (range 1.2 – 2.8%) over that of the single dose treatment. This may be explained by the fact that when pyrethroids are

Table 2. Frequency of chromosomal aberrations in mouse bone-marrow cells after oral administration of pyrethrins and λ cyhalothrin

Treatment and doses	Total number of examined metaphases (n) H ⁰	No. of metaphases with different types of chromosomal aberrations ^a							No. of metaphases with chrom. aberr. excluding gaps	Mean % chrom. aberr. excluding gaps $\pm$ S.E.
		Chromat. gap	Chromos. gap	Break	Frag.	M.A	Del.	RT	Polyp.	Endomit. (C-mitosis)
Single dose treatment										
I Control (corn-oil)	400	8	1	2	4	1	—	—	—	2
II Pyrethrins										
22.5 mg kg ⁻¹ b.wt.	400	16	—	6	1	—	—	—	—	3
45 mg kg ⁻¹ b.wt.	400	18	2	2	5	2	—	—	—	4
90 mg kg ⁻¹ b.wt.	400	23	7	6	2	1	3	—	—	4
180 mg kg ⁻¹ b.wt.	400	32	2	8	4	2	3	6	—	6
III Control (non-treated)	400	7	—	2	4	1	—	—	—	1
IV λ Cyhalothrin										
0.63 mg kg ⁻¹ b.wt.	400	16	—	3	1	2	—	1	—	4
1.25 mg kg ⁻¹ b.wt.	400	26	1	1	3	5	2	1	1	6
2.5 mg kg ⁻¹ b.wt.	400	30	1	4	4	7	—	1	—	6
5 mg kg ⁻¹ b.wt.	400	24	5	2	7	9	1	6	—	8
Repeated dose treatment										
V Control (corn oil)	400	8	1	3	3	2	—	—	—	2
VI Pyrethrins										
22.5 mg kg ⁻¹ b.wt.	400	28	8	2	8	2	—	2	—	4
45 mg kg ⁻¹ b.wt.	400	33	4	—	9	3	1	5	1	4
90 mg kg ⁻¹ b.wt.	400	39	4	2	13	4	—	4	—	6
VII Control (non-treated)	400	7	1	3	4	1	—	—	—	1
VIII λ Cyhalothrin										
0.63 mg kg ⁻¹ b.wt.	400	36	4	5	5	2	2	3	—	4
1.25 mg kg ⁻¹ b.wt.	400	38	1	3	6	5	1	3	—	6
2.5 mg kg ⁻¹ b.wt.	400	38	1	5	11	1	3	6	1	6
Mitomycin C (positive control)										
1 mg kg ⁻¹ b.wt.	400	21	6	19	16	20	5	18	4	3

(n)=Number of animals per each treatment group=5, H⁰=Harvest time after the last treatment=24 h, ^aChromat. gap=Chromatid gap, Chromos. gap=Chromosome gap, Frag.=Fragment, M.A.=Metaphases containing more than one type of chromosomal aberrations, Del.=Deletion, RT=Robertsonian translocation, Polyp.=polyploid, Endomit.=Endomitosis. * Significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level (t-test).

orally administrated to mammals, they are converted by hydrolytic or oxidative attack to polar metabolites, which are then eliminated unchanged or as conjugates in the faeces or urine (Litchfield 1985).

Concerning the different types of the induced aberrations (Fig. 3c–f), gaps were found to be the most sensitive type induced after treatment with the 2 pyrethroids. The importance of recording gaps in assessing the mutagenic potential of compounds has been controversial and can be considered as a separate category of damage (Brøgger 1982). In the present results, gaps were recorded but not included in the total aberrations. Breaks, fragments, deletions, translocations were observed. The aberrations were mainly of chromatid type. Numerical chromosomal aberrations in the form of endomitosis (endomitotic reduplication) were recorded in all treated groups. Endomitotic reduplication is specialized process of doubling the chromosome material with the tendency of cells to pass from G2 to the next S-phase without undergoing cell division. Mitotic spindle disturbances are considered important to this process (Dean and Danford 1984). Agarwal *et al.* (1994) reported that other pyrethroids such as deltamethrin induced endomitotic reduplication in rat bone-marrow cells.

The genetic toxicity/mutagenicity studies concerning the natural pyrethrins or the synthetic pyrethroids have produced controversial result depending on the compound itself or the assay used. The pyrethrum extract was reported to give negative results, in the Ames test using *Salmonella typhimurium*, in chromosomal aberrations in CHO cells and in DNA synthesis in rat primary hepatocytes (Williams 1973). Further some pyrethroids were reported to be scarcely clastogenic in a variety of mammalian assays (FOA 1981, Polakova and Vargova 1983, Leahey 1985). On the contrary other pyrethroids were reported to be mutagenic in rodent bone-marrow (Chatterjee *et al.* 1982, Amer and Aboul-Ela 1985, Pati and Bhunya 1989, Amer *et al.* 1993, Agarwal *et al.* 1994), human peripheral lymphocyte cultures (Puig *et al.* 1989, Surralles *et al.* 1990), and in aquatic organisms (Campana *et al.* 1999).

The induction of chromosomal aberrations in mouse spermatocytes was also studied (Table 3). Single oral treatment with different doses of both insecticides had no significant effect except with the highest tested dose ($1/2 LD_{50}$) where the percentage was significant at $P < 0.05$. Such percentage reached 8.60 ± 0.47 and 9.20 ± 0.52 after treatment with pyrethrins and λ cyhalothrin respectively compared with 5.25 ± 0.48 for the control. Mitomycin C showed higher percentage of chromosomal aberrations (19.75 ± 0.60) in mouse spermatocytes than the 2 insecticides. Moreover groups of mice treated for 5 consecutive days with the different doses show that the incidence of chromosomal aberrations did not exceed two fold increase over that of the groups with single dose treatment. After treatment with different doses of both insecticides, structural aberrations (Fig. 3g–i) were the main types observed in mouse spermatocytes. Autosomal and quoesomal univalents were increased. However, the latter univalents were much more frequent. Breaks, fragments, gaps, and translocations in the form of chain IV were also observed. It should be mentioned in this context that the genotoxic effect of the tested pyrethroids in germ cells was lower than that induced by other insecticides *e.g.* organophosphorous (Fahmy and Abdalla 1998) and carbamate (Farghaly 1997), also chemical mutagens (Fahmy and Aly 2000) and heavy metals (Fahmy 1999) which induce great damage to germ cells.

Chromosomal abnormalities in mouse spermatocytes are expected to show their effect on sperm morphology (Devi and Reddy 1985). Although not widely used in genotoxicity studies, the sperm morphology test appears to be sensitive because sperm shapes are known to be genetically controlled by numerous autosomal and sex linked genes (Krzanowska 1976). The regular process of sperm development involves the activity of several genes and the formation of normal sperm heads involving intricate synchronous morphological and biochemical steps (Rattner 1972). A statistically significant increase in the percentage of morphologically abnormal sperm occurred in the mice treated with 5 consecutive doses of pyrethrins at 45, 90 $mg\ kg^{-1}$ b.wt. and λ cyhalothrin at 1.25, 2.5 $mg\ kg^{-1}$ b.wt. (Table 4, Fig. 4). Such percentage reached 6.80 ± 0.82 and 7.36 ± 0.39 after treat-

Table 3. Frequency of chromosomal aberrations in primary spermatocytes of male mice induced after oral administration of pyrethrins and λ cyhalothrin

Treatment and doses	Total number of examined metaphases (n) H ^a	No. of abnormal metaphases	Mean % abnormal metaphases $\pm$ S.E.	No. of metaphases with different types of chromosomal aberrations ^a						
				X-yu	A-u	A-u+X-yu	Break or Frag.	Gap	Chain	Polyp.
Single doses treatment										
I Control (corn-oil)	400	21	5.25 $\pm$ 0.48	15	4	2	—	—	—	—
II Pyrethrins										
22.5 mg kg ⁻¹ b.wt.	400	18	4.50 $\pm$ 0.64	10	7	1	—	—	—	—
45 mg kg ⁻¹ b.wt.	400	19	4.75 $\pm$ 0.48	15	2	1	1	—	—	—
90 mg kg ⁻¹ b.wt.	400	26	6.50 $\pm$ 0.29	19	3	—	2	2	—	—
180 mg kg ⁻¹ b.wt.	500	43	8.60 $\pm$ 0.47*	25	3	1	3	8	3	—
III Control (non-treated)	400	20	5.00 $\pm$ 0.40	15	4	—	—	1	—	—
IV λ Cyhalothrin										
0.63 mg kg ⁻¹ b.wt.	400	22	5.50 $\pm$ 0.64	17	4	1	—	—	—	—
1.25 mg kg ⁻¹ b.wt.	500	28	5.60 $\pm$ 0.50	23	3	—	—	—	—	—
2.5 mg kg ⁻¹ b.wt.	400	27	6.75 $\pm$ 0.62	16	4	3	1	1	2	—
5 mg kg ⁻¹ b.wt.	500	46	9.20 $\pm$ 0.52*	30	10	3	3	—	—	—
Repeated dose treatment										
V Control (corn oil)	400	25	6.25 $\pm$ 0.48	16	6	1	1	2	—	—
VI Pyrethrins										
22.5 mg kg ⁻¹ b.wt.	400	29	7.25 $\pm$ 0.75	16	9	1	1	1	1	—
45 mg kg ⁻¹ b.wt.	400	38	9.50 $\pm$ 0.64*	24	10	2	1	—	1	—
90 mg kg ⁻¹ b.wt.	400	47	11.75 $\pm$ 1.50**	25	10	1	5	—	6	—
VII Control (non-treated)	400	22	5.50 $\pm$ 0.60	19	3	—	—	—	—	—
VIII λ Cyhalothrin										
0.63 mg kg ⁻¹ b.wt.	600	45	7.50 $\pm$ 0.47	27	10	—	6	—	2	—
1.25 mg kg ⁻¹ b.wt.	400	42	10.50 $\pm$ 0.65**	29	7	5	—	1	—	—
2.5 mg kg ⁻¹ b.wt.	500	61	12.20 $\pm$ 0.96**	31	17	5	2	—	2	4
Mitomycin C (positive control)										
1 mg kg ⁻¹ b.wt.	400	79	19.75 $\pm$ 0.60***	35	16	9	12	2	5	—

(n)=Number of animals per each treatment group=5, H^a=Harvest time after treatment=24 h, ^a X-yu=X-y univalent, A-u=Autosomal univalent, Frag.=Fragment, Polyp.=polyploid.
 * Significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level (t-test).

Table 4. Sperm abnormalities in male mice induced after oral administration of pyrethrins and λ cyhalothrin

Treatment and doses	Mice No.	Number of sperm examined	No. of abnormal sperm	Mean % abnormal sperm $\pm$ S.E.	Types of sperm abnormalities (No.)							Tail abnormalities coiled
					Head abnormalities							
					Amorphous	Without hook	Triangle	Banana	Small	Big	Fork	
I Control (corn-oil)	6	6000	180	3.00 $\pm$ 0.24	64	46	14	5	10	1	—	40
II Pyrethrins												
22.5 mg kg ⁻¹ b.wt.	5	5000	151	3.02 $\pm$ 0.90	45	42	22	19	9	3	—	11
45 mg kg ⁻¹ b.wt.	8	8000	349	4.36 $\pm$ 0.56*	95	58	45	26	16	29	2	78
90 mg kg ⁻¹ b.wt.	7	7000	476	6.80 $\pm$ 0.82**	132	34	83	14	6	15	2	190
III Control (non-treated)	5	5000	140	2.80 $\pm$ 0.39	36	41	34	6	9	4	—	6
IV λ Cyhalothrin												
0.63 mg kg ⁻¹ b.wt.	6	6000	243	4.05 $\pm$ 1.30	68	49	42	9	14	3	—	58
1.25 mg kg ⁻¹ b.wt.	6	6000	283	4.72 $\pm$ 0.63*	85	35	57	31	21	6	—	48
2.5 mg kg ⁻¹ b.wt.	7	7000	515	7.36 $\pm$ 0.39**	149	67	87	90	33	14	3	78
Mitomycin C (positive control)												
1 mg kg ⁻¹ b.wt.	325	5000	634	12.68 $\pm$ 1.20***	160	128	42	10	10	8	—	276

* Significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level (t-test).

Fig. 4. Different types of sperm abnormalities induced in male mice after oral treatment with pyrethrins and λ cyhalothrin: a) normal sperm, b) without hook, c-e) amorphous, f) triangle, g) big head, h) small head, i) banana, j-l) coiled tail.

ment with the highest tested dose of both insecticides respectively compared with 3.00 ± 0.24 for the control. Further marked increase in the incidence of sperm abnormalities was observed after treatment with mitomycin C (12.68 ± 1.20).

It could be concluded that the natural pyrethrins and the synthetic λ cyhalothrin induced some clastogenic effects with the high tested doses but the effect was greatly lower than that induced by mitomycin C.

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