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CYTOGENETIC EFFECTS OF MALATHION INSECTICIDE ON JAPANESE QUAIL (*Coturnix japonica*).

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ABSTRACT: *The Japanese quail (Coturnix japonica) belongs to the order Galliformes family Phasianidae and improved for egg and meat production. The cytogenetic effect of Malathion residues in grains stored for different periods was evaluated in quail using three doses low, medium and high. The study included chromosomal aberrations analysis in bone-marrow and spermatocyte cells. The study aimed to evaluate the genotoxic effects of the fungicides (Malathion), which is used to protect crops from fungi. We fed Japanese quail on seeds treated with Malathion to show the effect on Mitotic and Meiotic aberrations. For these reasons, bone marrow, spermatocytes and chromosome aberration tests were carried out in quail. Chromosomal aberrations may due to lesions in DNA which lead to discontinuities of the double helix. The results demonstrated that the cytogenetic effect induced in different quail tissues by Malathion residues were dose-dependent.*

Keywords: *Stored grains; Malathion; Chromosome aberrations (quail); cytogenetic effects*

INTRODUCTION

The Japanese quail (*Coturnix japonica*) belongs to the order Galliformes family Phasianidae, and along with the chicken (Gallus), has been domesticated and improved for meat and egg production (Crawford 1990). Quail have been widely used not only as a meat and egg production animal, but also as an experimental animal because of its small body size and short generation interval (Schmid *et al.* 2000).

The organophosphorous insecticide Malathion (*S*-1, 2 bis (ethoxy carbonyl) ethyl *O,O*-dimethylphosphorodithioate) had been the stored grain insecticide of choice in different countries because of its high toxicity to a wide range of stored product pests and its relatively low mammalian toxicity (Zayed *et al.*, 1990, Borrero de Saiz 1990, Gozek and Artiran 1990). A potential genotoxic effect was observed in mice fed with stored grains treated with insecticides. The selectivity of Malathion is primarily due to mammals having

a high level of carboxyesterases enzymes that can hydrolyze Malathion and its metabolites to non-toxic intermediates which can be easily eliminated from cells, insects lack or have a low level of these esterases are severely affected by Malathion (Thompson *et al.,* 1989). The aerial application of Malathion was used over large urban areas in southern California as part of the 1990 Mediterranean Medfly. Eradication Program raised concerns over the potential of Malathion to cause genetic damage (Flessel *et al.,* 1993) Malathion was employed in major eradication programs against insect infestations in metropolitan areas of Florida, Texas and California (DHS 1991). On occupational settings, pesticide applicators exposed to technical grade Malathion and other insecticides are reported to exhibit higher levels of chromosome aberrations and SCEs (Rupa *et al.,* 1991).

A certain number of field studies have been done, obtaining an association between occupational exposure to complexes of pesticides and the presence of chromosomal aberrations as a factor which increases the risk of cancer (Garaj- Vrhovac and Zeljezic 1999). An association between occupational exposure to mixtures of pesticides and the presence of chromosomal aberrations sister chromatid exchanges ,micronuclei has been established in a number of cases (Rupa *et al.,* 1988, Rupa *et al.,* 1989, Kourakis *et al.,* 1992, Carbonell *et al.,* 1995, Ribas *et al.,* 1996) but not in others (Scarpato *et al.,* 1996 , Windham *et al.,* 1998). The widespread use of pesticides in public health and agricultural programs caused severe environmental pollution and potential health hazards. The World Health Organization (WHO) has classified pesticides according to their potential health risks (WHO, 2009).

In the present study we fed quails with beans and maize treated with Malathion to study the effect of Malathion as organophosphorus insecticide on chromosome abnormalities in both bone-marrow and spermatocytes. We aimed to evaluate the cytogenetic effects of Malathion, which is used to protect crops. Also, the cytogenetic effect of Malathion residues in grains stored for different periods of time was evaluated in quail using three doses concentrations of Malathion.

MATERIALS AND METHODS

The present study was carried out at the Animal and Poultry Production Department, Faculty of Agriculture (Damanhour), Alexandria University, throughout the period of 2005 to 2006. One experiment was conducted to study the effect of contamination by Malathion on some physiological and productive performance of Japanese quails and also to evaluate the cytogenetic effects of fungicides.

Experimental Design:

144 quails at sex ratio (3 female + 1 male) were distributed into 4 groups. Each group contained 3 replicates, each replicate contained (9 female + 3 male). They were randomly housed in individual laying cages and maintained on 16 hours light per day. Water and diet were introduced to bird's ad labium. Four rations were used in this experiment. The four rations were treated with different levels of Malathion (low, medium and high). Birds were fed for 8 and 15 weeks, which were the experiment period.

Chromosomal aberrations in quail bone marrow cells:-

Semen were injected with colchicines (4mg/kg), animals were scarified after 1h of injection .Bone marrow cells were collected by flushing in 0.075 M Kcl for 15 min, incubated at 37°c for 20 min. The material was then centrifuged at 1000 rpm for 5 min, fixed in centrifugation and fixation was repeated twice at an interval of 30 min.

The material resuspended in a small volume of the fixative (carnoy's solution) was dropped on the slides chilled, flame- dried and stained in the following day in 5% Gemsa stain at pH6.8.

Analysis of Spermatocytes

Spermatocytes were collected and 2.2% Na citrate were used as isotonic solution, remove tunica cut and squeeze tubules in Petri dish, filtration and transfer to centrifuge tubes, centrifugation 5 min at 1000 rpm, 1% Na citrate were used as hypotonic treatment, centrifugation 5 min at 1000 rpm, fixation 3: 1 ethanol: acetic acid, The fixation was repeated twice at an interval of 30 min the third fixation step should last at least 1h (refrigerated) and can be extended to the next day change the fixative for the last time just prior to slide making. Slides were stained with 5% Gemsa stain at pH 6.8.

Statistical analysis:

The statistical analysis of the experimental data was computed using analysis of variance procedure described in the SAS (1988), one fixed mathematical to:

$$Yij = M + Ei + eij$$

Where
Yij = Observation of the dependent variable
M = Overall mean
E i = Fixed effect at the I A level of treatment
Eij = the random error

Results

For Bone marrow

The metaphase analysis of bone marrow cells revealed various types of chromosomal aberrations consisted of chromatid break, chromosome break,

fragments, deletions and stickness. All acute doses (medium and high) of Malathion, induced significantly higher frequency of chromosome abnormalities compared to the control value (Table 1). Significant increase in the percentage of chromosomal aberrations was observed in quail fed with grains stored and treated with Malathion. It increased by prolongation of feeding period and the different dose. It reached its maximum of 19.2±0.21 for stickness in the high dose for quails fed on grains treated with Malathion for 15 weeks compared with low dose 12.5±0.91.

Table (1): Chromosomal abnormalities in quail's bone marrow cells after feeding quails with grains treated with Malathion.

Feeding with grains stored	Types of aberrations						
	Doses	Stickness	Gap	Fragment	Polyploidy	Chromatid break	Chromosome break
8 weeks	Control	0	0	0	0	0	0
	Low	10.3±0.64	0.1±0.21	0.1±0.22	0±0.0	0.9±0.74	0.7±0.32
	Medium	11.9±0.88	0.2±0.28	0.2±0.28	0.2±0.28	1.6±0.72	1.4±0.90
	High	16.4±0.97	0.4±0.35	0.6±0.72	0.3±0.45	2.2±0.69	1.9±0.49
15weeks	Control	0	0	0	0	0	0
	Low	12.5±0.91	0.3±0.34	0.2±0.28	0.1±0.22	3.5±0.42	2.10±0.35
	Medium	13.7±0.94	0.4±0.43	0.3±0.45	0.4±0.35	4.8±0.53	4.0±0.73
	High	19.2±1.21	0.6±0.55	0.5±0.57	0.5±0.56	7.15±0.65	4.91±0.81

On the other hand, stickiness showed in the quails fed on grains treated with Malathion for 8 weeks but lower than 15 weeks which was 16.4±0.97 for high dose and 10.3± 0.64 for low dose. The number of stickiness increased according to the increase in the dose and the period of feeding as shown in Table (1) and (Figure 1a, b, c, d, e, f, g and h.). Also, chromatid break and chromosome breaks were showed in the bone marrow cells but less than stickiness which was 7.15±0.65 after 15 weeks of feeding and 2.2±0.69 for 8 weeks feeding, but for chromosome break that was less than chromatid break in both treatments of feeding were 1.9±0.49 for high dose after 8 weeks feeding and 4.91±0.81 for high dose after 15 weeks feeding.

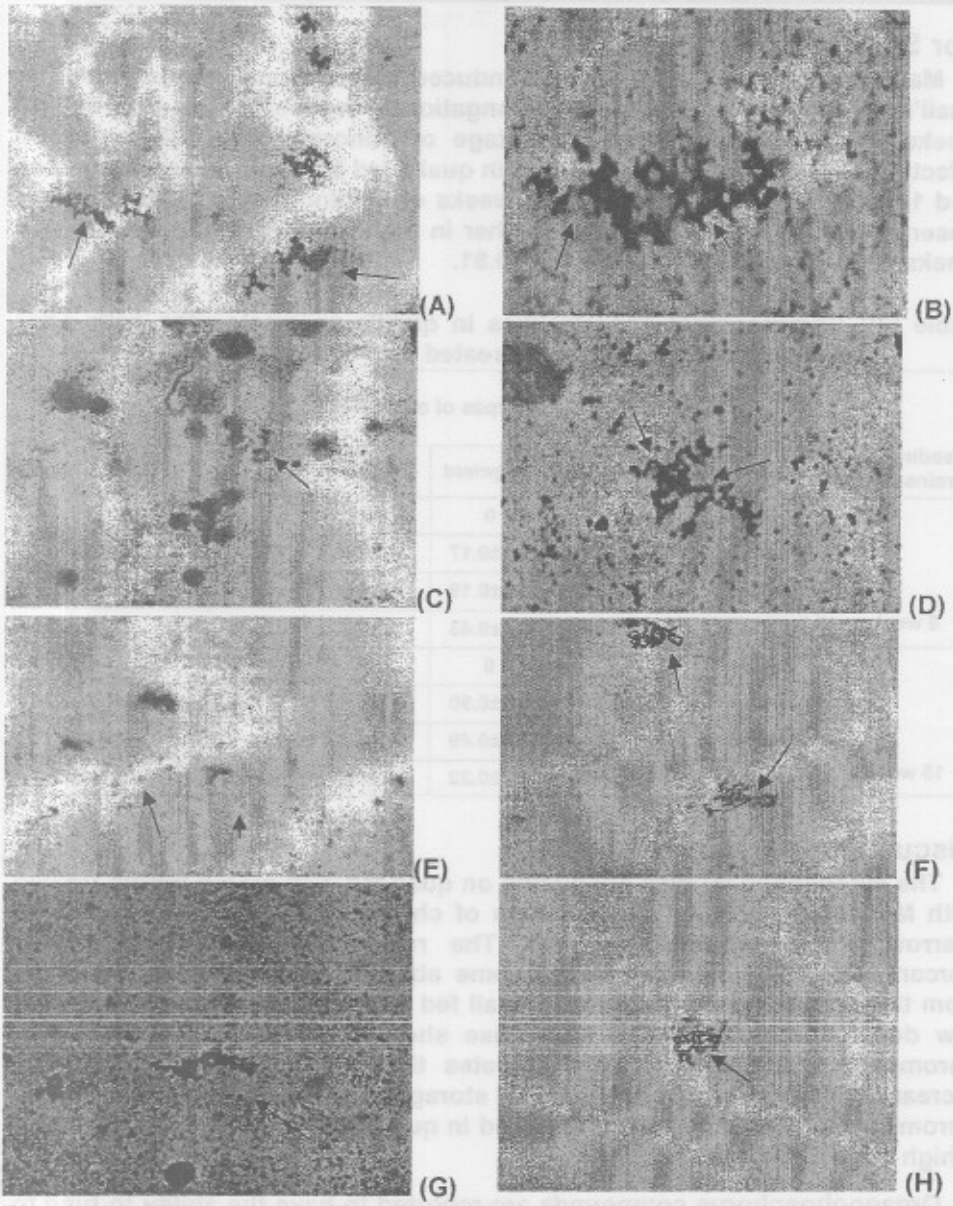

Figure (1): Chromosome aberration in Quail bone marrow and spermatocytes after feeding with Malathion (A) polyploidy, (B) Stickness, (C) Close ring, (D) Stickness & close ring and fragments, (E) Close ring & open ring , (F) Chromatid break, (G) Fragment, (H) Chromosome break & close ring

For Spermatocytes

Malathion residues in the grains induced chromosome abnormalities in quail's spermatocytes (Table 2). Prolongation of feeding period to 8 and 15 weeks affected a significant percentage of abnormalities. The maximum effect reaches 12.1±0.96 for open ring in quails fed for 8 weeks and high dose and 14.1±0.65 for high dose after 15 weeks of feeding. While close ring was observed for all doses but it was higher in high dose of Malathion after 15 weeks of feeding which reaches 4.91±0.81.

Table (2): Chromosomal abnormalities in quail's spermatocytes cells after feeding quails with grains treated with Malathion

Feeding with grains stored	Types of aberrations					
	doses	Stickiness	Fragment	Triploidy	Open ring	Close ring
8 weeks	Control	0	0	0	0	0
	Low	5.07±0.50	0.1±0.17	0±0.0	8.5±0.45	0.7±0.32
	Medium	5.07±0.56	0.2±0.19	0±0.0	10.3±0.72	1.4±0.90
	High	7.73±0.97	0.2±0.43	0±0.0	12.1±0.96	1.9±0.49
15 weeks	Control	0	0	0	0	0
	Low	4.53±0.53	1.4±0.90	0.1±0.19	7.5±0.42	2.10±0.35
	Medium	6.13±0.54	1.9±0.49	0.2±0.26	11.4±0.57	4.0±0.73
	High	7.20±0.80	0.7±0.32	0.3±0.31	14.1±0.65	4.91±0.81

Discussion

The cytogenetic studies conducted on quail fed with stored grains treated with Malathion revealed the induction of chromosome aberrations in bone marrow and spermatocytes cells. The results demonstrated that the percentage of the induced chromosome aberrations in the cells analyzed from the tissues was significant in quail fed with the grains treated with the low dose. The medium and high dose showed a significant increase of chromosome aberrations. This indicates that the residues of Malathion increased by increasing the period of storage. The maximum percentage of chromosome aberrations was observed in quail fed with the grains stored in a high dose of Malathion.

Organophosphorus compounds are reported to have the ability to bind to DNA (Wauchope *et al.*, 1992), and cause mutations (Rehana *et al.*, 1996 and Valkova *et al.*, 1993). Earlier studies have suggested only a weak interaction between Malathion and DNA. In these studies, Malathion was able to induce breakage in *E. coli* plasmid DNA (Wild 1975), alkylate nitrobenzylpyridine, a synthetic substrate (Imamura and Talcott 1985) and methylate DNA bases *in*

vitro (Wiaderkiewicz *et al.*, 1986).Malathion used in the present study is a technical grade product, and hence may contain impurities formed during manufacturing and storage. Among the impurities often found in Malathion are malaoxon, formed by oxidation of Malathion, and isoMalathion, formed by isomerization of Malathion (Berkman *et al.*, 1993). Malaoxon and isoMalathion have been reported to be mutagenic (Flessel *et al.*, 1993, and Pluth *et al.*, 1996). Therefore, possible contribution of impurities to the observed genotoxic effect of Malathion in the present study cannot be ignored. The present findings strongly indicate the potential of technical grade Malathion to induce genotoxicity and may be regarded as a potential germ cell mutagen. Therefore, application of Malathion that exposes large populations should be restricted.

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الآثار الوراثية الخلوية لمبيد الملاثيون علي السمان الياباني (كوترنكس جابونيكا).

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الملخص العربي

طائر السمان اليابانية (كوترنكس جابونيكا) ينتمي إلى عائلة دجاجيات النظام Phasianidae وهي محسنة لإنتاج البيض واللحوم. وتم دراسة تاثير مخلفات الملاثيون في الحبوب المخزنة علي المحتوي الوراثي الخلوي لفترات زمنية مختلفة. وتم تقييم السمان بإستخدام ثلاث جرعات (منخفضة , متوسطة , عالية) من مركب الملاثيون. وشملت الدراسة تحليل الكروموسومات في نخاع العظام والخلايا المنوية. تهدف الدراسة إلي تقييم الآثار للسمية الوراثية للمبيد الفطري الملاثيون والذي يستخدم لحماية المحاصيل من الإصابة الفطرية.

وأظهرت النتائج ظهور شذوذات كروموسومية شملت الشظايا الكروموسومية واللزوجة وكسور كروماتيدية وكذلك كروموسومية وذلك في نخاع العظام. في حين ظهرت حلقات مفتوحة وحلقات مغلقة في الخلايا المنوية. كذلك ظهرت علاقة طردية بين التركيزات والشذوذات.