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Study of Genotoxic and Cytotoxic effects of Malathion in Japanese Quail

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Abstract

The effect of Malathion was studied orally on Japanese quail males *(Cotrunix Cotrunix Japonica)*. The Median lethal Dose LD50 of malathion through 24 hours was found to be 163.6 mg / kg. Malathion was provided in doses of 75% 122.7 mg / kg b.wt., 50% 81.8 mg / kg b.wt., 25%40.9 mg/kg b.wt. LD50 of body weight plus cyclophosphamide 20 mg/kg b.wt as positive control and Corn oil as a negative control, The current study was carried out to detect the effects of malathion on cell cytotoxicity based on genetic cytotoxicity tests such as Micronuclei Test (MN for mature red blood cells in peripheral blood and immature blood cells in bone marrow for 18, 20 and 22 hours per treatment and Chromosomal Aberration (CA) test for immature red blood cells in bone marrow, And 24 hours for each treatment with subcutaneous injection of colchicine 0.5gm prior to 3-hour incubation. The results of the statistical analysis Suggested that a significant increase in $P \leq 0.05$of micronucleated mature red blood cells in peripheral blood and micronucleated immature blood cells in bone marrow for 18, 20 and 22 hours per treatment in the formation of micronuclei compared to negative control. The results of the study also Suggested that a significant increase in $P \leq 0.05$ on the induction of chromosomal Aberration of immature red blood cells in bone marrow.

Introduction

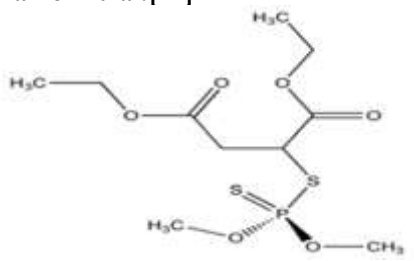
Over the past few decades, the toxicological effects of organophosphorus pesticides have increased because of the acute increase in their use in agriculture.[1,2] Environmental contamination by agricultural chemicals and industrial waste disposal results in adverse effects on reproduction of exposed birds. Pesticides have contributed from one side to dramatic increase in crop yields, and from the other side they may induce adverse ecotoxicological and hazardous health effects on a variety of living organisms, including birds [3]. Organophosphorus pesticides are widely used in agriculture and veterinary practice to control various pests. A number of long persistent organophosphates, which have been banned or severely restricted, are still used in many developing countries [4]. As birds have a high trophic level, they are vulnerable of accumulating large dosage of certain chemicals [5]. Some sub-lethal effects of pesticides were studied in birds with a view to identify characteristic biochemical response that may be useful for the monitoring of exposure to sub-lethal levels in the field [6]. Organophosphates have a remarkable acute toxicity due to inhibition of the cholinesterase enzyme and inducing acute neurological effects [7,8]. Malathion is one of the most commonly used organophosphorus insecticides and is the main cause of the most acute pesticide poisoning. [9] Its contamination may occur in poultry following its application to fruit, vegetables, grain, fiber and other crops. Contamination of poultry birds may also result from ingestion of treated cereals [10] or from the use of Malathion in the control of external parasites [11]. Malathion degrades into more toxic metabolites in the tissues like liver, kidney and brain and consequently poses a potential threat to public health due to the presence of pesticide residues in poultry meat [12]. The quail is a small migratory bird domesticated for two purposes, to produce egg and meat. And it's called in our Arabian countries in different names like (AL Saman and AL Salwa) most common name is (AL Salwa). Its one of the most important investable birds and became the focus of attention of researcher and investors. It's a domesticated bird

despite being a migratory bird. where it s migrate to Iraq during autumn.[13]. The present study was designed to investigate and explain the clastogenicity and aneugenecity of Malathion Japanese quail by using micronucleus (mn) test in peripheral blood swabs, and chromosome aberrations (CA) test in bone marrow.

Malathion is: *S*-1,2-bis(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate

Molecular formula: $C_{10}H_{19}O_6PS_2$

Structural formula :[14]



Materials and Methods

Experimental animals:

A total of 120 apparently healthy male Japanese quail (Coturnix coturnix japonica) weighing from 100 to 150 g, purchased from faculty of Agriculture salah Aldin University, applied for both LD50 and a treatment. LD50 medium lethal dose of OP (Malathion) with 50% concentration of malathion as an effective substance and study of toxic toxicological effects on animals was determined. The up and down method Dixon [15] was used to determine LD50 and 12 hours after cut off the feeding and drinking water from the birds before the experiment. The birds were orally Gavages with different doses of malathion with 1 ml of corn oil through a special injection syringe for 18,20,22 hours for Micronucleus Test and,24 hours for Chromosomal aberration.

Method of selection of potions and how to dosage:

The doses used for each group of animals were selected based on LD50 (163.6 mg/kg bw) According to the experimental design, the coefficients were divided into five treatments consisting of 15 animals and each treatment of 3 groups (A. B and C.) and each group of 5 animals. The amount of potions per treatment was as follows 75%, 50% and 25% LD 50 of malathion plus positive control group 20 mg / kg .bwt. (Cyclofosphamide) [16] and negative control group (corn oil). The oral dosage was administered by a modified syringe for the Gavage dosage. The dose is estimated according to tables [17].

Micronucleus and Chromosome Aberration Tests:

Periphrial Blood and Bone-marrow preparations were made and stained by May grunwald- Giemsa stain according to the method described in schmid [18]. The presence of micronucleated polychromatic erythrocytes was visually scored (at least2000 for periphrial blood and 1000 for bon marrow per Bird) by optical microscopy using a Leica bright field microscope. Cells were considered to be micronucleated when they contained neatly defined chromatin corpuscles with diameter of less than one third the diameter of the cell nucleus and stained equal or denser than the nucleus of the cell from which the micronucleated cell had developed [19, 20]. The experimental and control micronucleus frequency for each specimen within and between different bird groups were compared using the paired Tukey-test. Chromosome aberration from bone-marrow cells were prepared following the method of [21]. Stained with Giemsa stain well spread metaphases were analyzed per each animal for scoring different types of aberration.

Tretment

1- Induction test of micronucleus in peripheral blood cells and bone marrow. after successive oral administration of Malathion for 18,20, 22 hours of 75% 122.7 mg / kg bwt., 50% 81.8 mg / kg b.wt., 25% 40.9 mg / kg b.wt. LD50 of body weight

2- Chromosomal Aberration (CA) test for immature red blood cells in bone marrow after successive oral administration of Malathion for 24 hours of 75% 122.7 mg / kg bwt., 50% 81.8 mg / kg b.wt., 25% 40.9 mg / kg b.wt. LD50 of body weight.

Statistical

The values were statistically analyzed using the Statistical Package for Social Sciences (SPSS) statistical analysis program and the results presented in the mean arithmetic mean ± standard error. The results of the present study were statistically analyzed by the ANOVA test for the purpose of detecting significant differences by comparing the arithmetic averages of the different experimental groups and using the TUKEY test. At the probability level $P \leq 0.05$.

Results and discussion

The study showed that the malathion had a genetically toxic effect [22]. Table 1: illustrates Mean difference ± standard error in mature red blood cells containing micronucleus in peripheral blood swabs through a significant increase in the number of mature red blood cells containing micronucleus of blood samples (MNEs .P.B.S). for 18hoours three doses of malathion 75% 122.7 mg / kg b.wt., 50% 81.8 mg / kg , 25% 40.9 mg / kg b.wt. LD50 of body weight 92952±7.20*, 92952±15.00*, 92952±18.40*, and also an increase in the mean difference in treatments Respectively for three doses for 20 hours, 2.73±233.20*, 2.73±337.40*, 2.73±396.80* . for the same doses. There is also an increase in the mean difference in treatments Respectively for three doses for 22 hours 2.90±227.20*, 2.90±326.40*, 2.90±352.80*. for the same doses. Compared with negative control Figure (1-2) reveal mature red blood cells containing micronuclei treated with malathion In the peripheral blood smear (A) single mn. (B) bi MNi. (C) Tri MNi. Table 2: illustrates Mean difference ± standard error for a total number of micronucleus. (MNi .P.B.S). in mature red blood cells containing micronucleus in peripheral blood swabs for 18, 20, 22 hours for three doses of malathion 75%

122.7 mg / kg b.wt., 50% 81.8 mg / kg , 25% 40.9 mg / kg b.wt. LD50 of body weight there was an increase in the mean difference between treatments for18 hours were as follows; 1.32±8.80*, 1.32±19.20*, 1.32±22.60* and for 20 hours were as follows; 4.92±385.20*, 4.92±513.00*, 4.92±620.40* and for 22 hours as follows 5.67 ± 365.80*, *5.67 ± 502.80, *5.67 ± 550.80.compared with negative control. Table 3 : illustrates Mean difference ± standard error in Immature red blood cells containing micronucleus in Bone Marrow swabs .(MNPCE. B.M). Mean differences between the three doses were increased in a number of immature red blood cells containing micronucleus for three doses of malathion 75% 122.7 mg / kg b.wt., 50% 81.8 mg / kg , 25% 40.9 mg / kg b.wt. LD50 of body weight for 18 hours 2.10 ± 7.20*, 2.10 ± 12.20*, 2.10 ± 18.40* Respectively. There is also an increase in the mean difference in treatments Respectively for three doses for 20 hours of three doses of malathion 83427 ± 7.80* , 83427 ± 15.20* , 83427 ± 19.40*. There is also an increase in the mean difference in treatments Respectively for three doses for 22 hours of three doses of malathion were as follows 97570 ± 8.80* , 97570 ± 17.00 *, 97570 ± 21.00* Figure (1-4) micronucleated polychromatic erythrocytes. (MNPCEs) May- Grunewald + Gimesa, 100 X. (a) single mn. (b) bi MNi. and Figure (1-5) micronucleated polychromatic erythrocytes. Bone Marrow (MNPCEs) (C) Tri MNi.

Table 4: illustrates Mean difference ± standard error in Immature red blood cells containing micronucleus in Bone Marrow swabs.(MNi. B.M.) the total number of micronucleus, there was an increase in the mean difference between the three treatments doses of malathion 75% 122.7 mg/kg b.wt., 50% 81.8 mg / kg , 25% 40.9 mg / kg b.wt. LD50 of body weight for 22 hours were as follows 1.70 ± 11.00*, 1.70 ± 21.60*, 1.70±27.20*. The results of the statistical analysis referred to a significant increase in P≤ 0.05 of micronucleated mature red blood cells in peripheral blood and micronucleated immature blood cells in bone marrow for 18, 20 and 22 hours per treatment in the formation of micronuclei compared to negative control. Table: Shows Types of chromosome aberrations Structural and numerical chromosomal aberrations in males of Japanese quail in both of negative and positive control groups and groups treated with different doses of the malathion insecticide and the results of the study of structural and numerical chromosomal Aberration for 24 hours of three treatments for malathion were shown in bone marrow , Also an increase in the average of the significant differences between treatments % 25, %50, %75, Of the Median Lethal Dose LD50 rrespectively for structural chromosomal abnormalities, It was found to increase as the dose increased 0.76 ± 2.6* , 0.76 ± 7.2* , 0.76 ± 14.20* . As well as structural chromosomal Aberration ,there is also an increase in the mean difference between 25%, 50% and 75% Of the midterm dose LD50 rrespectively. Median Lethal Dose 2.0±13.00*, 2.0±36.20*, 2.0±70.00* . The results of the study also argued that a significant increase in P≤ 0.05 on the induction of chromosomal Aberration of immature red blood cells in bone marrow**.** Figure (2-1) illustrates Structural and Numerical chromosome aberrations in treated and untreated bird A and B normal Chromosome brushes C- Ring and fragment D- ring Chromosome, E- deletion F- Robertsonian translocation, G- Centromeric gaps ,H - Centromere attenuation, I–Aneuploidy J-euploidy, K-bi centromere.

Table 1 : Focuses on Mean difference ± standard error in mature red blood cells containing micronucleus in peripheral blood swabs .(MNEs .P.B.S).

After 22 hour		After 20 hour		After 18 hour		No. of examind cells	Treatment / Dosage Mg / kg. Body weight
S.E.	M.d.	S.E.	M.d.	S.E.	M.d.		
2.90	-	2.73	-	92952	-	2000	Corn Oil
2.90	234.20*	2.73	254.00*	92952	29.00*	2000	Cychlophosphomide 20
2.90	227.40*	2.73	233.20*	92952	7.20*	2000	25 %LD50 Malathion 40.9
2.90	326.00*	2.73	337.4*	92952	15.00*	2000	50 %LD50 Malathion 81.8
2.90	352.00*	2.73	396.80*	92952	18.40*	2000	75 %LD50 Malathion 122.7

M = bone marrow P.B.S = peripheral blood c, b, a = animal sacrifice after 18, 20, 22 hours of treatment, Md = mean difference S.E. = Average standard error, test-Tukey

Table 2: Focuses on Mean difference ± standard error in mature red blood cells containing micronucleus in peripheral blood swabs .(MNi .P.B.S).

After 22 hour		After 20 hour		After 18 hour		No. of examind cells	Treatment / Dosage Mg / kg. Body weight
S.E.	M.d.	S.E.	M.d.	S.E.	M.d.		
5.67	-	4.92	-	1.32	-	2000	Corn Oil
5.67	350.40*	4.92	381.40*	1.32	38.40*	2000	Cychlophosphomide 20
5.67	365.80*	4.92	385.20*	1.32	8.80*	2000	25 %LD50 Malathion 40.9
5.67	502.80*	4.92	513.00*	1.32	19.20*	2000	50 %LD50 Malathion 81.8
2.90	352.00*	2.73	396.80*	92952	18.40*	2000	75 %LD50 Malathion122.7

M = bone marrow P.B.S = peripheral blood c, b, a = animal sacrifice after 18, 20, 22 hours of treatment, Md = mean difference S.E. = Average standard error, test-Tukey

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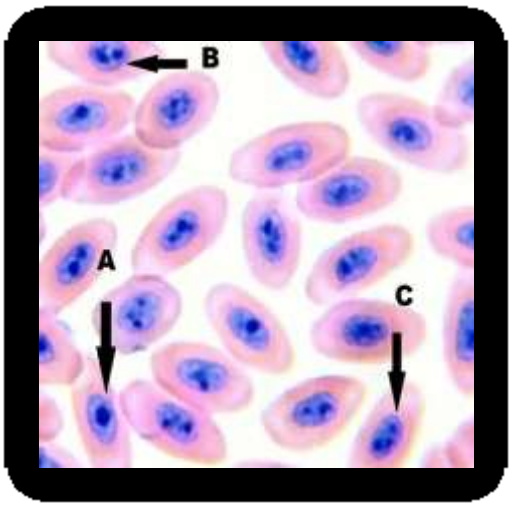

Figure (1-1)) Presents the mature red blood cells without micronucleus Normal blood smear negative control May-gruwald and Geims 100 X

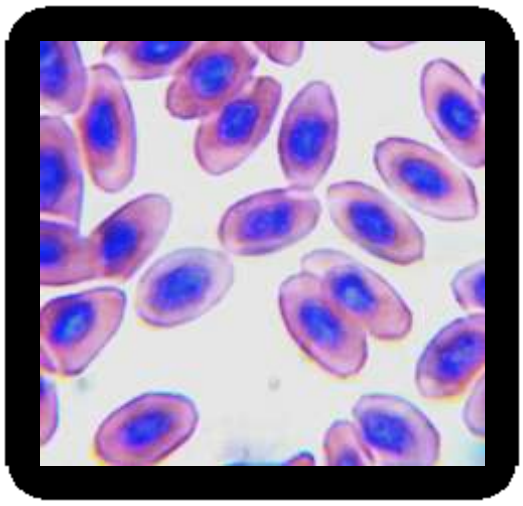

Figure (1-2) Presents mature red blood cells containing micronuclei treated with malathion In the peripheral blood smear (A) single mn. (B) bi MNi. (C) Tri MNi

Table 3: Focuses on Mean difference ± standard error in Immature red blood cells containing micronucleus in Bone Marrow swabs .(MNPCE . B.M).

After 22 hour		After 20 hour		After 18 hour		No. of examind cells	Treatment / Dosage Mg / kg. Body weight
S.E.	M.d.	S.E.	M.d.	S.E.	M.d.		
97950	-	83427	-	2.10	-	1000	Corn Oil
97950	34.00*	83427	33.20*	2.10	29.00*	1000	Cychlophosphomide 20
97950	8.80*	83427	7.80*	2.10	7.20*	1000	25 %LD50 Malathion 40.9
97950	17.00*	83427	15.20*	2.10	12.20*	1000	50 %LD50 Malathion 81.8
97950	21.00*	83427	19.40*	2.10	18.40*	1000	75 %LD50 Malathion 122.7

M = bone marrow P.B.S = peripheral blood c, b, a = animal sacrifice after 18, 20, 22 hours of treatment, Md = mean difference S.E. = Average standard error, test-Tukey

Table 4: Focuses on Mean difference ± standard error in Immature red blood cells containing micronucleus in Bone Marrow swabs .(MNi . B.M.)

After 22 hour		After 20 hour		After 18 hour		No. of examind cells	Treatment / Dosage Mg / kg. Body weight
S.E.	M.d.	S.E.	M.d.	S.E.	M.d.		
1.70	-	1.34	-	1.32	-	1000	Corn Oil
1.70	43.60*	1.34	44.80*	1.32	38.40*	1000	Cychlophosphomide 20
1.70	11.00*	1.34	10.00*	1.32	8.80*	1000	25 %LD50 Malathion 40.9
1.70	21.60*	1.34	19.40*	1.32	19.20*	1000	50 %LD50 Malathion 81.8
1.70	27.20*	1.34	24.20*	1.32	22.60*	1000	75 %LD50 Malathion 122.7

M = bone marrow P.B.S = peripheral blood c, b, a = animal sacrifice after 18, 20, 22 hours of treatment, Md = mean difference S.E. = Average standard error, test-Tukey

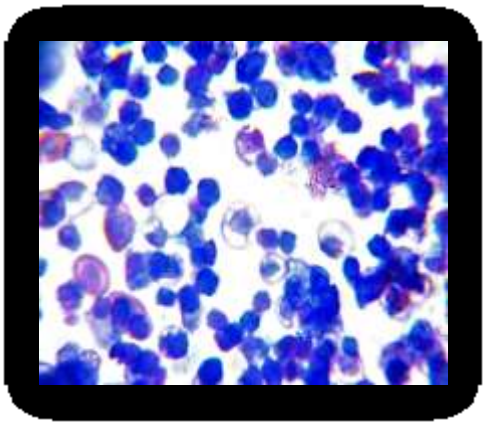

Figure (1-3) Annucleated polychromatic Erethrocytes immature red blood cells (PCEs) Bone marrow

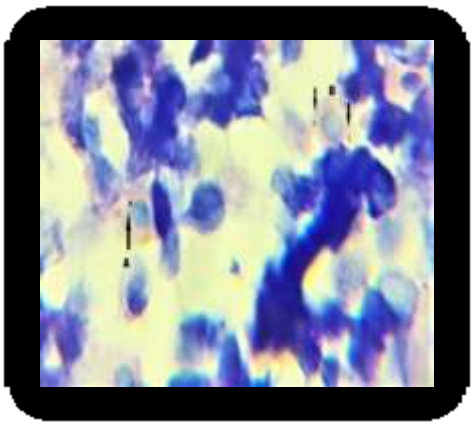

Figure (1-4) micronucleated polychromatic erythrocytes. (MNPCEs) May- Grunewald + Gimesa, 100X (a) single mn. (b) bi MNi.

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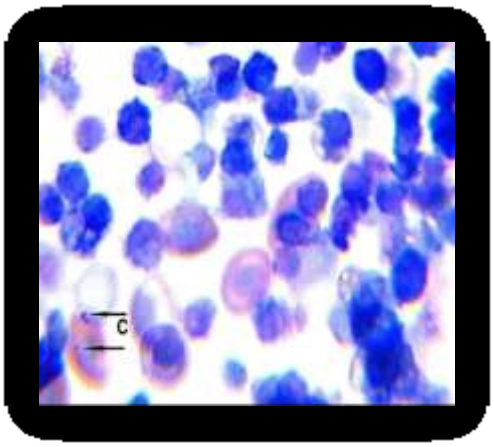
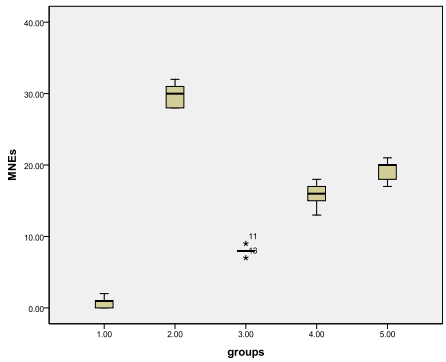
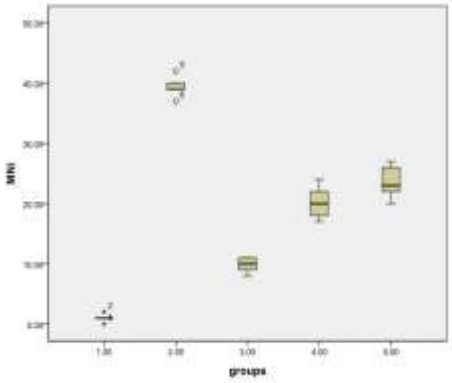


Figure (1-5) micronucleated polychromatic erythrocytes. Bone Marrow (MNPCEs) (C) Tri MNi

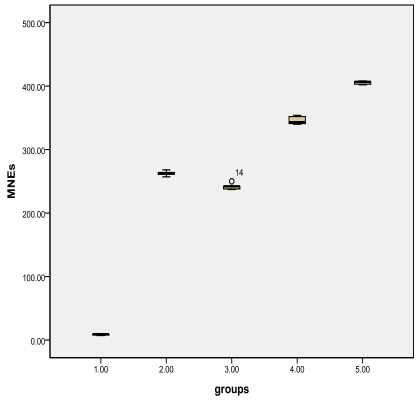


Boxplot MNEs Blood Smear 18 hours Figure (1)

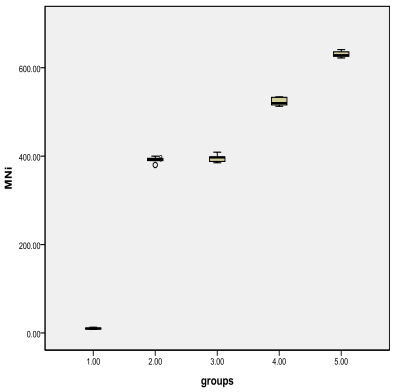


Boxplote MNi Blood Smear 18 hours Figure (2)

R Statistics

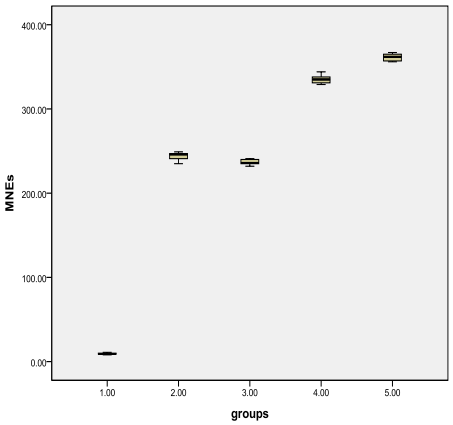


BoxplotMNEs Blood Smear 20 hoursFigure (3)

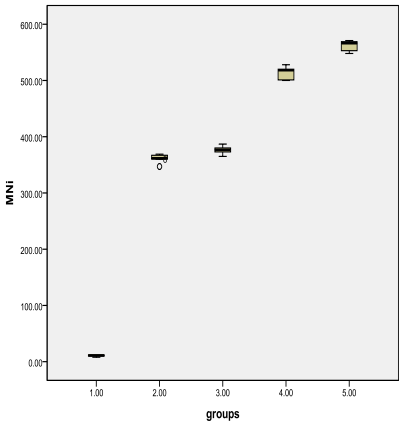


Boxplot MNi Blood Smear 20 hours Figure (4)

R Statistic



Boxplot MNEs Blood Smear 22 hours Figure (5)



Boxplot MNi Bood Smear 22 hours Figure (6)

Table 5 : Focuses on Types of chromosome aberrations Structural and numerical chromosomal aberrations in males of Japanese quail in both of negative and positive control groups and groups treated with different doses of the malathion insecticide

Total anomalies Numerical	Cells with numerical anomalies		Cells with chromosomal anomalies									Tretment Mg/Kg .bwt
S.E .±M.D	euploid %	Aneuploid %	Total Structural Aberration S.E .±M.D	Assoc %	atten %	Rob %	r %	g %	frag %	b %	del %	
–	66.6	33.3	-	-	-	-	-	33.3	–	33.3	33.3	Corn Oil
0.76±11.40*	8.6	46.5	2.0±61.20*	6.8	28.8	2.6	0.9	14.5	15.5	15.2	17.1	Cychlophosphomide 20
0.76±2.6*	62.5	37.5	2.0±13.00*	1.5	25	1.5	1.5	20.5	20.5	17.6	14.7	25 %LD50 Malathion 40.9
0.76±7.2*	56.4	43.6	2.0±36.20*	5.4	20.6	1.0	1.0	19.0	19.0	16.3	20.1	50 %LD50 Malathion 81.8
0.76±14.20*	54.1	45.6	2.0±70.00*	7.4	28.9	1.7	1.4	14.4	15.4	15.0	16.1	75 %LD50 Malathion 122.7

Md = mean difference S.E. = Average standard error, test-Tukey p≤0.05

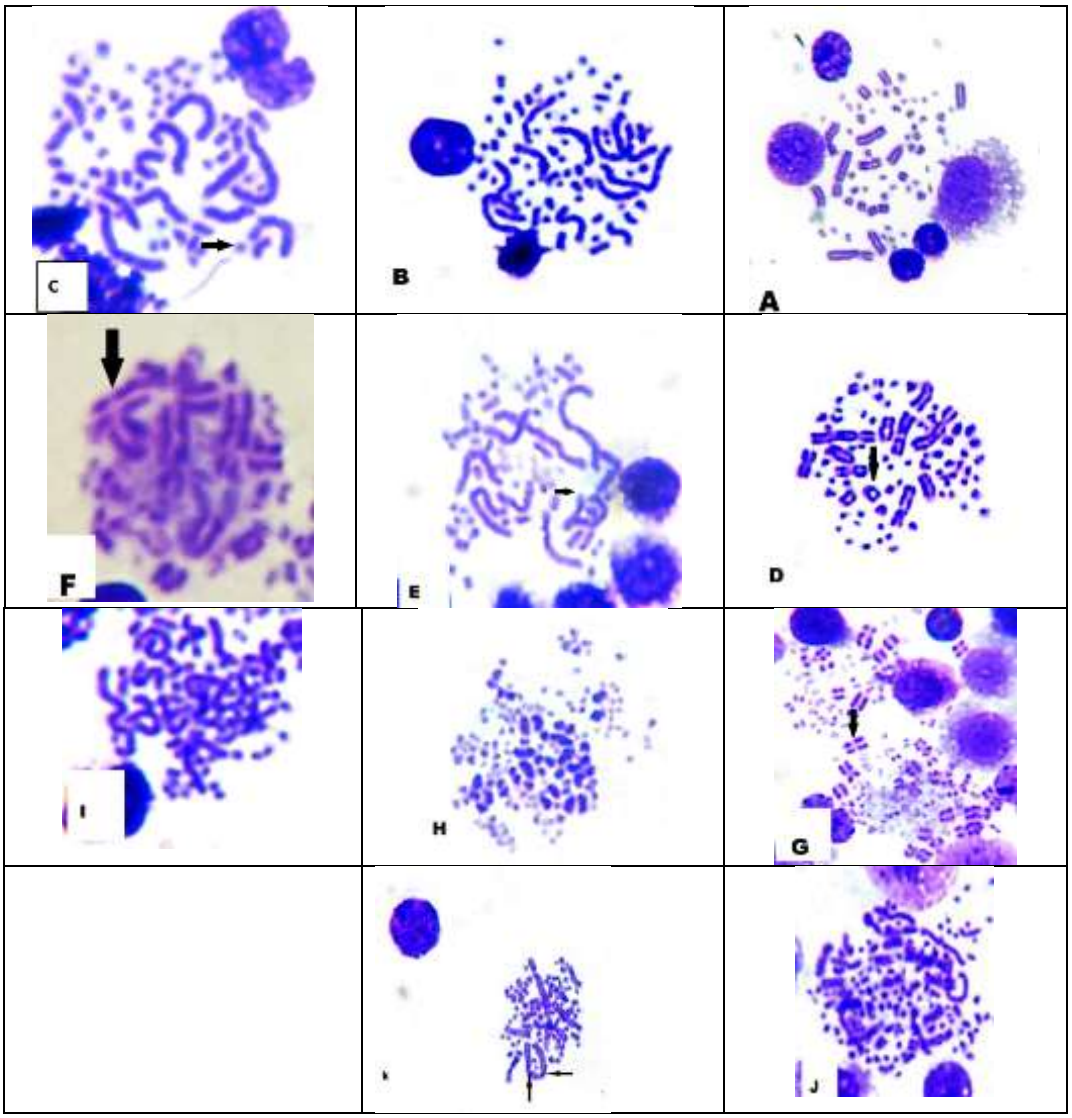


Figure (2-1) Presents Structural and Numerical chromosome aberrations in treated and untreated bird A and B normal Chromosome brushes C- Ring and fragment D- ring Chromosome , E- deletion, F- Robertsonian translocation , G- Centromeric gaps ,H - Centromere attenuation , I – Aneuploidy, J- euploidy , K- bi centromere

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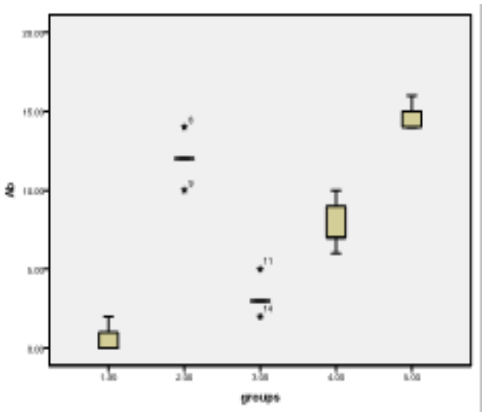
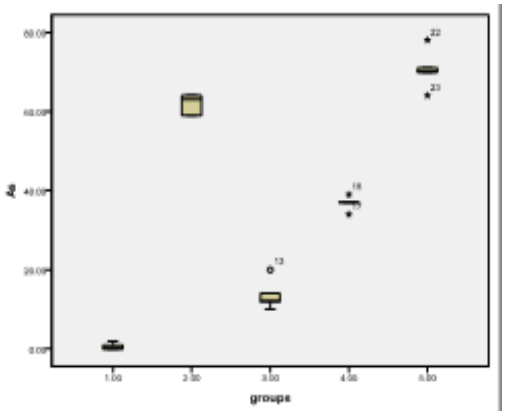


Boxplot Numerical Aberration Figure(7)



Boxplot Structural Aberration Figure(8)

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دراسة تاثير السمية الخلوية الوراثية للملاثيون في السمان الياباني

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الملخص

خلال العقود القليلة الماضية، ازدادت التأثيرات السمية الإيكولوجية للمبيدات الحشرية الفسفورية العضوية بسبب الزيادة الحادة في استخدامها في الزراعة، صممت هذه الدراسة للبحث عن تأثير Malathion فمويا على الذكور السمان الياباني (***Cotrunix Cotrunix Japonica***)، وجد أن الجرعة المميتة النصفية (LD50) للملاثيون خلال24 ساعة هي 163.6 ملغ / كغ .تم توفير Malathion بجرعات من 75 ٪ 122.7 ملغم/ كغم، 50 ٪ 81.8 ملغم اكغم، 25 ٪ 40,9 ملغم/ كغم من LD50 من وزن الجسم بالإضافة الى عقار سيكلوفوسفومايد 20 ملغم \ كغم من وزن الجسم كسيطرة موجبة وزيت ذرة كسيطرة سالبة ،واجريت الدراسة الحالية، للكشف عن تأثيرات الملاثيون السمية الخلوية الوراثية اعتمادا على الاختبارات السمية الخلوية الوراثية مثل فحص النواة الدقيقة (Micronuclei Test, MN) لخلايا الدم الحمر الناضجة في الدم المحيطي وخلايا الدم الحمر الغير الناضجة في نقي العظم ولمدة 18، 20 و22 ساعة لكل معاملة. واختبار زيغ الكروموسومي (Chromosomal Aberration CA) لخلايا الدم الحمر الغير الناضجة في نقي العظم. ولمدة 24 ساعة لكل معاملة مع حقن الكولشيسين تحت الجلد قبل التضحية بالطيور ب3 ساعات. واظهرت نتائج التحليل الاحصائي ارتفاع معنوي عند $P\leq 0.05$ لخلايا الدم الحمر الناضجة في الدم المحيطي وخلايا الدم الغير الناضجة الحاوية على النوى الدقيقة في نقي العظم ولمدة 18، 20 و22 ساعة لكل معاملة في تكوين الأنوية الدقيقة مقارنة مع السيطرة السالبة، وكذلك أظهرت نتائج الدراسة أيضا ارتفاع معنوي عند احتمالية $P\leq 0.05$ على استحثاث الزيغ الكروموسومي لخلايا الدم الحمر الغير الناضجة في نقي العظم .

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