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Evaluation of Hematological, Cytogenetical and Biochemical Effects of Malathion and Spinosad on Male Albino Rats

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Abstract: Adult male albino rats feeding on stored wheat grains treated with malathion and spinosad were used as experimental animals to evaluate the hematological, cytogenetical and biochemical effects of both insecticides. Wheat-bound malathion and spinosad were fed to rats at 8 and 16 ppm in the diet for 90 consecutive days. The tested concentrations of both insecticides induced observable significant decrease in the hematological indices (i.e. RBC's, HCT and PLT). However, the other indices such as HGB, MCV and MCH were significantly decreased in malathion and spinosad-treated rats with the higher concentration only as compared to the untreated control group. On the other hand, WBCs recorded significant increases in the higher concentration of both insecticides. Results of the cytogenetical effects indicated that the tested concentrations of both insecticides showed significant increase in the average number of chromosomal aberrations in rat's bone marrow cells. The maximum effect was recorded in rats fed on grains treated with both malathion concentrations and the higher concentration of spinosad. The effect of both insecticides on the genetic material (DNA content) showed significant increase, although non significant increase was observed at the low concentration of spinosad. Amount of total proteins in rat liver were significantly decreased comparing with control as a result of treatment with both insecticides. Gel scanning with standard molecular weights revealed variations in the number and intensity of protein bands among the control and the tested concentrations. All concentrations revealed an increase of protein bands than those in the control group, except the higher malathion concentration which showed decrease in bands number. PAGE analysis of the esterases isozymes exhibited the same effects for malathion and spinosad as well as control group in the number of bands, although the intensity of these bands were differed. The obtained results indicate that malathion and spinosad residues in stored wheat grains have potential genotoxic effect on male albino rats under the conditions tested, moreover, malathion was able to exhibit more pronounced effects than spinosad.

Key words: Hematology · Chromosomal Aberrations · Biochemical · Genotoxicity · Spinosad · Malathion · Albino Rat

INTRODUCTION

Securing adequate sources of food is one of the most urgent problems today in the great majority of developing nations. Among cereals, wheat is the most frequently cultivated species and the most consumed in the world. Loss of cereal grains in storage can range from 10 to 20% of overall production and a primary factor in this loss is the depredations of stored product insect pests [1]. Stored grain pests cause both quantitative and qualitative damage to wheat grain [2].

The organophosphorus compound malathion is used extensively throughout the world; especially in developing countries, to protect grains in storage [3]. Malathion has 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 [4]. Also, one of the most promising alternatives to residual pesticides in stored grains is the use of spinosad, which is a metabolite of the actimomycete Saccharopolyspora spinosa [5]. Spinosad has low mammalian toxicity and is very effective against

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a wide range of pest species [6, 7]. Spinosad has received U.S. Environmental Protection Agency approval for its use as a grain protectant in 2005 [8], as an alternative to traditional grain protectants. Since then, spinosad has been shown to provide highly effective and long-lasting control of numerous key stored product pests on various grains [9, 10].

The use of pesticides is one means of preventing some losses during storage. However, the choice of pesticides for control of stored-grain pests has resulted in serious adverse health effects [11, 12]. Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition [13]. Blood analysis is crucial in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes and diseases investigation [14]. The complete blood count (CBC) is an important and powerful diagnostic tool as it can be used to monitor the severity of manifestations of illness [15].

Genotoxic potential is a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage [16,17]. Pesticides have been considered potential chemical mutagens whereas experimental data revealed that various agrochemical ingredients possess mutagenic properties [18]. Chromosomal aberrations analysis is among the most extensively used markers of genotoxic effects of pesticides. Chromosomal aberrations are particularly dangerous to the cell, because the physical discontinuity of the chromosome may cause loss of genetic information and even cell death if a housekeeping gene is involved [19]. Also, chromosomal aberrations may be used as an early warning signal for cancer development and it has been suggested that the detection of an increase in chromosomal aberrations, related to an exposure to genotoxic agents, may be used to estimate cancer risk [20].

Biochemical changes were used as indications of exposure and/or effects of pesticides [21]. The great advantage of biochemical markers is providing evidence of the state of pollution in a comprehensive way based on the synergistic and antagonistic effects of all contaminants involved. Most of the toxic effects are due to poisoning of metabolism. Biochemical values indicate the intensity of the toxic action of the pesticides [22].

Consequently, the objective of this study was to investigate the effect of sub-chronic exposure to malathion and spinosad on the hematological parameters as well as their potential to induce cytological and biochemical effects.

MATERIALS AND METHODS

Chemicals: Malathion (Nasr lathion®, 1%WP) was provided by El-Nasr Chemical Industries Co. Egypt. Malathion [O,O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate]. Spinosad (Tracers®, 24% SC) was provided by Dow AgroSciences Company (Tracers® is a commercial formulation containing spinosyns A and D (Dow AgroSciences Company). Spinosyn A is 2-[(6-deoxy-2, 3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy)-13-[(5- dimethylamino) tetrahydro-6-methyl-2 H-pyran-2-yl) oxy)- 9-ethyl-2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16btetradecahydro- 14-methyl-1 H-as-indaceno (3, 2-d)oxacyclododecin-7, 15-dione. Spinosyn D is 2-((6-deoxy-2,3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy)-13-((5-(dimethylamino) tetrahydro-6-methyl-2 H-pyran-2-yl) oxy)-9-ethyl 2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16btetradecahydro-4, 14-dimethyl-1 H-as-indaceno (3, 2-d) oxacyclododecin-7, 15-dione. All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Tested Animals: Adult male albino rats (Sprague-Dawely), *Rattus norvegicus var. albus*, weighting 160-185 g were purchased from the Biological Products & Vaccines Holding Company, Helwan Farm, Cairo, Egypt. Rats were kept under the laboratory conditions of 25±5°C and 65±5% R.H. for two weeks as an acclimatization period. They were housed in metal cages (35x25x20 cm) and maintained on *ad libitum* diet and water. Rats were sacrificed after feeding on stored wheat grains treated with different concentrations of both tested insecticides. All treatments and procedures were in accordance with the protocol of National's Animal Care and Use Committee and Guidelines for the Care and Use of Experimental Animals and in accordance to Helsinki Declaration.

Experimental Design: Rats used in this study were divided randomly into five groups, each of ten rats. The first group was fed on pesticides-free wheat diet and

was considered as a control. The second and third groups were fed on wheat grains treated with 8 ppm of malathion (the recommended concentration used for grains storage) and 16 ppm (double of the recommended concentration). The fourth and fifth groups received wheat grains treated with 8 and 16 ppm of spinosad. Pesticides-free wheat (*Triticum aestivum* L.) was used in preparing of the toxicated wheat grains diet as described by Eissa and Zidan [23]. Feeding administration was lasted for 90 successive days.

Parameters Studied

Signs of Toxicity: During the experimental period, rats were under observation for general appearance, behavior, symptoms of toxicity and mortality. Signs of toxicity were conducted at least twice daily during the study.

Hematological Examination: At the end of the experimental period, rats were weighed, slaughtered and blood samples were individually collected from each rat immediately after slaughtering in dry clean centrifuge tubes and taken on heparin as anticoagulant (1-2 IU/ml) for hematological studies. The studied parameters have been assessed in respect of Complete Blood Count (CBC), comprised Red Blood Cell Count (RBC's), Haematocrit (HCT), Haemoglobin concentration (HGB), total Platelet Count (PLT), White Blood Cell Count (WBC's), Mean Cell Volume of RBC's (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC). The CBC was measured by Hematology analyzer (MEDONIC CA 620, Sweden).

Cytogenetical Analysis: Five rats of each experimental group were separated and sacrificed after two hours from injection with 4 mg/Kg B.wt. colchicine [24]. Metaphase chromosomes from bone marrow were prepared using the method described by Rabello-Gay and Ahmed [25] and stained with Giemsa. Fifty well spread metaphase cells per animal were assayed and scored for chromosomal aberrations. Structural and numerical chromosomal aberrations were scored using the light microscope at 1000× magnification.

Biochemical Studies

Determination of Dna Content: DNA content was determined in a known weight of livers (100 mg) in three independent replicates for each treatment. Total genomic DNA was isolated as described by Tinwell *et al.* [26] and its concentration was measured spectrophotometerically. The concentrations of DNA were computed and expressed as μg of DNA/100 mg of tissue.

Determination of Total Protein: The total protein content was measured for three replicates for each treatment. Liver samples (200 mg) were homogenized according to El-Fadly *et al.* [27]. The homogenate was centrifuged at 12000 rpm for 15 minutes. The obtained supernatant was used for total protein quanification.

SDS-Polyacrylamide Gel Electrophoresis Assay: Total protein banding patterns were determined electorphoretically using SDS-polyacrylamide gel according to Laemmli [28]. SDS-PAGE was performed as slabs; 4% and 7.5% for stacking and separating gels, respectively. Samples were submitted with lower range of protein molecular weight marker (Stock No. SDS-7 and Technical Bulletin No. MWS-877L) followed by staining with Coomassie Brilliant Blue R-250.

Esterase Isozymes Assay: Esterase (Est) isozymes were detected using 7.5% polyacrylamide gel electrophoresis according to the procedures described by Davis [29]. The gel was stained in a solution of α- and β-naphthyl acetate (as a substrate) and fast blue RR (as Diazo coupler) in 0.1 M of phosphate buffer pH 6.5 [30].

Statistical Analysis: All values were expressed as mean±standard error (SE). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by LSD tests using the computer program package SPSS; version 9.0 and the significance of difference was set up at ($p < 0.05$).

RESULTS AND DISCUSSION

Signs of Toxicity: No obvious signs of toxicity were noticed during the experimental duration in the behavioral activity or external appearance in any of the treated rats at any of the tested concentrations for both insecticides. Furthermore, no mortality was occurred. Mansour *et al.* [31] found that there were no signs of toxicity were noted in rats treated with spinosad. Spinosad is a neurotoxin with a novel mode of action and acting as an agonist at the post-synaptic cholinergic ion channels and GABA-gated ion channels [6], for this reason signs of toxicity were recorded.

Table 1: Hematological changes in rats feeding for 90 days on wheat grains treated with two different concentrations of malathion and spinosad.

			Malathion				Spinosad			
	Control		8 ppm		16 ppm		8 ppm		16 ppm	
Parameters	Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control
RBC's (× 10^6/µl)	5.43±0.13^a	100	5.13±0.12^b	94.48	4.17±0.07^d	76.79	5.00±0.06^{bc}	92.08	4.73±0.03^c	87.11
HGB (g/dl)	16.86±0.12^a	100	15.07±0.09^b	89.38	10.42±0.11^c	61.81	15.27±0.23^a	90.57	12.47±0.37^b	73.96
HCT (%)	50.60±2.76^a	100	45.20±0.26^b	89.32	31.25± 0.33^c	61.75	45.80±0.70^b	90.51	37.45±1.13^b	74.01
PLT (10^3/mm^3)	211.67±13.64^a	100	177.00±1.53^b	83.62	146.00±12.29^c	68.98	170.21±7.64^b	80.41	169.67±3.28^{bc}	80.16
WBC's, (× 10^3/µl)	4.90±0.38^c	100	4.57±0.26^c	93.27	9.43±0.58^a	192.45	4.22±0.06^c	86.12	6.87±0.40^b	140.20
MCV (îm^3)	93.18±6.34^a	100	88.13±1.58^a	94.58	75.06±1.95^b	80.55	89.98±2.24^a	96.57	79.13±2.42^b	84.92
MCH (pg)	31.04±0.36^a	100	29.38±0.53^a	94.65	25.02±0.65^c	80..60	28.15±1.10^{ab}	92.14	26.34±0.81b^c	84.86
MCHC (g/dl)	33.32±2.85^a	100	33.33±0.00^a	100.03	33.33±0.00^a	100.03	33.33±0.00^a	100.03	33.28±0.05^a	99.89

Means having the same superscript letter(s) across each row are statistically insignificant (p> 0.05).

Effect on Hematological Parameters: Results in Table (1) indicate the presence of biologically meaningful differences in hematological parameters of malathion and spinosad-treated rats. There was a significant reduction in RBC's, HCT and PLT of malathion and spinosad-treated rats at both concentrations. However, the other hematological indices such as HGB, MCV and MCH were significantly decreased only at the higher concentration of both insecticides comparing with the control group. On the other hand, WBC's recorded significant increases in malathion and spinosad-treated rats at the higher concentration. Generally, these effects were significantly more pronounced in malathion-treated rats at the higher concentration comparing with the same concentration of spinosad.

Hematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies induced by industrial compounds, drugs, dyes, heavy metals, pesticides and several others [32, 33]. Red blood cells (known as erythrocytes) are very important for the transport of oxygen from the lungs to the tissues and haemoglobin concentration is directly correlated with RBC's count [34]. In this study, the reduction in erythrocyte counts and consequently haemoglobin concentration may be attributed to an increased rate of breakdown of red cells and/or the toxic effect of pesticides on bone-marrow. In general, anemia; reduction in the number of red blood cells or of haemoglobin in the blood can reflect impaired synthesis of haemoglobin (eg. in iron deficiency) or impaired production of erythrocytes (eg. in folic acid or vitamin B12 deficiency [35]. Also, many laboratories have reported the induction of anemia with experimental insecticidal exposure of animals [36].

Our findings are in agreement with the results reported by Yano *et al*. [37], who found that male rats given 0.2% spinosad for 13 weeks had significant decreases in HGB concentration (60%) and RBC's count (11%) relative to control. Stebbins *et al*. [38] reported that erythrocytic parameters (RBC's count & hemoglobin concentration) were decreased in male mice given 0.036% spinosad after 3 and 12 months. The organophosphorus insecticide chlorpyrifos caused decrease in RBC, HGB and HCT, which might be due to the effect of pesticide on blood-forming organs suggesting the anaemic condition of the treated animals. The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocytes destruction in hemopoietic organs [39]. Patel *et al*. [40] have reported an induction of DNA damage in hematopoietic system, viz. spleen, bone marrow and lymphocytes, showing that chlorpyrifos induce chromosomal aberrations and micronucleus formation in mouse bone marrow. On the other hand, the values of RBC, MCV and PCV were higher; in rats treated with 50 mg malathion a.i.kg-1 b.w. than control after 4 weeks of treatment [41]. Also, malathion-treated group had significantly higher RBCs, HGB and HCT in all groups compared with control group and lower significant in WBCs [42].

The reduction in MCH may be due to destruction of RBC (size and shape) and decrease in Hb synthesis and haemoglobin content. These symptoms imply the microcytic hypo chromic anemia. Decrease in MCH was observed in rats treated with various insecticides such as endosulfan, malathion, methyl parathion, phosphomidon, monocrotophos and fenvalerate [43].

The WBC's formed in the bone marrow are found either in the blood or migrate to key organs such as the spleen, lymph nodes, or gut. The increase in total leukocytes count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue [44]. Such lymphocyte response might be due to the presence of toxic substances which induce tissue damage and severe disturbance of the non-specific immune system leading to increased production of leukocytes. The observed effects of spinosad insecticide, which represented by increase of

Table 2: Types of structural and numerical chromosomal aberrations induced in bone marrow cells after feeding rats for 90 days on wheat grains treated with two different concentrations of malathion and spinosad.

					Structural aberrations												Numerical aberrations				
			Aberrant cells																		
Treatment		No. of examined cells	No.	%	Gap (G)	Break (B)	Fragment (F)	Centric fusion (CF)	End-to-end association (E)	Deletion (D)	Ring chro mosome (R)	Centric separation (CS)	Stickiness (S)	Total No.	Mean ± SE	Hypo ploidy	Hyper ploidy	Total No.	Mean ± SE
Control		250	20	8	2	2	5	9	3	3	0	10	4	38	7.6 ± 0.75^{c}	5	0	5	1.0 ± 0.45^{c}
Malathion	8 ppm	250	100	40	15	12	17	12	3	24	4	36	21	144	28.8 ± 1.39^{a}	42	2	44	8.8 ± 0.37^{a}
	16 ppm	250	105	42	13	25	23	16	16	11	2	28	31	165	33.0 ± 3.11^{a}	46	2	48	9.6 ± 0.75^{a}
Spinosad	8 ppm	250	89	35.6	11	10	28	11	5	18	1	16	9	109	21.8 ± 2.76^{b}	16	1	17	3.4 ± 0.93^{b}
	16 ppm	250	100	40	8	0	13	8	5	27	1	71	19	152	30.4 ± 2.86^{a}	44	0	44	8.8 ± 0.86^{a}

Means having the same superscript letter(s) across each column are statistically insignificant (p> 0.05)

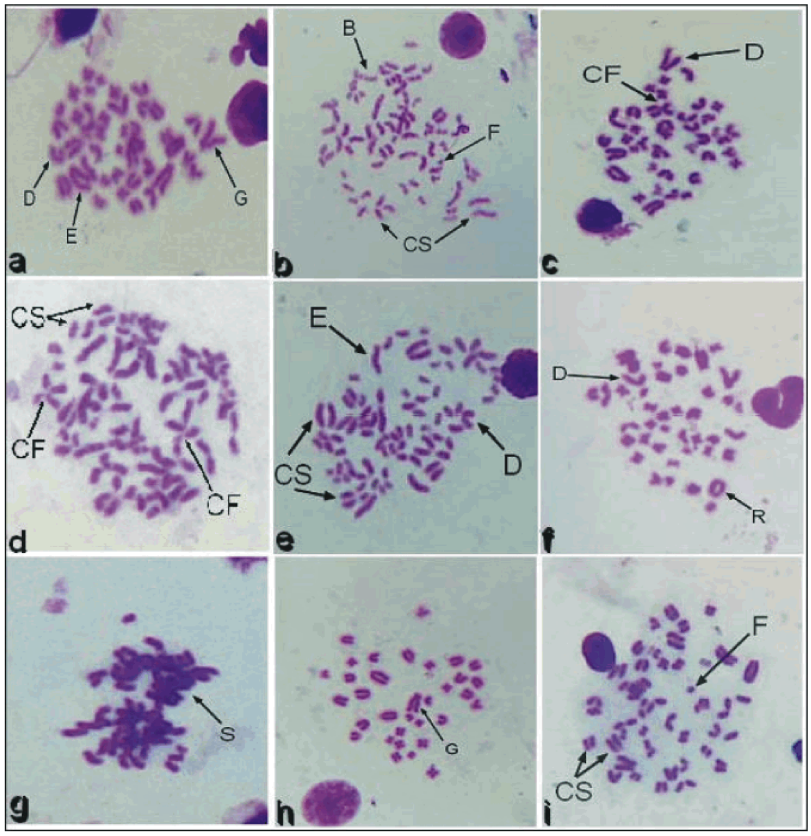

Fig. 1: Metaphase spread from rat bone marrow cells; after 90 days of feeding on wheat grains treated with malathion and spinosad, showing different types of structural and numerical chromosomal aberrations.

(a) Gap, deletion and end-to-end,
(b) Break, fragment and centric separation,
(c) Deletion and centric fusion,
(d) Centric fusion and centric separation,
(e) End-to-end, deletion and centric separation,
(f) Deletion and ring,
(g) Stickiness,
(h) 33 chromosomes with gap,
(i) 43 chromosomes with fragment and centric separation.

WBC's in the blood of the treated rats are generally in agreement with the results of several investigations. Yano *et al.* [37] reported that after rat exposure to spinosad, white blood cells count of females from the 0.1% group was 39% higher than the controls after 18 months, they also noted that this difference was likely related to inflammation of the lung and thyroid gland in these rats. Stebbins *et al.* [38] found that WBC's counts of male and female mice given 0.036% spinosad and females given 0.024% spinosad, were 2-2.5 times higher than the control after 12 months.

Chromosomal Abnormalities in Rat Bone-marrow Cells: Table (2) and Fig. (1) present the structural and numerical chromosomal aberrations that induced in bone marrow cells after feeding rats for 90 days on wheat grains treated with malathion or spinosad at the concentrations of 8 and 16 ppm for each. The structural chromosomal aberrations were found to include gaps, breaks, fragments, centric fusions, end-to-end associations, chromatid deletions, chromosomal rings, centric separations and stickiness. Moreover, numerical aberrations were shown in the form of hypoploidy and hyperploidy compared to the normal number of chromosomes (42 chromosomes).

Results revealed that the average number of chromosomal aberrations was found to be statistically significant in all malathion and spinosad treatments. It was accounted to 38 and 5 aberrations; with an average of 7.6 ± 0.75 and 1.0 ± 0.45 in control group for structural and numerical aberrations, respectively. The highest average number of structural aberrations (33.0 ± 3.11) was detected as a result of the exposure to the higher concentration of malathion. The induced aberrations by this concentration differed significantly from the control group (7.6 ± 0.75) as well as the lower concentration of spinosad (21.8 ± 2.76), while its effect was not significant as compared with the other concentration of malathion and the higher concentration of spinosad.

The same trend was observed for numerical aberrations, the highest increase was observed at 16 ppm malathion treatment which did not differ significantly from the other concentration of malathion and the higher concentration of spinosad. The numerical aberrations observed in bone marrow cells were hypoploidy. Hyperploidy were observed at a low frequency.

Although treatments with both malathion concentrations were not significantly different in the average number of structural aberrations, it could be observed that breaks, fragments, centric fusions, end-to-end associations and stickiness; at the high concentration, were higher than the low concentration. On the other hand, gaps, deletion, rings as well as centric separations revealed the highest values in the lower concentration.

After treatment with both spinosad concentrations, the average value of structural aberrations was significantly differ from control, this difference was higher for the higher concentration which did not differ significantly from both malathion concentrations. Meanwhile, the frequencies of deletions, centric separations and stickiness were highly elevated due to the treatment with 16 ppm spinosad as compared with the lower concentration (8 ppm). It could be observed that gaps, breaks, fragments and centric fusions, were the most frequently induced aberrations due to treatment with the lower concentration of spinosad and they were decreased in their frequencies following treatment with the higher concentration.

Thus, animals treated with either malathion or spinosad for 90 days showed a significant increase in the average number of aberrations compared with control group and this effect was higher in malation than spinosad for most recorded aberrations. This increase was in a concentration dependent manner for both structural and numerical aberrations. These results indicated that malathion and spinosad at both concentrations have side effects on structural and numerical aberrations. This might be due to that both insecticides contain clastogenic compound, which was capable to induce chromosomal aberrations in rat bone marrow cells. These results agree well with the earlier findings of Mansour *et al.* [45], who indicated that cytogenetical effects of malathion and spinosad were observed in a dose-dependent manner, while malathion exhibited more pronounced effect than spinosad. These results were supported by Amer *et al.* [46] and Giri *et al.* [47], who appeared that malathion induced a dose-dependent significant increase in the chromosomal aberrations in mouse bone marrow cells. In contrast, some studies showed that malathion produced no cytogenetical effects in a variety of test systems [48]. This is consistent with the conclusions of EPA [49], which recorded that although the results of some tests *in vitro* on malathion were positive, malathion was not genotoxic *in vivo*. The results which belong to the effect of spinosad and its side effects did not agree with EPA [50], who stated that spinosad has no mutagenic activity based on the results of some *in vivo* and *in vitro* genotoxicity tests. This suggest that cytogenetic activity of spinosad may refer to the spinosad's chemical structure and/or certain impurities in the commercial formulation [51].

Table 3: Effect of pesticides on DNA and total protein contents in control and treated rat's livers.

Treatments		DNA (µg/100 mg)	Total Protein (µg/100 mg)
Control		84.66 ± 0.78 [d]	272.17 ± 40.29 [a]
Malathion	8 ppm	133.60 ± 1.79 [b]	157.17 ± 8.37 [bc]
	16 ppm	152.73 ± 0.29 [a]	91.17 ± 14.68 [c]
Spinosad	8 ppm	87.00 ± 0.29 [d]	172.00 ± 23.18 [b]
	16 ppm	99.91 ± 0.53 [c]	163.50 ± 22.14 [bc]

Means having the same superscript letter(s) across each column are statistically insignificant (p> 0.05)

Effect of Pesticides on Biochemical Parameters in Liver
Effects on Dna and Total Protein Content: DNA content has been found to be increased in all treatments compared with control. It was increased as malation and spinosad concentrations increased (Table 3). The highest increase was recorded following the treatment with 16 ppm malathion. Non significant increase has been observed at the low concentration of spinosad compared to control, whereas the higher concentration was significantly increased. In general, both spinosad concentrations showed DNA contents lower than malathion concentrations. This suggested that this change in DNA content may be due to differential effects of pesticides or its metabolite(s) on synthesis of DNA in liver cells of the rats [52]. Thus, it can be concluded that the marked increase in the DNA content upon exposure to pesticides may be due to that most cells in tissue is driven into a proliferative phase with a resultant increase in the DNA content in the S-phase [53]. The biological consequences of increase in DNA content increase remains unexplored [54].

The results of total protein revealed a significant decrease in the total protein content (µg/100 mg tissue) at all insecticide treatments compared with the control treatment (272.17 ± 40.29). The highest significant decrease of 91.17 ± 14.68 was observed with the higher concentration of malathion, while the lower concentration of malathion and the higher concentration of spinosad showed similar decrease effect with 157.17 ± 8.37 and 163.50 ± 22.14, respectively. Protein content in organisms is known to respond to a wide variety of stresses [55]. However, decrease of the protein contents was a clear response to different pesticides. It was proposed that certain pesticides caused a significant reduction in protein level in plants [55]. In this regard, treatment of *Gambusia affinis* with malathion had induced a highly significant ($P < 0.01$) decrease of the protein contents of the liver, testis and ovary as elucidated by Hassanein [56]. Our results were in agreement with those of Hussain *et al.* [57], who reported that spinosad decreased the total protein contents in adult beetles. Reduction in the total protein may be due to its breakdown to amino acids and entering them to TCA cycle as a keto-acid for energy production [58].

Effects on Protein Banding Patterns: Comparing the treatments with its corresponding control (Fig. 2), several expressed protein bands were visualized after exposure to different concentrations of malation and spinosad. In addition to several up- and down-regulated proteins, new protein bands were appeared when compared to the corresponding control. SDS protein profile showed fourteen common bands in control and all treatments, although there were changes in bands intensity. The results showed that two sets of bands were detected with relative mobility's (R_f) of 0.11 and 0.43 in the control and all treatments, except in the 16 ppm malathion treatment. On the other hand, the band with R_f of 0.03 was detected in both low concentrations of malathion and spinosad, while it was absent in the control and the other treatments. It was also noticed that the bands with R_f of 0.23, 0.34, 0.68 and 0.86 were detected in lower malathion and both spinosad treatments, while it was absent in the control and the higher concentration of malathion. With regard to the band with R_f of 0.21, the results showed that it was only found in both spinosad treatments but was absent in the control and malathion treatments, this band can be considered as a potential marker associated with spinosad pesticide.

Also, it could be observed that the treatment with the higher malathion concentration decreased bands number (15 bands), this was constant with the results of total protein content which revealed the highest decrease compared with the other treatments. This agreed well with the results of Galal [59], who reported the appearance of a definite decrease in protein bands number as response to stress conditions. Whereas, there are four basic differences in terms of protein content under the stress conditions: 1. Production of some new proteins not present in the untreated group; 2. Inhibition of some proteins that are produced by the untreated group; 3. Increase in the level of expression of some proteins; 4. Decrease in the level of expression of some proteins that are present in the untreated group. All the four differences were directly associated with the response of rats to malathion and spinosad stress conditions in the present investigation. An obvious increase in the protein profiling pattern was observed which could be the result of 8 ppm malathion and both concentrations of spinosad stress.

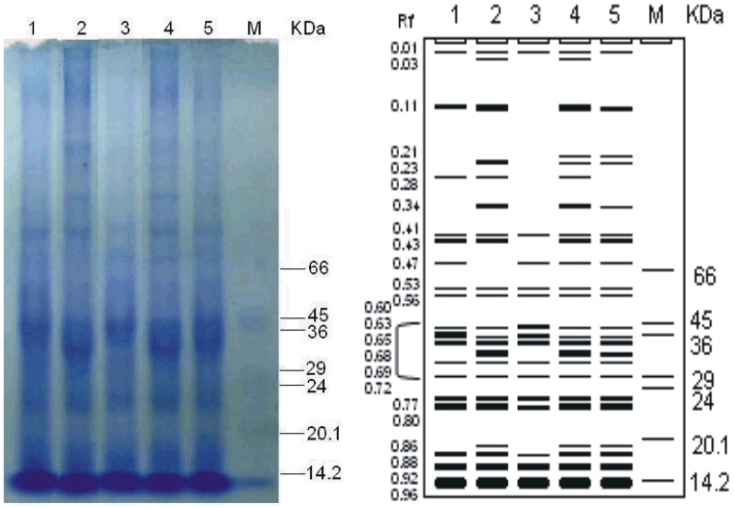

Fig. 2: Effects of pesticides on banding patterns of total proteins from rat's livers as analyzed on SDS-PAGE at the end of the experiment. Lane 1: control, Lane 2 & 3: malathion 8 & 16 ppm, respectively; Lane 4 & 5: spinosad 8 & 16 ppm, respectively, and lane M: represents the molecular weight marker.

These results tend to agreement with those of Rajendran *et al*. [60], who reported that the production of novel proteins or the increased production of already existing proteins are due to stress response. In other words, the appearance of extra bands due to the treatment with an insecticide indicates that resulting proteins are probably responsible for the detoxification of the insecticide [61]. In addition, it was noticed that, no differences between the lower concentration of malathion and the higher concentration of spinosad in bands number (22 bands). The alterations in the electrophoretic profiles of liver proteins are indicative of the ability of both malathion and spinosad to alter the gene expression in treated rats. Among the two tested pesticides, malathion expressed the most deleterious effect on the protein synthesis, remarkably inhibiting protein production in liver.

Disappearance of some bands in this study could be traced back to the induction of cytological abnormalities like deletion that lead to the loss of some of the genetic material. Therefore, some electrophoretic bands could have disappeared due to the deletion of their corresponding genes [62]. On the other hand, appearance of new characteristic bands could be explained on the basis of mutational event at the regulatory system of unexpected gene(s) that activate it [63].

Further, the obtained results indicate that the stress protein analysis is a promising alternative and more sensitive method for measuring toxic effects on the organisms at sublethal levels. Thus it can suggest that the proteomic profiling is a sensitive tool for environmental stress diagnosis and that the stressed proteins could be used as biomarkers for environmental pollution identification. As reported before by Duke *et al.* [64], biomarkers of exposure for specific pesticides or pesticide classes can be determined in part with gene expression profiling. Gene expression signatures for pesticides with unknown side effects have recently been suggested as a means of defining pesticide action and discovering pesticide alternatives.

Effects on Esterases Patterns: An attempt was made to study the changes on the level of esterase enzyme that are related to the reduction and detoxification of chemical toxins. Esterases are proteins that are defined by their ability to catalyze the hydrolysis of ester bonds within lipophilic compounds [65]. Because most insecticides are esters of substituted phosphoric, carbamic, or cyclopropanecarboxylic acids, they are subjected to degradation by esterases [66]. Therefore, esterases are the most significant enzymes for insecticide detoxification.

PAGE analysis of the esterase preparation showed a smear of bands suggesting the presence of numerous isozymes which are common with liver esterases [67]. A total number of ten bands was detected, since all treatments exhibited the same effects on esterase isozymes in the number of bands (Fig. 3). In addition, the same effect on the bands intensity was appeared which indicate that the effect of malathion and spinosad treatments was identical as well as control group, except

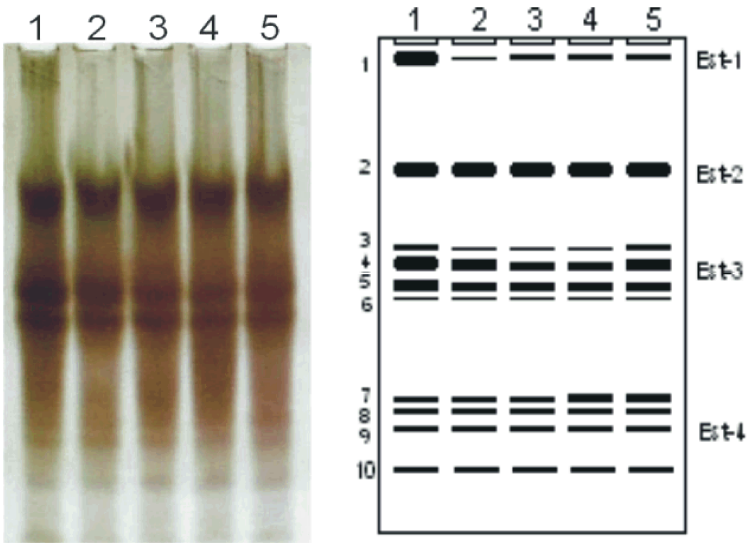

Fig. 3: Electrophoretic profiles of esterase (Est) isozymes from liver of rats at the end of the experiment. Lane 1: control, Lane 2 & 3: malathion 8 & 16 ppm, respectively; Lane 4 & 5: spinosad 8 & 16 ppm, respectively.

for bands No. 1, 3, 4 and 7 which appeared different intensity than control. Bands were assigned to four main zones of esterase isozymes (Est-1, Est-2, Est-3 and Est-4). The first and second zones (Est-1 and Est-2) were appeared as a single band. The band in Est-1 was decreased in its intensity in both malation and spinosad treatments as compared to control, especially for 8 ppm malathion which appeared very faint than the others. However, band No. 2 was extra darkly stained band in all treatments and control, this might be due to post-translational modification of the formed enzymatic polypeptide chain. The third zone (Est-3) involved bands No. 3, 4, 5 and 6. The treatment of 16 ppm spinosad was found as the control activity for band No. 3, while the rest treatments showed lower activity for this band. The weak staining ability could be due to the low expression of the corresponding band. Band No. 4 had the highest intensity for control (very dark), while it was noticed that the intensity of this band altered to dark as a result to the effects of 8 ppm malathion and 16 ppm spinosad treatments and appeared with lower activity as a results to 16 ppm malation and 8 ppm spinosad treatments. Band No. 5 showed lower activity than control for all treatments which altered from very dark to dark. The fourth zone (Est-4) appeared as having four sets of bands (No. 7, 8, 9 and 10). Band No. 7 was appeared in both spinosad treatments with higher intensity (dark) than control and malathion treatments (faint). The appearance of this band with high intensity could be due to the high gene expression of this isozyme, which might be considered as marker for spinosad pesticide. The three rest isozyme bands; No. 8, 9 and 10, having similar enzymatic activity in all treatments as will as control.

Changes in the band intensity could be interpreted as a result of certain mutational events that would have occurred in the regulator genes, which would lead to inhibition, attenuation or constitutive gene expression. Therefore, the corresponding bands become faint or more intense. The recorded changes in band intensity could also be attributed to the cytological abnormalities induced by malathion or spinosad treatments. This conclusion is in accordance with Asita and Makhalemele [68], who concluded that the increase in band intensity could be due to gene duplication.

Several experiments showed that malathion affects activity of esterases [69] suggesting that a metabolite of malathion (possibly the oxygen analog, malaoxon) was the actual inhibitor of malathion-esterase and that technical grade samples of malathion may contain a small quantity of this inhibitor. On the other hand, Shakoori and Saleem [70] revealed that there is general tendency towards enhanced enzyme activities after insecticide application which is perhaps due to increased concentration of enzymes following induction at the gene level. The endogenous level of various enzymes increased to meet the condition of stress developed by the insecticide toxicity. Hussain *et al.* [57] found that there was 40.27% decrease at a lower concentration (LC_{10}) and 21.16% increase at a higher concentration (LC_{20}) of spinosad, in the total esterases activity in PAK adults of *T. castaneum*,

while in FSS-II adults, both the concentration levels showed non-significant effect on the activity of the enzyme.

CONCLUSION

In conclusion, the presence of both commercial insecticides; malathion and spinosad residues in treated stored wheat grains seems to be toxic for haematological parameters in rats (especially RBC's, HGB and WBC's). Both insecticides showed an accumulative effect on the induction of chromosome damage and biochemical changes in male albino rats. However, the effect of malathion was much pronounced than spinosad. This may be due to the metabolic biotransformation of malathion to malaoxon or the presence of malaoxon and/or isomalathion. On the other hand, spinosad toxicity is consistent with altered phospholipid metabolism, resulting in cellular phospholipidosis, as well as other unspecified impurities and unidentified inert ingredients in the commercial formulation of this insecticide. This means that this agent may induce a malignancies in individuals exposed to it. Accordingly much more care should be taken in using these insecticides, particularly malathion, which is contained in most agricultural pesticides used in the developing countries. Therefore, these findings have to be taken in consideration when using malathion or spinosad as a grain protectant for storage. Our findings open the door to future studies examining the toxicological potential of spinosad in health.

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