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Research Article
Assessment of DNA Damage and Biochemical Responses in *Rhyzopertha dominica* Exposed to Some Plant Volatile Oils

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Abstract

Objective: This study was carried out to investigate the insecticidal activity of *Citrus aurantium* (Rutaceae), *Eruca sativa* (Brassicaceae), *Zingiber officinale* (Zingiberaceae) and *Origanum majorana* (Lamiaceae) volatile oils (EO) and organophosphate insecticide (Chlorpyrifos) against *R. dominica*. **Materials and Methods:** The wheat grains were treated by plant oil extracts and chlorpyrifos with different concentrations, then they were given as food for the insect pests to record mortality percentages and identify the LC_{25}, LC_{50} and LC_{90} for each one. Also, different enzymes, DNA concentration and DNA damage for treated insects were estimated. **Results:** The data was showed that the activity ratios of AChE, acid, alkaline phosphatase, lactate dehydrogenase and Phenol Oxidase (PO) ranged from 0.97-0.75, 1.08-0.71, 1.09-0.60, 1.39-0.57 and 1.37-0.67, respectively. The DNA tail (%), tail moment, olive-tail moment and tail length were all greater in case of treatment with chlorpyrifos compared with the control. **Conclusion:** The tested plant oils could play a significant role as safer insecticide than chemical one. Tested plant oils could be used as a practical tool of integrated pest management program as insecticides for the stored-grain insects.

Key words: *C. aurantium*, *E. sativa*, *Z. officinale*, *O. majorana*, enzymes activities, comet assay, DNA damage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The stored grain products pest *Rhyzopertha dominica* is a destructive pest[1]. Control of this weevil has been usually carried out through the use of conventional pesticides such as chlorpyrifos[2] but resistance to this insecticide has been reported and it affect genetic content[3-5]. Natural plants offer an alternative source of insect-control agents because they contains a wide range of bioactive secondary chemicals, many of which are selective and/or have little or no harmful effect on non-target organisms as well as environment[6]. Alternative methods for reducing infestation by *R. dominica* in store wheat grains include the use of botanical oils[7], plant oil extracts had been used in many studies in order to control *R. dominica*[8-10]. Also, botanical oils and their secondary metabolites have attracted attention as a very potential pest control agents, due to their insecticidal, repellent and/or anti-feedings properties[11-19].

On the other hand, it was found that the natural oils of the plant origin which are used in the insect pest control affect the enzymatic profiles[20]. Also, esterases (ESTs) are one of the major detoxifying enzymes in insects and at least one of them is involved in detoxification of insecticides[21]. Acid phosphatase (ACP) and alkaline phosphatase (ALP) hydrolysis phosphomonoesters under acid or alkaline conditions, respectively[22,23]. Alkaline phosphatase (ALP) is a brush border membrane marker enzyme which is active in tissues such as intestinal epithelial cells and malpighian tubules[24] and hemolymph[25]. Thus, decreased levels of ACP at higher concentrations of azadirachtin may be due to a reduced phosphorous liberation for energy metabolism, decreased rate of metabolism as well as decreased rate of transport of metabolites[23]. Lactate dehydrogenase may be involved in carbohydrate metabolism also it has been used as an indicative criterion of exposure to chemical stress or toxic materials[26,27]. Moreover, it was concluded that, the lactate dehydrogenase is expressed at high levels when the cells become distressed and damaged[28]. The AChE may be considered as a key enzyme that terminates nerve impulses which catalyze the hydrolysis of neurotransmitter and acetylcholine in the nervous system of various organisms[29].

Nathan *et al*.[30] demonstrated that the *Azadirachta indica* extract significantly inhibited the activity of AChE compared with the control. The ChE inhibition is considered as the biomarker of exposure to pesticides such as the organophosphates (OPs) compounds which are used worldwide for pest control[31,32]. The ChE can't hydrolyse the choline esters due to the effect OPs pesticides which destroy ChE activity by phosphorylating a specific serine within the ChE catalytic centre[31,33]. Phenol Oxidase (PO) enzymes that have tyrosinase-like activity can hydroxylate tyrosine (EC 1.14.18.1) and also can oxidize o-diphenols to quinones (EC 1.10.3.1) so called o-phenol-oxidases[34]. The quinones produced by PO due to polymerization and melanin synthesis in the final stages of nodulation and encapsulation against invading microorganisms, after undergoing to a series of enzymatic and non-enzymatic reactions[35]. Indeed, insect PO are synthesized as zymogens called pro PO which are activated by proteolytic cleavage at a specific site in response to infection or wounding[36].

Many factors affect the protein synthesis, in the time in which the protein synthesis is necessary for the body growth and reproduction[37]. The genotoxicity and carcinogenicity in the cell genome are caused by genotoxic agents some of the lethal or sub-lethal effects induced by some xenobiotic substances. Among recently used methods to identify DNA damage is the comet assay Single Cell Gel Electrophoresis (SCGE). The comet assay provides a rapid, sensitive and inexpensive method to detect DNA strand breaks in individual eukaryotic cells[38]. Despite of some difficulties in obtaining cell/nuclei suspension, this method has been used to detect and evaluate DNA damage caused by double strand breaks, single strand breaks, alkali labile sites, oxidative base damage and DNA cross-linking with DNA or protein. It has been successfully applied to cells of various animal groups[39]. Only a few studies have been reported on DNA damage in insects, including *R. dominica*[40].

The present study aimed to investigate the possible insecticidal activity of four essential oils (*Citrus aurantium*, *Eruca sativa*, *Zingiber officinale* and *Origanum Majorana*) and organophosphate insecticide (Chlorpyrifos) as well as some biochemical activity aspects, i.e., lactate dehydrogenase, phenol oxidase, acetylcholinesterase (AChE), alkaline phosphatases, acid phosphatase, total proteins, DNA concentration and DNA damage by comet assay (SCGE) in *R. dominica*.

MATERIALS AND METHODS

Plant material and oils extraction: Natural plants from different families were used, *Citrus aurantium* (Rutaceae), salad rocket *Eruca sativa* (Brassicaceae), ginger *Zingiber officinale* (Zingiberaceae) and marjoram *Origanum majorana* (Lamiaceae). These plants were collected during 2016 from Sinai region and identified at the herbarium of the Department of Biology, Umm Al-Qura University, Makkah, Saudi Arabia, then cleaned, dried, ground into a powder

form and kept in tightly closed containers under laboratory conditions. Plant samples were prepared according to clevenger[41]. The output oil extracts were stored in a dark and clean glass bottles at 4°C for the experimental trials.

Insecticide: Organophosphate insecticide; chlorpyrifos (C9H11Cl3NO3PS) which were used in this study obtained from local nurseries marketing.

Chemicals: The chemicals which are used in this study were obtained from Sigma-Aldrich company, except the following: DNA tag polymerase (Promega, USA), dNTPs (Boehringer Mannheim), DNA extraction reagents, agarose gel (Qiagen), oligonucleotides as random primers (Operon Tech., Inc., USA), DNA maker for agarose gel electrophoresis (Gibco BRL) and loading dye solution (Fermentas, Lithvuania).

Insect bioassay: In order to evaluate the toxicity of plant oils (*Citrus aurantium*, *Eruca sativa*, *Zingiber officinale* and *Origanum majorana*) as natural products and chlorpyrifos insecticide against *Rhyzopertha dominica*. Wheat grains mixed with serial dilutions of *C. aurantium E. sativa*, *Z. officinale* and *O. majorana* oils were 4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625% (w/w) and other serial dilutions of the recommended insecticide called chlorpyrifos was 0.1, 0.08, 0.06, 0.04 and 0.02% to record mortality percentages and identify the LC_{25}, LC_{50} and LC_{90} for each compound[42]. Three replicates (10 adults/replicate) of *R. dominica* adults for each concentration in this study, *R. dominica* adults were homogeneous in age and size. The control (untreated check) test was run in parallel with the serial concentrations.

Enzyme assay: Effect of essential oils and chlorpyrifos insecticide on the activities of enzymes [Lactate dehydrogenase (LDH), Phenol Oxidase (PO), acetylcholinesterase (AChE), alkaline phosphatase (ALP) and acid phosphatase (ACP)] also total proteins in *R. dominica* were measured after treatment with LC_{50} for each compound. The *R. dominica* were treated with LC_{50} of test essential oils for 48 h and preserved in refrigerator until analysis. However, the total proteins were determined by the method of Bradford[43]. Moreover, lactate dehydrogenase (LDH) determined and described according to the method which were recommended by the German Society for clinical chemistry[44]. Also, phenol oxidase activity was determined according to Ishaaya[45]. While, AChE (acetylcholinesterase) activity was measured according to the method described by Simpson *et al.*[46] using acetylcholine bromide (AChBr) as substrate. On the other hand, acid and alkaline phosphatase were measured according to the method described by Powell and Smith[47] using disodium phenyl phosphate as substrate.

DNA extraction and purification: The molecular experiments were carried out at the Department of Biology, Al-Jamom College, Umm Al-Qura University, Saudi Arabia. The genomic DNA was extracted from *R. dominica* using, purification of total DNA from insects using the DNeasy blood and tissue kit (Qiagen.com, Cat. No. 69504) according to QIAGEN's protocol.

DNA concentration: The DNA quantity was estimated by measuring the absorbance at 260 nm, adjusting the A_{260} measurement for turbidity (measured by absorbance at 320 nm), multiplying by the dilution factor and using the relationship that an A_{260} of 1.0 = 50 μg mL^{-1} pure dsDNA. Concentration (μg mL^{-1}) = (A_{260} reading–A_{320} reading) × dilution factor × 50 μg mL^{-1}. Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume. The DNA yield (μg) = DNA concentration × total sample volume (mL). The most common purity calculation is the ratio of the absorbance at 260 nm divided by the reading at 280 nm. The DNA which has an A_{260}/A_{280} ratio of 1.7-2.0 considered good-purity. The ratio can be calculated after correcting for turbidity (absorbance at 320 nm).

Single Cell Gel Electrophoresis (SCGE) assay: The DNA strand breakage of adult *R. dominica* treated with LC_{50} of oily extracts as well as the chemical insecticide was assessed using single-cell DNA comet assay. Single cell gel electrophoresis was used for the measurement of DNA damage as described by Singh *et al.*[48] with minor modifications.

Evaluation of DNA damage: The DNA damage was visualized with fluorochrome stain of DNA with the fluorescent microscope and a 40X objective (depending on the cell size). A Komet analysis system (Andor Technology Ltd.) linked to a CCD camera to measure the length of DNA migration Tail Length (TL) and the percentage of migrated DNA (DNA%). To distinguish between populations of cells differing in size nuclear diameter was measured and calculated tail moment. About 50-100 randomly selected cells are analyzed per sample (at least 25 cells per slid and 3 slide per treatment were evaluated).

Statistical analysis: Mortality percentages were corrected according to Abbott's formula[49]. The regression lines were potted and fitted on log dosage paper according to Finney[50]. On the other hand, the toxicity index and relative potency of the tested insecticides accounted according formula of Sun[51]. Data from all experiments were subjected to analysis of variance (ANOVA) and means separated using Turkey's test.

RESULTS AND DISCUSSION

Mortality and concentration-response in *R. dominica*: The data illustrated in Table 1 showed that the LC_{50} values of different concentrations of plant oils namely; *C. aurantium*, *E. sativa*, *Z. officinale* and *O. majorana*, also, the conventional insecticide (Chlorpyrifos) against the laboratory strain of *R. dominica* were 1.79, 1.31, 2.39, 3.22 and 0.47 (w/w) g kg^{-1} of wheat grain, respectively. These results clearly showed that the *E. sativa* was the most effective plant oil against the laboratory strain of *R. dominica*. While *O. majorana* oil was the least efficient one according to the LC_{50} values, the four phytochemical oils and the chemical insecticide could be descendingly arranged as follow: Chlorpyrifos, *E. sativa*, *C. aurantium*, *Z. officinale* and *O. majorana*. The susceptibility of the insects also was dependent on oil extract concentrations and decreased as the concentrations were increased (Fig. 1). As well as, the values of LC_{50} and LC_{90} for adult insects indicated the ability of those extracts to control of *R. dominica*. These results get along with these of Kiran and Prakash[8], who concluded that, essential oils and its major compound methyl salicylate showed 100% mortality at 150 µg mL^{-1} against *R. dominica* after exposure for 24 h.

Susceptibility index and relative potency levels: By comparing the toxicity of the tested materials with each other, it was found that, chlorpyrifos was considered as the standard toxicants and then was given the arbitrary index value of 100 U. When compared with the chlorpyrifos according to Sun's method, the susceptibility index of the other four phytochemical oils, *C. aurantium*, *E. sativa*, *Z. officinale* and *O. majorana* at LC_{50} level were 26.25, 35.87, 19.66 and 14.59%, respectively as toxic as the toxicity of chlorpyrifos against *R. dominica* (Table 1). Regarding, the susceptibility index values at LC_{50} level, it was found that chlorpyrifos was distinctly potent, while *E. sativa* and *C. aurantium* were intermediate, whereas *Z. officinale* and *O. majorana* were extremely the least effective compounds. It was clear that the rates of efficiency of the tested compounds on the basis of the toxicity index at LC_{50} level were similar to that of LC_{90} level (Table 1). Relative potency level can be used also as a conventional method to

Table 1: LC values, slope, relative potency and toxicity index of *Citrus aurantium*, *Eruca sativa*, *Zingiber officinale*, *Origanum majorana* and chlorpyrifos against *Rhyzopertha dominica*

Compound	LC_{50}	LC_{90}	Lower limit	Upper limit	Slope	Relative potency	Toxicity index
C. aurantium	1.79	9.10	1.51	2.1	1.817	1.79	26.25
E. sativa	1.31	5.13	1.11	1.51	2.162	2.45	35.87
Z. officinale	2.39	9.90	2.08	2.75	2.076	1.35	19.66
O. majorana	3.22	13.53	2.79	3.73	2.054	1.00	14.59
Chlorpyrifos	0.47	1.64	0.42	0.53	2.382	6.85	100.00

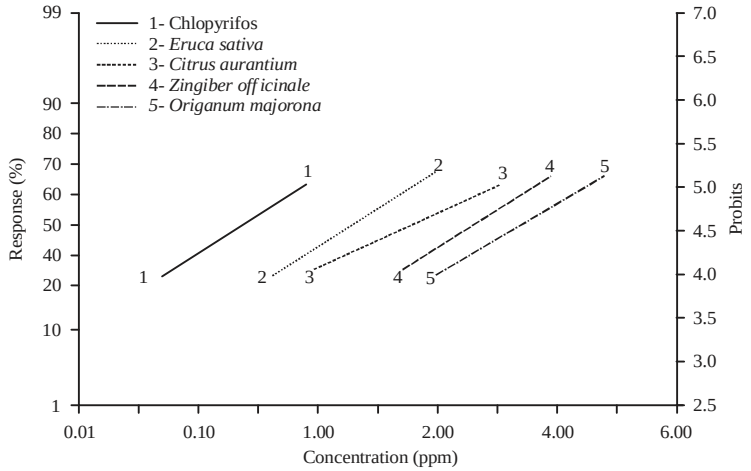

Fig. 1: Mortality of *Rhyzopertha dominica* after 48 h of treatment with the *Citrus aurantium*, *Eruca sativa*, *Zingiber officinale*, *Origanum majorana* and chlorpyrifos

Table 2: Biochemical assays of detoxification enzyme activities (Acetylcholinesterase, acid phosphatase, alkaline phosphatase) of *Rhyzopertha dominica* adults after 48 h of treatment with LC_{50} of essential oils and chlorpyrifos

	Enzyme activity after 48 h			Activity ratio*		
Compound name	AChE (µg AChBr min^{-1} g^{-1} b.wt.)	Acid phosphatase ($U\times10^3$ g^{-1} b.wt.)	Alkaline phosphatase ($U\times10^3$ g^{-1} b.wt.)	AChE	ACP	ALP
Control	363.6±18.5	585.3±11.9	827.6±17.6	-	-	-
C. aurantium	335.0±11.1[c]	417.3±16.5[d]	499.0±9.5[e]	0.92	0.71	0.60
E. sativa	272.6±11.0[e]	585.3±14.0[b]	533.6±14.8[d]	0.75	1.00	0.64
Z. officinale	297.6±5.8[d]	563.3±6.6[b]	802.0±16.6[b]	0.82	0.96	0.97
O. majorana	352.6±10.2[b]	484.6±15.0[c]	636.6±17.5[c]	0.97	0.83	0.78
Chlorpyrifos	344.3±4.9[bc]	635.0±15.8[a]	899.6±19.5[a]	0.94	1.08	1.09

Within columns, Mean±SD followed by a same letter do not differ significantly (Tukey's test, $p<0.05$), [a]Not significantly different ($p<0.05$), [b]Significantly different ($p<0.05$), [c]Highly significantly different ($p<0.01$), [d]Very highly significantly different ($p<0.01$), *Activity ratio: Enzyme activity in treated stages/Enzyme activity in control

make a comparison between the toxicity degrees of different toxicants to any pest. The potency levels of tested materials are expressed as the number of folds and compared with the least effective compound included in the evaluation against the tested insect.

Hence, the number of folds representing the potency levels was obtained by dividing the LC_{50} of *O. majorana* oil which were considered the standard compounds by the corresponding LC_{50} of each tested insecticide. The relative potency levels at LC_{50} values expressed as a number of folds as shown in Table 1, indicated that chlorpyrifos, *E. sativa*, *C. aurantium* and *Z. officinale* were 6.85, 2.45, 1.79 and 1.35 times more effective than *O. majorana* oil (3.22), respectively. It could be concluded that those results show the similarity and adequacy of the methods, i.e., toxicity index and relative potency levels in evaluating and comparing the efficacy of the toxicants against *R. dominica*. These results are going in line with those of Bajracharya *et al*.[2], Tripathi *et al*.[9] and Bekele and Hassanali[10].

Enzyme activities: The second part of the present study was carried out to explore the possible effects of different phytochemical insecticides on the enzymatic activities [Lactate dehydrogenase (LDH), Phenol Oxidase (PO), acetylcholinesterase (AChE), alkaline phosphatase (ALP) and acid phosphatase (ACP)] also total proteins and DNA concentration estimated on *R. dominica.*

Botanical insecticides and acetylcholinesterase: When comparing between activities of acetylcholinesterase in *R. dominica* after treatment with plant oils and chlorpyrifos it was found that, a significant elevation in AChE activity was observed in case of control. Whereas it was reduced descendingly in case of treatment with LC_{50} of *O. majorana*, chlorpyrifos, *C. aurantium*, *Z. officinale* and *E. sativa*, respectively. Also, the activity ratios of AChE ranged from 0.97-0.75 compared with the control (Table 2). These results coincided with Kennedy[31] and Gallo and Lawryk[33] who demonstrated that, OPs pesticides destroy ChE activity by phosphorylating a specific serine residue within the ChE catalytic center, under this condition the enzyme can't hydrolysis the choline esters. Hence, the inhibition of ChE activity considers the biomarker of exposure to pesticides such as the organophosphate (OP) compounds used worldwide for pest control[31,32]. Also, these results are going in line with that of Kiran and Prakash[8] who demonstrated that the plant essential oil caused inhibition of AChE activity which is ranged between 6.12 and 27.50%.

Botanical insecticides and detoxifying enzymes: On the other hand, in case of acid and alkaline phosphatase, it was significantly increased by chlorpyrifos, medium with *Z. officinale* and *O. majorana* and reduced by *C. aurantium*. Also, the activity ratios of acid and alkaline phosphatase were ranged from 1.08-0.71 and 1.09-0.60, respectively, compared with the control (Table 2). The obtained results of detoxification enzymes revealed were due to their mode of action, similar conclusion stated by Mates[52]. Also, it was found that, acid phosphatase (ACP) and alkaline phosphatase (ALP) hydrolysis phosphomonoesters under acid or alkaline conditions, respectively[22,23]. On the other hand, it was concluded that the ALP is actually involved in the reaction which called trans-phosphorylation[22], adenosine triphosphatases (ATPases) are essential for the transport of many organic materials in the cell that's why any impairment in their activity will affect the physiological operations in the insect gut[53]. Also, these results are in harmony with Terriere[54] who stated that these results indicated that this enzyme may play role in detoxifying tested compounds as a self-defense.

Botanical insecticides and lactate dehydrogenase and phenol oxidase: By comparing between activities of lactate dehydrogenase (LDH) and Phenol Oxidase (PO) in *R. dominica*

Table 3: Biochemical assays of lactate dehydrogenase, phenol oxidase, total proteins and DNA concentration of *R. dominica* adults after treatment with LC_{50} of essential oils and chlorpyrifos

	Enzyme activity after 48 h						
					Activity ratio*		
Compound name	Lactate dehydrogenase ($U \times 10^3$ g^{-1} b.wt.) (LDH)	Phenol oxidase (OD $U \times 10^3$ min^{-1} g^{-1} b.wt.) (PO)	Total proteins (mg g^{-1} b.wt.) (TP)	Con. of DNA (ng μL^{-1})	DH	PO	Total proteins
Control	242.6±20.2	3440.3±53.4	19.40±0.6	259.0	-	-	-
C. aurantium	337.0±15.1[a]	2302.0±28.05[e]	14.76±0.6[c]	111.6	1.39	0.67	0.76
E. sativa	138.0±8.185[d]	2732.6±38.5[d]	10.86±0.4[e]	25.5	0.57	0.79	0.56
Z. officinale	160.3±10.0[d]	3191.3±112.2[c]	15.30±0.3[c]	93.6	0.66	0.93	0.79
O. majorana	229.0±8.18[c]	4697.6±124.1[a]	17.46±0.4[b]	170.6	0.94	1.37	0.90
Chlorpyrifos	273.0±15.39[b]	3494.3±132.1[b]	14.02±0.4[c]	106.3	1.13	1.02	0.72

Within columns, Mean±SD followed by a same letter do not differ significantly (Tukey's test, $p<0.05$), [a]Not significantly different ($p<0.05$), [b]Significantly different ($p<0.05$), [c]Highly significantly different ($p<0.01$), [d]Very highly significantly different ($p<0.01$), *Activity ratio: Enzyme activity in treated stages/Enzyme activity in control

Table 4: DNA damage in *Rhyzopertha dominica* after treatment with LC_{50} of *Citrus aurantium*, *Eruca sativa*, *Zingiber officinale*, *Origanum majorana* and chlorpyrifos as reflected by Single Cell Gel Electrophoresis (SCGE) assay

	SCGE assay parameters			
Tested materials	DNA tail (%)	Tail moment (μm)	Olive-tail moment (μm)	Tail length (μm)
Control	08.36±0.80	01.82±0.46	02.10±0.52	16.22±3.69
C. aurantium	29.84±0.81[e]	40.21±0.88[c]	22.04±1.36[c]	67.24±1.11[c]
E. sativa	37.08±0.56[c]	46.56±1.32[b]	31.32±1.87[b]	86.47±0.28[b]
Z. officinale	32.33±1.40[d]	36.85±1.14[d]	12.89±2.65[e]	42.66±3.01[e]
O. Majorana	42.74±1.23[b]	21.25±0.67[e]	18.22±2.01[d]	56.11±0.45[d]
Chlorpyrifos	67.18±3.21[a]	69.42±1.57[a]	52.12±1.68[a]	110.52±3.65[a]

Within columns, Mean±SE followed by a same letter do not differ significantly (Tukey's test, $p<0.05$), [a]Not significantly different ($p<0.05$), [b]Significantly different ($p<0.05$), [c]Highly significantly different ($p<0.01$), [d]Very highly significantly different ($p<0.01$)

after treatment with LC_{50} of plant oils and chlorpyrifos it was found that, it was significantly increased by *C. aurantium* and *O. majorana*, respectively, medium with chlorpyrifos, while it was decreased with *E. sativa* and *Z. officinale*. The activity ratios of lactate dehydrogenase and Phenol Oxidase (PO) were ranged from 1.39-0.57 and 1.37-0.67, respectively, compared with the control (Table 3). The results revealed in this study indicated that the potential of these enzymes considered as an indicative criterion in *R. dominica* toxicity. These results are going in line with that of Brown *et al*.[28] who concluded that lactate dehydrogenase (LDH) is an enzyme who expressed at high levels when the cell exposed to stress and/or damaged. Also, lactate dehydrogenase used as an indicative criterion of exposure to chemical stress or toxic materials[26,27]. The LDH is a parameter which widely used in toxicology and in clinical chemistry in order to diagnose cell, tissue and organ damage[55]. Also as reported in the available study, LDH activity has been disturbed by plant extracts and insecticides[6,56] and the same in lepidopteran by hexaflumuron[57]. The current results of disturbed LDH activity in of *R. dominica* by the different extracts indicate that the toxic compounds contained in these extracts might be affecting the synthesis or functional levels of LDH, directly or indirectly by altering the cyto-morphology of the cells. Also, the induction or inhibition of the LDH activities as recorded in the present study might be on molecular levels, referred to depression or mutations of the regulating genes responsible for biosynthesis of polypeptide chains that building this enzyme[58,59].

Botanical insecticides and total protein levels: On the other hand, when comparing between the total protein levels in *R. dominica* after treatment by LC_{50} of plant oils and chlorpyrifos for 48 h, it was found that, the total protein was largely decreased with *E. sativa* (Very highly significantly different) also, it decreased after treatment with *C. aurantium, Z. officinale* and chlorpyrifos (Very significant different) and significantly decreased with *O. Majorana* compared with control. These results are in agreement with Shakoori and Saleem[60] who reported that the increasing protein content or fat body of some insect species may be due to the synthesis of proteinases needed for insecticide detoxification. Also, changes in protein content probably reflect the balance between synthesis and degradation of structural and functional nutrients during metamorphosis as well as a response to insecticide detoxification[6].

DNA damage assessment using comet assay: The results obtained in Table 4 and Fig. 2 showed the effects of LC_{50} of oily extracts of *C. aurantium*, *E. sativa*, *Z. officinale*, *O. majorana* and chlorpyrifos on genomic DNA (DNA damage) of *R. dominica* adults which were determined by

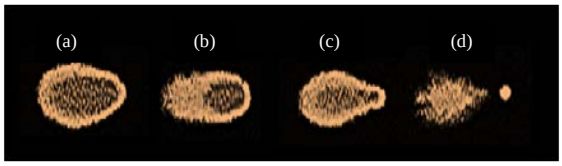

Fig. 2(a-d): DNA damage was quantified by visual classification of cells into four categories (0-3), (a) Corresponding to tail size with 0 representing no damage, (b) Representing low level damage, (c) Representing medium level damage and (d) Representing high level damage

comet assay. The oily extracts chlorpyrifos caused a significant increase in DNA damage as indicated by a greater migration of DNA fragments on agarose gel and can be arranged descendingly as following: Chlorpyrifos, *E. sativa*, *C. aurantium*, *Z. officinale* and *O. majorana* compared to control. The nuclei with a clear tail like extension which were observed indicated that the cells of the insect were damaged and DNA strands also were broken. The typical DNA comet for *R. dominica* showed rounded nuclei of control, while maximum length of tail formed and migration of DNA in this tail after treatment with different compound concentration, the DNA damage of *R. dominica* fed on wheat exposed to LC_{50} concentrations 1.79, 1.31, 2.39, 3.22 and 0.47 (w/w) g kg^{-1} of *Citrus aurantium*, *Eruca sativa*, *Zingiber officinale*, *Origanum majorana* and chlorpyrifos was analyzed quantitatively by comet assay and expressed as Tail Length (TL), DNA% and tail moment (Table 4). It was found that chlorpyrifos caused a very high significant increase in the values of TM. While, *E. sativa* caused a very significant increase in the values of TM.

On the other hand, *C. aurantium* caused significant values of TM followed by *Z. officinale* and *O. majorana*. Regarding Tail Length (TL), it was found that chlorpyrifos caused a very high significant increase followed by *E. sativa* which caused very significant increase in TL values followed by *C. aurantium* and *Z. officinale*, while *O. majorana* oil has the lower significant increase in TL values effect. Also in case of DNA% it was found that chlorpyrifos caused a very high significant increase in DNA% values, whereas *E. sativa* was caused very significant increase and a lower significance increase caused by *O. majorana*. These results are in same line with Al-Joubori *et al.*[61] who concluded that, treatment with mixture of plants extracts significantly reduced DNA fragmentation in both DNA extracted from WBC and renal cortex in all treatment groups.

Generally, the analysis of variance of the data revealed by comet assay showed that, the LC_{50} of the different compounds used in *R. dominica* control had a clear significant effect on the DNA damage (TL, DNA% and TM) compared with the control. All these results indicated a significant compound dependent effect in all test parameters of DNA damage assay; also it was found that the DNA damage was dependent on the insecticide which is used. These results are in agreement with those of Hasan *et al.*[40], Siddique *et al.*[62], Todoriki *et al.*[63] and Augustyniak *et al.*[64].

CONCLUSION

The present results showed a significant alterations in enzymatic system (acetylcholinesterase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and phenol oxidase), total protein and DNA concentration after treatment with LC_{50} of *C. aurantium*, *E. sativa*, *Z. officinale*, *O. majorana* and chlorpyrifos against *R. dominica* after 48 h, these alteration were varied according to the material were used and their mode of action. On the other hand, DNA damage assessment showed that the LC_{50} of the different compounds used in *R. dominica* control had a clear significant effect on the DNA damage (TL, DNA% and TM) compared with the control. All these results indicated a significant compound dependent effect in all test parameters of DNA damage assay, also it was found that the DNA damage was dependent on the insecticide which is used. Thus, we can depend on the plant oils as biocontrol agent integrated pest management program *R. dominica* in stored products.

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