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STUDY OF SOME MOLECULAR BIOMARKER (COMET ASSAY) IN ONE SPECIES SNAIL UNDER EFFECT THE PESTICIDES

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Abstract

In this study, the molecular biomarker (comet assay) was used as a technique in order to study the effect of the pesticides on some aquatic organism, since the exposure to the pesticides in the environment are very widely. (*Cerithium tenellum*) was selected as one species of snail in al-Hilla river, collected from al-Hilla river and exposed to three concentration from insect pesticides (0.001 ppm, 0.015ppm, 0.02ppm). After 24 hours, the observation was conduct for the selected concentration and it was found that the first concentration of the pesticide caused slow movement in species but the second and three concentration caused death of the species. During the reading of the (DNA) through technique (comet assay), depending on the comet length and tail moment, the first concentration the ratio damage of (DNA) was (36), while the second concentration (47) and third concentration was (92), these results refer to the first concentration which has a slight effect, while second and third concentration its effect was deadly. From the results, the main conclusion is that the concentrations (0.015 ppm, 0.02 ppm) highly recommend not to use in agriculture or aquatic environment, because of its effect on organism sand aquatic environment specially if the exposure was daily to the pesticides.

Keywords : Molecular biomarker (comet assay), *Cerithium tenellum*

Introduction

There are many groups of organisms such as fish, amphibians, invertebrates, plants or microorganisms in the fresh water represent as a community in it and pesticides can have harmful effects on these organisms. The effects caused by the pesticide are dangerous on the organism and the community described by interaction between all organism in environment such as competition or predation and indirect effects (Preston, 2002)

The comet assay has been used as an important tool for monitoring genotoxicity in aquatic environments (Lemos *et al.*, 2005) there are large amounts of pesticides application globally and given the fact that they are prepared to be harm to all organism, there is a high probability for counteractive environmental effects also on non-target pollutant (Oerke EC *et al,* 2004)

Among the tests for genotoxicity, the micronucleus test has been widely utilized in fish to determine exposure to water pollutants, in environment as well as under experimental laboratory conditions (Minissi *et al.*, 1996). Farmers are using a variety of pesticides indiscriminately and a sharp increase was observed during the last decades (Rahman, 2013). The application of pesticides may lead to contamination of the aquatic environment through several ways including: spray drift, runoff, and leaching (van den Brink *et al.*, 2009). A chemical and biological monitoring will be accomplished to assess the exposure concentrations and the ecological risks of these pesticide concentrations in the field using (Bollmohr *et al.*, 2009; Preston, 2002).

The aim of this study: To knowledge the harmful which it happens the pesticide in aquatic organism, and therefore aquatic environment.

Methods and Materials

1. Sampling Sites and Collection: Collected samples from species snail: **(***Cerithium tenellum)* from al hilla river and exposure to three concentration from insect pesticides in three baskets respectively (0.001 ppm, 0.015 ppm, 0.02 ppm).

Phylum	:	Mollusca,
Class	:	Gastropoda. (cuvier 1795),
Sup class	:	Gaenogastropoda.
Super family	:	cerithioidea,
Family	:	cerithiidae.
Genus	:	*Cerithium.*
species	:	C. *tenellium* (G.B. Souerbyll 1855)

Fig. 1: Snail (*Cerithium tenellum*)

2- Single Cell Gel Electrophsoresis:

By comet assay kit: OxiSelect™ Comet Assay Kilt (3-Well Slides, 75 Tests)

CELL BIOLABS, INC. STA-351. Using this kit by alkalin conditions order producer in the kit

Tissue Preparation: from body fluid (Mussel Hemolymph)

After ending all steps of kit, it possible determination

Tail length= length of tail measured by ocular micrometer (μm)

DNA Tail Moment (%) = the product of distance and normalized intensity integrated over the tail length, Σ(L**x**. %DNA**x**)

Comet Assay Index = (width of head / length of tail)

Classes of damage = according to comet assay index :1.2 – 2 (Low damage), 2.1-3 (Medium damage), up to 3 (High damage).

Fluorescence microscope determines the exact shape of the glow in the DNA, and determination comet length and tail moment according to comet assay program on computer.

3- Statistical Analysis

The significance of the differences was evaluated using the oneway ANOVA by comparing comet %, tail length and tail moment of samples.

Fig. 2: Three concentration of pesticides

Table 1: The criteria of DNA damage in the species *(Cerithium tenellum)* according to Comet Assay in the different concentration.

Concentration (3)			Concentration (2)			Concentration (1)			pesticides
350.8±105	Comet L.	Damage 92	2.70±121.8	Comet L.	Damage 47	1.20± 70.1	Comet L.	Damage 36	(*Cerithium tenellum*
46.2±40	Tail L µm		15.5±25.5	Tail Lµm		36.9± 22	Tail L µm.		
32.9±15.2	Mome nt%		36.8±17.4	Moment%		1.98±14.69	Moment %.		
756±2	Comet L.	Control 7	42.3± 2	comet L.	8	56.2±3	Comet L.	Control 9	
7.8±3	Tail Lµm		9 ±1	Tail Lµm		±30	Tail Lµm.		
1.2±2	Moment%		0	Moment%		0± 0	Moment%		

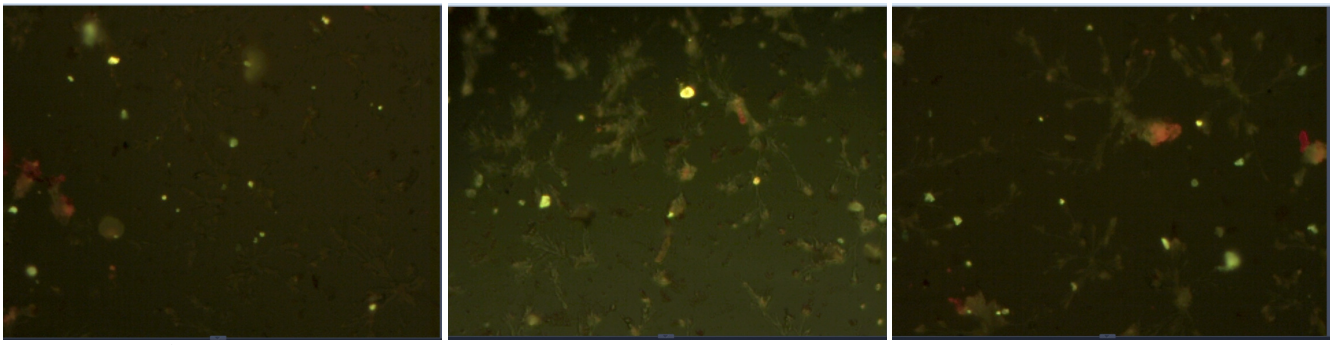

Fig.3 : The damage of DNA in the comet assay in snail (*Cerithium tenellium)* in (1) concentration

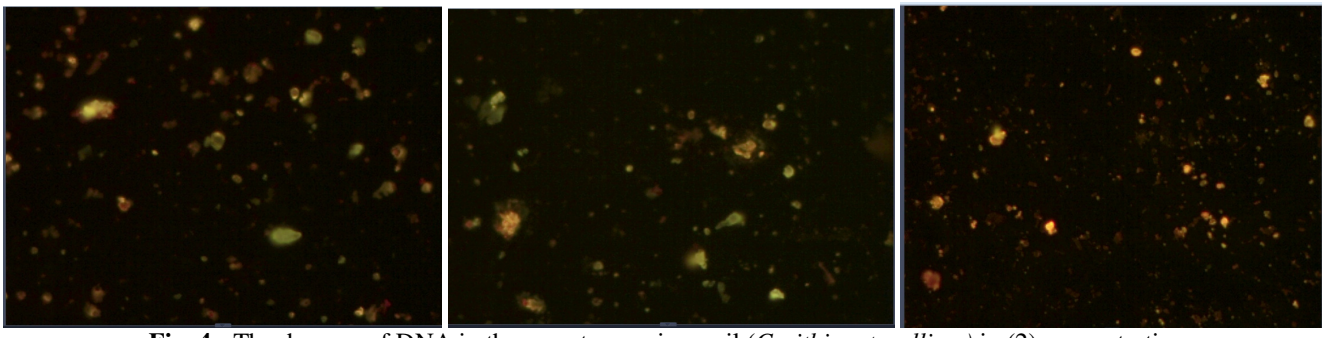

Fig. 4 : The damage of DNA in the comet assay in snail (*Cerithium tenellium)* in (2) concentration

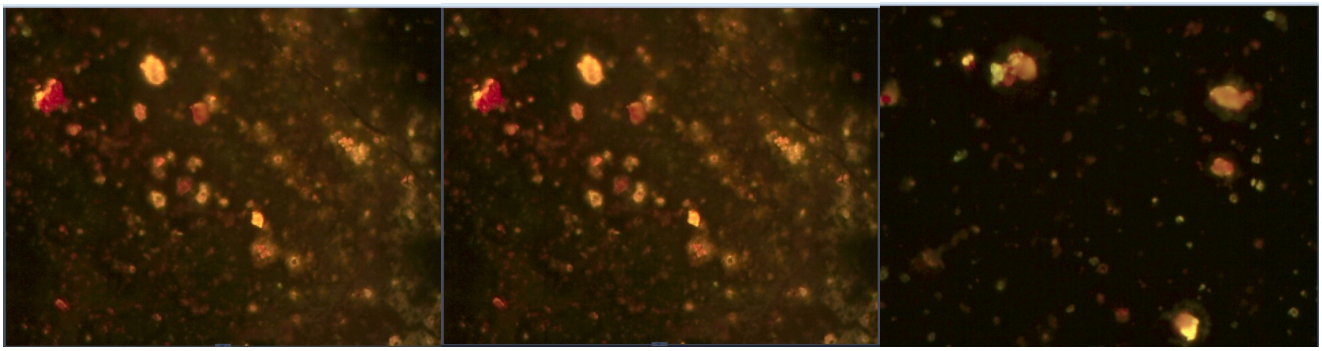

Fig. 5 : The damage of DNA in the comet assay in snail (*Cerithium tenellum)* in (3) concentration

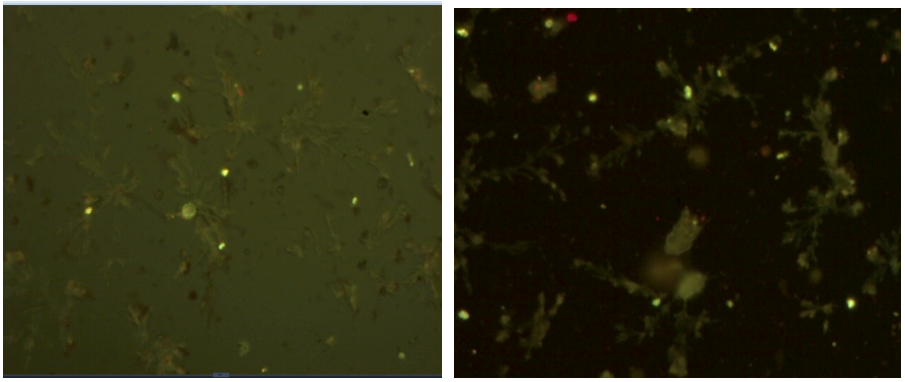

Fig. 6 : The of DNA in the normal cell (control) by comet assay in snail (*Cerithium tenellium)*

Discussion

In recent years, there has been an increasing interest in the effects of toxicants on aquatic organism due to the importance of this organism in rivers, ponds and estuaries, exposed to wastes of the productive activity. Chemical contamination of water may affect molecular (genetic alterations) that can be used as markers of DNA alterations in environmental pollution. (Jha, 2008).

The snail one from species that lives in fresh water and sensitivity to any change in characteristics of water, because chemical and physical pollutant or any toxins for example (pesticides), which can be genotoxicity (Anderson, 1989).

These results cnfirmed the pesticides the effect is obvious, in first concentration was ratio damage of DNA (36) : comet length (70.1), tail length (22.1µ m), but the snail in this concentration stayed alive, only movement slow. This refer to the dose of pesticides Non-lethal, the average dose refers to the amount of pesticide to which an animal is subjected (orally, dermally, or through inhalation). When the dose is low, but Its toxicity is higher chemical may be more harmful, and sometimes a large dose its toxicity is lower chemical. Dosages can be weighted as the weight of toxicant per unit (kilogram) of body weight (expressed as mg pesticide/kg of body weight) or as the focus of toxicant in the water or food supply (usually expressed as parts per million, ppm or parts per billion, ppb) (Johnson *et al.*, 1980). Many pollutants present in the aquatic environment not only endanger the survival and physiology of the organism's present.

It also induces genetic alterations which may lead to mutations and cancer (Russo *et al.*, 2004) Either in second concentration: the ratio damage of DNA (47), comet length (121.8), tail length (25.5µ m), and after period 24 hour, these snail died. this refer to the dose of pesticides was lethal.

The third concentration was more harmful concentrations because the ratio of damage very high of DNA (92), comet length (105.1), tail length (40 µ m), and after period 24 hour, these snails died. This refer to the dose of pesticides was lethal. A Killer dose is the quantity of pesticide required to cause death. Because not all organisms of a species die at the same amount (some are more tolerant than others), Perfect quantity a toxicity amount measurement, called a Lethal Concentration 50 (LC_{50}), is used. This is the amount of a pesticide that kills 50% of a test community of organism within a set period, usually 24 to 96 hours.

Dosage can be measured as the weight of toxicant per unit (kilogram) of body weight (expressed as mg pesticide/kg of body weight) or as the concentration of toxicant in the water or food supply (usually expressed as parts per million, ppm or parts per billion, ppb) (Rosenberg, 1975).

All these results to Indicate that all pesticides are chemicals contaminated with the aquatic environment, it has the effect of either a simple or deadly poison to living organisms. Where the effect reaches the level of deadly genetic contamination on another organism in the aquatic environmental.

The best concentration possible to use is less than (0.001 ppm), that it no any effect on another organism.

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