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Hematological, physiological and genotoxicological effects of Match 5% EC insecticide on *Biomphalaria alexandrina* snails

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ABSTRACT

Freshwater snails are used as brilliant biomarkers of aquatic ecosystem pollution by chemical compounds. The objective of this study is to highlight the ecotoxicological impacts of the insecticide Match 5%EC (its active ingredient is lufenuron 5% EC) on *Biomphalaria alexandrina* snails the intermediate host of *Schistosoma mansoni* in Egypt. The present investigation recorded a remarkable molluscicidal effect of lufenuron 5% EC on these snails and there was a decrease in total number of their hemocytes after exposure. Three morphologically distinct populations of circulating hemocytes were identified (round small cells, granulocytes and hyalinocytes) and results showed that some hyalinocytes had a shrunk nucleus and some were degenerated. Significant increase of transaminases (ALT and AST), while, a decrease of the total protein and albumin content in hemolymph was recorded. The results of alkaline comet assay in the present study demonstrated that lufenuron 5% EC has a genotoxic effect especially when its concentration increases. It can be concluded that *Biomphalaria alexandrina* snails can be used as bio monitor to screen the deleterious effects of lufenuron 5% EC insecticide as a cause of the environmental pollution, and this insecticide can be used in controlling schistosomiasis because of its molluscicidal effects on *B. alexandrina* snails.

1. Introduction

Pesticides have been used all over the world to help in reduction of pests (Lance et al., 2016). The use of these pesticide has increased due to industrial and intensive agricultural activities (Tataji and Kumar, 2016). They have toxicological impacts on natural ecosystems, especially aquatic systems (Moustafa et al., 2016). Lufenuron 5%EC is a benzoyl urea insect growth regulator and is used for the control of Lepidoptera and Coleoptera larvae. It prevents insects from molting by inhibiting the chitin synthesis in its cuticle (Fonseca et al., 2015). In water column of the sprayed ditch sections with Lufenuron 5%EC, the water dissipation time (50%) reaches about 2 days (López-Mancisidor et al., 2008; Brock et al., 2016), and its concentration reaches of 3 μg/L (Brock et al., 2010), while, its peak concentrations never surpasses 0.1–0.2 μg/L (López-Mancisidor et al., 2008).

Lufenuron 5%EC shows toxic impacts on crustaceans, fish, aquatic insects, nematodes, annelids and zooplankton, where, it negatively affects the biological parameters of *Colossoma macropomum* fish, a vertebrate animal, when exposed acutely to 0.7 and 0.9 mg/L (Soares et al., 2016). In environmental toxicology, the main research challenge is replacing mammals with invertebrates (Guilhermino et al., 2000). These Invertebrates are excellent bio indicators of environmental pollutants because they represent more than 90% of the aquatic species and can diminish the usage of higher vertebrates for bioethics reasons (Cheung and Lam, 1998). Freshwater snails are sensitive to environmental contaminations, so, they are good bio indicators of pollution of freshwater environments (Silva et al., 2017). *Biomphalaria alexandrina* snails are found throughout the Nile River basin and irrigation canals in Egypt (Fahmy et al., 2014). These snails beside being a major food source for many vertebrate and invertebrate predators (Dillon, 2000), are also, obligate intermediate hosts for many trematodes that cause diseases in humans like schistosomiasis (Le Clec'h et al., 2016). In this way, the present study was designed to evaluate the ecotoxicological mechanisms of lufenuron in the freshwater snail *B. alexandrina* as bio indicator of environmental pollution and study how it affects its biological systems.

Snails have their own immune system, which consists of both cellular and humoral components (Larson et al., 2014; Le Clec'h et al., 2016). Hemocytes are the main line of cellular defense (Larson et al., 2014), where, they participate in several internal defense-related activities (Fried, 2016) and release soluble compounds including agglutinins and antimicrobial peptides (Mitta et al., 2000). Examination of

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hemocytes monolayers revealed the presence of three morphologically different cell types, round small (undifferentiated), hyalinocytes and granulocytes (Mohamed, 2011). These granulocytes are the most dominant, more responsive and highly phagocytic type, while, hyalinocytes are responsible for wound repair (Barçante et al., 2012). The immunological responses and molecular aspects in *B. alexandrina* snails have been proposed as biomarkers of exposure to environmental pollutants (Mohamed, 2011). The aminotransferase enzymes are essential group of enzymes in the gluconeogenesis pathway, they have an important role in the linking of the amino acids and carbohydrate metabolism (Hamed et al., 2012) and are specific indicators of hepatocellular damage (Awad et al., 2014). Proteins and albumin are responsible for hemolymph osmotic balance, which regulates water distribution in intravascular compartments (Fahmy et al., 2014). Also, albumin level and albumin/globulin (A/G) ratio are indicators of the condition of parenchyma (Mohamed et al., 2012).

The genetic material is damaged by many environmental agents, which lead to up-regulation of several genes involved in different repair pathways (Silva et al., 2007). Comet assay is a sensitive method for direct visualization of DNA damage on the level of a single cell (Azqueta et al., 2009). DNA strand breaks are measured by various methods (e.g. DNA tail moments, percentage of DNA in tail… etc.), and they are increased after exposure to well-known DNA damaging chemicals compared with controls (Ye et al., 2012). Some recent studies link DNA strand breaks in aquatic animals to effects on the immune system, reproduction, growth, and population dynamics (Lee and Steinert, 2003; Sharaf El-Din et al., 2016). In Egypt, lufenuron 5%EC is used in agriculture under trade name Match 5% EC. So, in the current study, residual/ environmental concentrations of this insecticide were evaluated in order to investigate its effects on hematological, biochemical and molecular parameters of *B. alexandrina* snails, the intermediate host of *S. mansoni*.

2. Materials and methods

The experiments were carried out according to the National Institute of Health Guide for Care and Use of Laboratory Animals (The authors declare that no experiments were performed on humans).

2.1. Experimental animals (snails)

Biomphalaria alexandrina snails (8–10 mm) provided from Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt were used. Snails were kept in plastic aquaria (16 × 23 × 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/L) and covered with glass plates. They were maintained in air conditioned room at 25 °C and fluorescent light was reflected 30 cm over them during day time. Oven dried lettuce leaves and blue green algae (*Nostoc muscorum*) were used for feeding and water in the aquaria was changed weekly. Pieces of polyethylene sheets were put into the aquaria to collect egg masses.

2.2. Synthetic insecticide

Match 5% EC: Lufenuron 5% EC insecticide (Registration No., 609) was purchased from Syngenta (AGRO-Egypt).

2.3. Bioassay tests

2.3.1. Molluscicidal screening

A stock solution of 1000 ppm was prepared from the insecticide on the basis of V/V using dechlorinated tap water. To calculate LC_{50} and LC_{90}, Five serial dilutions were prepared (0.5, 1, 2, 3, and 4 mg/l). The dilution was carried out by using dechlorinated water and ten snails were incubated for each concentration (WHO, 1983). Another snail group of the same size was dipped in dechlorinated water only as control. Three replicates were used, each of 10 snails (6–8 mm), for each concentration. The exposure period was 24 h then, the snails were removed from the experimental test solution, and washed thoroughly with dechlorinated tap water, and recovery period was 24 h, this was done for two weeks and every week, a fresh stock solution was prepared. The percentages of observed mortalities were recorded.

2.3.1.1. Effect of sublethal concentration on hemocytes. To collect the hemolymph, a small portion of the snail shell which situated directly above the heart was removed and a capillary tube was inserted into the heart according to Nduku and Harrison (1980).

a. Total hemocytes count: The number of hemocytes was counted using a Bürker- Turk hemocytometer (der Knaap et al., 1981) by using 10 µl of hemolymph of each group.
b. Hemocytes differentiation: Hemocytes monolayers were prepared by placing 10 µl of hemolymph on a glass slide and were allowed to adhere the glass surface for 15 min at room temperature. Hemocytes were fixed with absolute methanol for 5 min and then stained with 10% Giemsa stain (Aldrich) for 20 min (Abdul-Salam and Michelson, 1980). Then examined under microscope.
c. Biochemical assays: Activities of aspartate and alanine aminotransferase (AST; ALT) and in snail's hemolymph were determined using the (Reitman and Frankel, 1957) technique. Total protein was determined according to Doumas (1975). Albumin was determined according to Gustafsson (1976). Calculation of globulins was determined by subtracting the amount of albumin from the total protein (Rawi et al., 1995).

2.3.2. Comet assay

Snails (8–10 mm) were subjected to LC_0, LC_{10} and LC_{25} of Match 5% EC for 48 h and then DNA damage was measured by single cell gel assay (Singh et al., 1988) and (Grazeffe et al., 2008) as follows:

Cell suspensions are harvested by centrifugation and re-suspend cells at 1 × 105 cells/ml in ice cold 1X PBS (phosphate buffer saline). This suspension was stirred for 5 min and filtered, then, 100 µl of this cell suspension was mixed with 600 µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE (Tris/Borate/EDTA)), pH 8.4, containing 2.5% SDS (sodium dodecyl sulphate) for 15 min. The slides were placed in the electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA, staining with ethidium bromide 20 µg/ml at 4 °C. The DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope at 510 nm. The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40× increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-stained (Ethidium Bromide Staining) DNA using a 40x objective on a fluorescent microscope.

3. Statistical analysis

Lethal concentration values were defined by Probit analysis (Finney, 1971) and analysis of data was carried out by Student's *t*-test for comparing the means of experimental and control groups (Murray, 1981).Values were expressed as mean ± S.D., and the obtained data were analyzed using the Graph Pad Prism 6.04 software for Windows (Graph Pad Software, San Diego, California, U.S.A.; 1992–2014).

4. Results

The molluscicidal activity of the tested insecticide Lufenuron 5%EC (Match 5%EC) against adult *B. alexandrina* snails after 24 h of exposure followed by another 24 h for recovery is recorded as LC_{50} 2.04 mg/l and LC_{90} 3.33 mg/l.