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Influence of Atrazine and Roundup pesticides on biochemical and molecular aspects of *Biomphalaria alexandrina* snails

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ABSTRACT

The excessive use of pesticides in agriculture has sparkled the interest of scientists in investigating the harmful effects of these compounds. The present study evaluates the pesticides Atrazine and Roundup (glyphosate) on biochemical and molecular aspects of *Biomphalaria alexandrina* snails. The results showed that LC_{10} of these two pesticides caused considerable reduction in survival rates and egg production of treated snails. Additionally, Atrazine proved to be more toxic to *B. alexandrina* snails than Roundup. In treated snails, glucose concentration (GL) in the hemolymph as well as lactate (LT) and free amino acid (FAA) in soft tissues of treated snails increased while glycogen (GN), pyruvate (PV), total protein (TP), nucleic acids (DNA and RNA) levels in snail's tissues decreased. The activities of glycogen phosphorylase (GP), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), succinic dehydrogenase (SDH), acetylcholinesterase (AChE), lactic dehydrogenase (LDH) and phosphatases (ACP and ALP) enzymes in homogenate of snail's tissues were reduced in response to the treatment with the two pesticides while lipid peroxide (LP) and transaminases (GOT and GPT) activity increased ($P < 0.001$). The changes in the number, position and intensity of DNA bands induced by pesticides may be attributed to the fact that pesticide can induce genotoxicity through DNA damage. It was concluded that the pollution of the aquatic environment by Atrazine and Roundup pesticides, would adversely affect the metabolism of the *B. alexandrina* snails, and have adverse effects on its reproduction.

1. Introduction

The use of pesticides may contaminate the irrigation and drainage systems during agriculture activities and pests' control, and then negatively affect the biotic and abiotic components of the polluted water course [1,2]. Some pesticides are highly persistent in the environment, and can affect and contaminate both aquatic and terrestrial species [3,4].

Atrazine is one of the most widely used pesticides for the control of both grasses and weeds in many crops and in non-agricultural situations such as on railways, highways and industrial sites. Due to high runoff potential, Atrazine is the pesticide most frequently detected in aquatic ecosystems and its concentration can exceed the general quality standard for surface water [5]. However, due to its persistence in aquatic environments, Atrazine may occasionally cause sublethal effects in various aquatic organisms [5]. Furthermore, its physical and chemical properties allow its accumulation in the phytoplanktons, invertebrates and fish species. This process may induce chronic toxic effects to the aquatic species [6]. Acute and chronic toxicity of Atrazine in freshwater invertebrates is well documented [7].

Roundup is a commercial pesticide with active compound glyphosate used in a broad spectrum in agricultural applications for weed control [8]. Due to its high water solubility and extensive usage, especially in shallow water systems, the exposure of non-target aquatic organisms to this pesticide is a concern [9]. It may be exceptionally dangerous for aquatic ecosystems through high water solubility [10].

Previous studies showed that glyphosate may affect plants, fishes, amphibians, arthropods and snails by causing physiological, immunological and biochemical alterations [11,12]. Certain pesticides persist as residues in the environment for only a few days, there may be a cumulative effect on aquatic organisms because the electrophonic nature of some pesticide that affects the various enzymes responsible for normal metabolic processes [13].

The aim of this study was to determine the effects of the pesticides, Atrazine and Roundup (glyphosate) on the biochemical and molecular aspects of *Biomphalaria alexandrina* snails.

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2. Materials and methods

2.1. Snails

Laboratory bred *B. alexandrina* snails (3–12 mm) from Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), were used.

2.2. Pesticides

2.2.1. Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 97.8% purity) was obtained from Cluzeau Info Labo (Ste Foy La Grande, France) (Fig. 1). With molecular formula $C_8H_{14}ClN_5$, molecular weight of 215.68 g/mol, melting point 173–175 °C, boiling point 200 °C, 473 K, 392 °F and density 1.187 g/cm^3. Solubility in water (33 ppm) (Fig. 1).

2.2.2. Roundup

Roundup (glyphosate concentration 120 g/l in the form of glyphosateisopropylamine salt 162 g/l). Roundup herbicide was used in the liquid commercial form was supplied by Monsanto Company (St. Louis, MO, USA). With molecular formula $C_6H_{17}N_2O_5P$, molecular weight of 228.183 g/mol, melting point 200 °C and density 1.218 g/cm^3 (Fig. 1).

2.3. Bioassays tests

2.3.1. Molluscicidal screening

The efficiency of the pesticides (Atrazine and Roundup) against adult snails (6–12 mm) was determined according to WHO [14]. A stock solution (1000 ppm) was prepared using dechlorinated water on the basis of concentration/volume and a series of concentrations was prepared from each experimental pesticide that would permit the computation of LC_{10}, LC_{50}, and LC_{90}. Exposure and recovery periods were 24 h each [15,16]. Mortality rates were recorded and SPSS was used to computer program under windows.

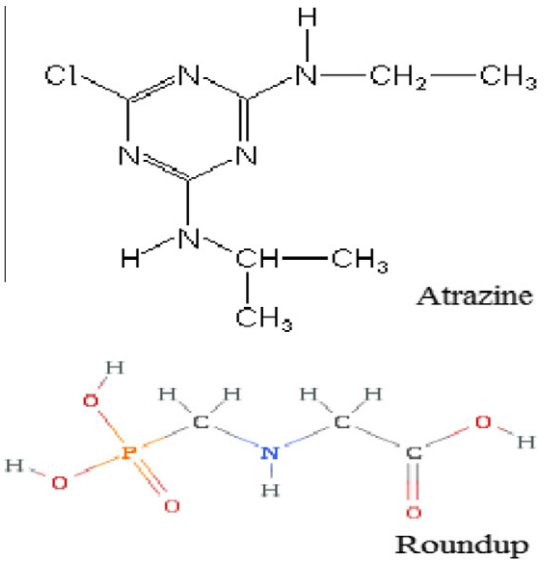

Fig. 1. Chemical structure of pesticides Atrazine and Roundup.

2.4. Effect of exposure to LC_{10} from the two pesticides on mortality rate, egg normality and egg hatchability of snails

For studying the effect of exposure to LC_{10} for Atrazine and Roundup on mortality rate, egg normality and egg hatchability for 6 weeks, 150 healthy mature snails (8–12 mm diameter) were randomly divided into 3 groups (each of 50 snails). The 1st group was continuously exposed to LC_{10} of Atrazine and 2nd group was exposed to LC_{10} of Roundup. The 3rd group was left unexposed under the same laboratory conditions as control. Pesticide solutions were prepared every 24 h to avoid the effect of storage. Dead snails are removed daily from aquaria and the mortality rate was calculated. The containers of treated and untreated snails were provided by thin plastic sheets for egg deposition. In addition to lettuce, tetramine (fish food) was added twice weekly. The egg clutches were weekly collected and examined microscopically every week. Egg masses with deformed or dead embryos, masses containing different developmental stages, empty egg case or more than one embryo in one egg were considered as abnormal [17]. The egg clutches were transferred into container containing dechlorinated water and the control ones. The percentage of egg hatching and hatchability were recorded every week.

2.5. Effects of LC_{10} from the two pesticides on snails' biochemical parameters

Snails were randomly divided into three groups (50 snails each). The 1st and 2nd groups were continuously exposed to LC_{10} of Atrazine and Roundup pesticides for 4 weeks. The third group of snails was left unexposed under the same laboratory conditions as control. Surviving snails were subjected to withdrawal of their hemolymph. Hemolymph samples were collected [18] by removing a small portion of the shell and inserting a capillary tube into the heart.

The hemolymph pooled from 10 snails was collected in a vial tube (1.5 ml) and kept in ice-box. For preparation of tissue homogenates of both exposed and unexposed snails, 1 g of snail's soft tissues from each group was homogenized in 5 ml distilled water at pH 7.5. A glass homogenizer was used and the homogenate was centrifuged for 10 min at 3000 rpm, then the fresh supernatant was used. All physiological parameters were determined spectrophotometrically using kits purchased from BioMerieux Company, France.

2.5.1. Assay methods

Glucose concentration (GL) (mg/ml hemolymph) in snails' hemolymph was measured according to Trinder [19]. Lactate (LT) was estimated according to Barker and Summerson [20] as modified by Huckabee [21]. Lactate content was expressed as mg lactic acid/g of tissue. Total protein level (TP) was estimated according to the folin–phenol method of Lowry et al. [22], total protein content was expressed as µg protein/mg of tissue. Glycogen (GN) was measured according to Van der Vies [23]. Glycogen content was expressed as mg glycogen/g of tissue. The pyruvate level (PV) was measured according to Friedemann and Haugen [24]. The pyruvate content was expressed as µmol of pyruvate/g of tissue. Total free amino acid level (FAA) was measured according to Spies [25]. The homogenate (50 mg/ml, w/v) was prepared in 96%. Total free amino acid content was expressed as lg/mg of tissue. Nucleic acids (DNA and RNA) were estimated according to Schneider [26], using diphenylamine and ordinal reagents, respectively. Nucleic acids content was expressed as µg/mg of tissue.

Glycogen phosphorylase (GP) [27], glucose-6-phosphatase (G-6-Pase) [28], superoxide dismutase (SOD) [29], catalase (CAT) [30], glutathione reductase (GR) [31] lipid peroxides (LP) [32], lactic dehydrogenase (LDH) [33], succinic dehydrogenase (SDH) [34],