



Effect of Endosulfan Pesticide on the Oxygen Consumption Rate of Three Species of Snails Collected from Middle Part of Shatt Al-Arab River

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Abstract: The present study has been achieved to estimate the acute toxicity effect of pesticide (endosulfan) (organochlorine) and its sublethal concentrations effect on the oxygen consuming rate of three various snails species (*Melanoides tuberculata*, *Radix auricularia* and *Theodoxus jordani*) collected from Shatt Al-Arab river along the region extended from Abu-Al-Khasib to Garmat-Ali during 2018. The 24 hr LC₅₀ indicated that the *M. tuberculata* was more resistant, while *R. auricularia* and *T. jordani* were more effective to endosulfan and showed no difference. The study indicated that the employ of various species of snails, with their differing degree of sensitivity to the same toxicant, might be a useful tool in aquatic environmental toxicological investigations. The sublethal concentrations exposure (24 hr LC₅₀) of pesticide had no considerable influence on the rates of oxygen consumption for each snail species. However, a decrease in the capability of snails to preserve respiratory independence through hypoxia was observed 24 hr after exposure to this dose.

Keywords: Pesticide, Snails, Shatt Al-Arab river, Acute toxicity, LC₅₀

Introduction

Important data exists about the sensibility of aquatic organisms like fishes, crustaceans, and mollusks toward pesticides and their impacts on various physiological factors from around the world (Hose & Van den Brink, 2004; Schafer *et al.*, 2007; Archambault & Cope, 2016; Finnegan *et al.*, 2017). However, comparatively few data have been found on the bioassays accomplished with Shatt Al-Arab river species in general and on snails in

particular. Many species of snails are found in the Shatt Al-Arab river, and due to the bio-physiological differences among these species (Farid, 2007), different snail species are likely to have different sensitivities to toxic substances.

Respiration is an important phenomenon of life and the rate of oxygen consumption reflects the internal metabolic activities of animals (Jadhav *et al.*, 2012). In aquatic

animals, respiration plays a major role in controlling the conversion of energy. Therefore, the metabolic responses of organisms due to changes in the surrounding environment refer to the adaptive capacity of the organism (Zimmer *et al.*, 2014; Alexis *et al.*, 2017). The increase in population leads to pollution of the environment that reduces the oxygen available in the water media. Agricultural, industrial and domestic effluents lead to degradation of aquatic organisms (Mateo-Sagasta & Burke, 2010; Bhuiyan *et al.*, 2013; Ahmed *et al.*, 2015). Environmental pollution from pesticides is an important issue that attracts wide public attention. Among them, organophosphate and organochlorine pesticides are routinely used in agriculture (Schreinemachers & Tipraqsa, 2012). It has been shown that respiration in the presence of the pesticide through gills will be rapid in aquatic animals at high temperature, because the demand for oxygen increases during low solubility (Mane *et al.*, 2012). Several studies have been conducted on this subject (Kamble & Shinde, 2012; Mane *et al.*, 2012; Burrige, 2013; Xu & Liu, 2014). Respiration is a vital process in which organisms obtained and used energy through oxidative metabolism. Aquatic animals have to pass large quantities of water over their respiratory surface when they are relatively exposed to high risk of toxic substances (Burrige, 2013). Considering this point of view, the aim of current study was conducted; (i) to investigate whether different species of Shatt Al-Arab river snails could exhibit differences in sensitiveness to organochlorine pesticide (endosulfan). (ii) to determine the impact of endosulfan sublethal concentration on the snails oxygen consumption.

Materials and Methods

Adult and uniform size snails, *Melanoides tuberculata* (Müller), *Theodoxus jordani* (Soweby) and *Radix auricularia* (Linneus) were collected from middle part of Shatt Al-Arab river along the region extended from Abu-Al-Khasib to Garmat-Ali during 2018 for bioassays. Specimens were relocated to the fish laboratory of Veterinary Medicine College, University of Basrah at $20 \pm 1^\circ\text{C}$ under aerated conditions and 12 hr:12 hr light/dark cycle. The ventilation was stopped after acclimation for 24 hr.

Natural river water collected from the Shatt Al-Arab river was utilized to prepare dilutions in the biological experiments, where the water was filtered (through $0.45 \mu\text{m}$ Whatman sterile membrane filter) and boiled (to kill the microorganisms) prior to use. The quality parameters of the control/dilution river water fixed in the experiments during the test period are $6.8\text{-}6.9 \text{ mg.l}^{-1}$ (dissolved oxygen), $7.5\text{-}7.9$ (pH), $23.3\text{-}27.5^\circ\text{C}$ (temperature), $5.1\text{-}5.3\%$ (salinity), and $1155\text{-}1187 \mu\text{mos cm}^{-1}$ (conductivity).

The mortality rate was determined within 24 hr of exposure to endosulfan using the method developed by the USEPA (2002) with some modifications (in size of containers, types of organisms and pesticide). Glass containers ($10 \times 7 \times 20 \text{ cm}^3$) were utilized for tests. Endosulfan stock solution (95% purity, Hindustan Insecticides Limited, India) was prepared using acetone as a solvent and a dilution agent to make up a stock solution of endosulfan concentration at 40 g L^{-1} . The stock solution is placed in chiller at 4°C . After initial testing, the concentration range from $0\text{-}20 \text{ mg.l}^{-1}$ was chosen for toxicity tests. In volumetric flasks, different concentrations (2, 5, 10, 15, and 20 mg.l^{-1}) of pesticide were prepared utilizing natural river water and

suitable amounts of stock solution. Each test was achieved with five different concentrations of endosulfan as well as a control consisting the same acetone quantity such as in the highest pesticide concentration used. Each glass container is loaded with 1 L of proper concentration of pesticide. Three replicated containers consisting 10 snails were employed for each concentration. The last line was let go as intermediate transport containers to avert dilution of the pesticide solutions in experimental containers when snails were exposed. Three containers were utilized for each species of snails filled and covered with a glass lid. Snails weren't fed through tests. Mortality of snails were registered if no response was detected and dead animals were removed when found. LC₅₀ values and confidence limits 95% were estimated by plotted the percentage of dead snails after 24 hours of exposure against the concentrations of pesticides according the method of Huang (2001).

Before measuring the oxygen consumption rates, a set of each studied snail was subjected to pesticide concentration (sublethal) of 24hr LC₅₀ at 25 ±1°C for 24 hr through which they didn't eat. Other set of snails of each species were employed as control treatment and preserved in the same experiential conditions in clean Shatt Al-Arab river water and in clean river water consisting acetone at a concentration similar to that found in the experiential assay. The influences of pesticide exposition and oxygen tenseness on the oxygen consuming rates of various snails species were established using Gilson differential respirometer (Gilson Medical Electronics, Wisconsin, USA).

Prior being transmitted to the flasks, the snails were washed again with clean river water at the proper salinity to assure that the

contamination of bacteria was in low state. The snails (three individuals) were placed in a chain of 100 ml flasks and then placed in the test temperature to thirty min through that period the flasks were constantly shaken at a speed of forty-five vibrations in min. Control treatment that doesn't contain snails is utilized of each test run. After equilibration, oxygen consuming was estimated each thirty to forty-five min at two to four hours period. After the finish of each test, the snails were isolated form flasks and the lengths of the snails total body were measured by the ruler. The flasks were washed with concentrated cleaner (PCC/54) (USA) to reduce bacterial pollution.

The oxygen consumption rates of snails was estimated at the acclimatization temperature of 20°C. The rates of oxygen consuming were estimated from list traces of ten Torr in oxygen tenseness (Po₂) periods after fling from the oxygen consumption rate mean of water obtained from control runs.

The oxygen consumption weight specific rates (Mo₂) in normoxic statuses were determine from the Po₂ changes chart traces at time (100 Torr) via dividing the oxygen consuming (total) of each snails set by the individuals number in the volumetric flask and through their dry weight mean. The dry weight (total) of snails in each flask was taken follow oven drying in 24 hr at 60°C. Finally, the oxygen consumption weight specific rates were explicated as μmol O₂ mg⁻¹ h⁻¹ (Mo₂) (Varó *et al.*, 1993).

One way ANOVA and the test of Tukey's multiple range ($p \leq 0.05$) were used to compared the LC₅₀ values. The data of oxygen consumption obtained from subjected the snails to pesticide concentrations (sub-lethal) (24 hr LC₅₀) were compared by Two

way ANOVA. The analysis factors were species and treatment (Bruce & Bruce, 2017).

Results

Fig. (1) represents the 24 hr LC₅₀ mean values of pesticide endosulfan for three species of snails studied. The mean 24 hr LC₅₀ values were 4.23, 7.64 and 3.98 mg.l⁻¹ for *T. jordani*, *M. tuberculata* and *R. auricularia* respectively. One way ANOVA analysis method exhibited that there were statistically significant differences ($p \leq 0.05$) among snail species were exist. The analysis by Tukey's multiple range test showed that *M. tuberculata* was the most resistant to pesticide. No statistically significant differences ($p > 0.05$) in the LC₅₀ values of other snails species (*T. jordani* and *R.auricularia*) were found.

Exposure of snails species to 24 hr LC₅₀ effected on the relation between Mo₂ and Po₂

(Fig. 2). It was noted that the capacity of all species of snails to preserve their Mo₂ nearly stable over a broad range of Po₂ reduced while they were subjected to pesticide. The critical of Po₂ (Pc) values (in

which the independence of respiratory can't be preserved for a long time)are registered of three species of snails that were in the range of 40-60 Torr to control treatment and treatment of control with acetone sets. However, the Pc excesses of 60-80 Torr when each snail species was subjected to pesticide.

Values of Mo₂ of each snail species and each treatment in normoxic statuses are shown in Fig. (3). The analysis of two-way ANOVA showed no statistically significant differences ($p > 0.05$) were exist for Mo₂ for snails at each treatment used (to the same species or to the various species of snails that have been studied).

Discussion

Three different species of snails from the Shatt Al-Arab river have a different sensibility to organochlorine endosulfan pesticide. The values of 24 hr LC₅₀ showed that *M. tuberculata* was the most resistant to the pesticide, while *T. jordani* and *R. auricularia* were lower tolerant. No statistically significant differences in the values of 24 hr LC₅₀ calculated of *T. jordani* and *R. auricularia* were found.

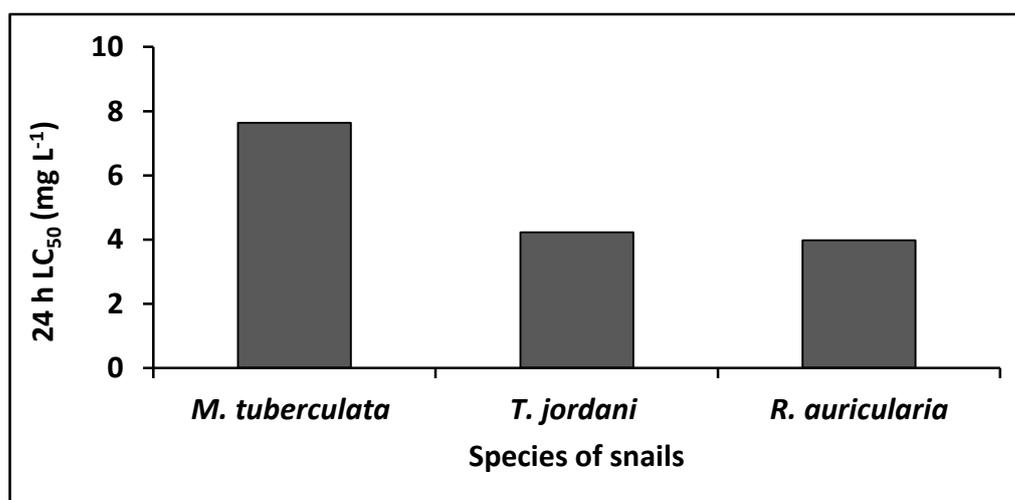


Fig. (1): The mean values of 24 h LC₅₀ of Shatt Al-Arab river snails.

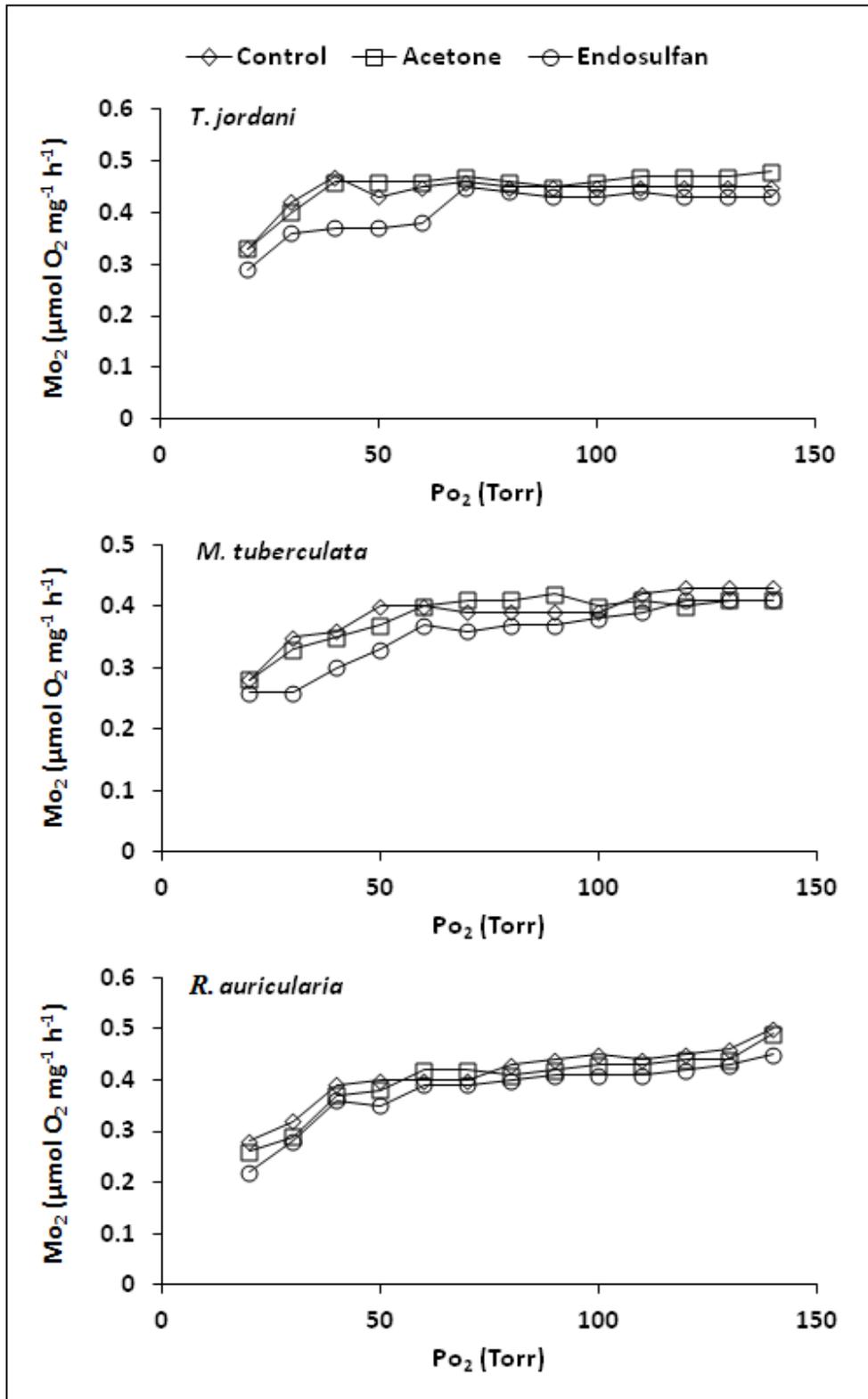


Fig. (2): Effect of endosulfan on the relationship between Mo_2 and Po_2 for Shatt Al-Arab river snails.

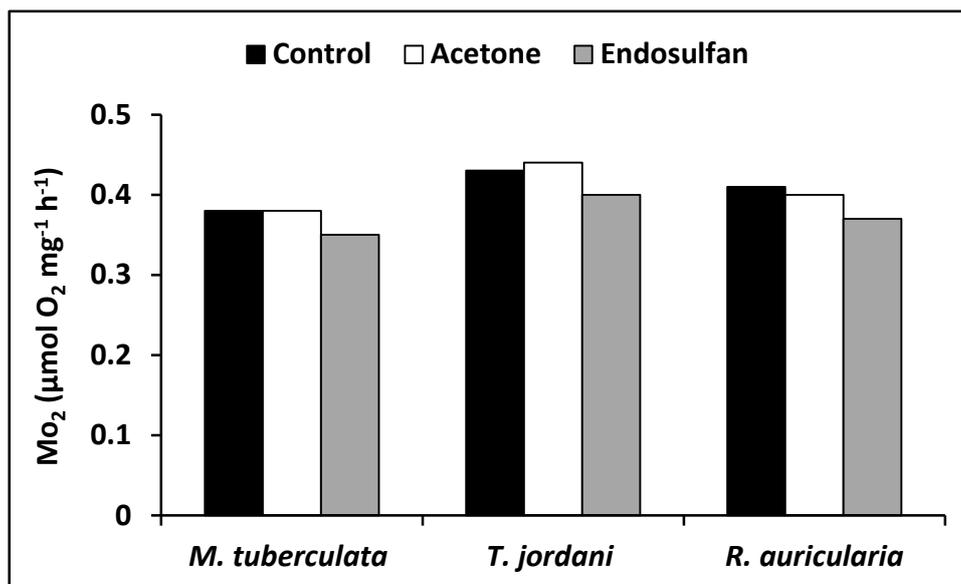


Fig. (3): The mean values of Mo_2 of Shatt Al-Arab river snails for each treatment.

There was no prior study of endosulfan toxicity on snail species. As comparison with other species subjected to pesticide. Jayaraj *et al.* (2016) reported the acute toxicity of many organochlorine pesticides to many living organisms. The researchers found that organisms were highly sensitive to these pesticides after different periods of exposure. WHO (1984) documented that 24 hr LC_{50} was 0.30 mg.l^{-1} for *Procambarus clarkia* subjected to pesticide endosulfan. Nebeker *et al.* (1983) found that 96 hr LC_{50} was 0.34 mg.l^{-1} of *Daphnia magna* effected by endosulfan, while Ferrando *et al.* (1992), which also worked on *D. magna*, reported that the 24 hr LC_{50} of the species was 0.62 mg.l^{-1} and the 48 hr LC_{50} was 0.487 mg.l^{-1} as found by (Barry *et al.*,1995).

The values of 24 hr LC_{50} for *T. jordani* and *R.auricularia* were less than those reported by Fernández-Casalderrey *et al.* (1993) of *Brachioms plicatilis* 24 h LC_{50} of 5.60 mg.l^{-1} and *B. calyciflorus* 24 hr LC_{50} of 5.15 mg.l^{-1} subjected to pesticide endosulfan. The data of the current study display that *T. jordani* and

R.auricularia are more sensitive to endosulfan than *B. plicatilis* and *B. calyciflorus*. Whereas, *M. tuberculata* is lower susceptible to endosulfan than the two species mentioned above .

M. tuberculata, *T. jordani* and *R.auricularia* are common snails in Shatt Al-Arab river. Therefore, the use of different species of snails with different sensitivity to the same toxic substance in the toxicity test could be worthy in assessing the toxicity of toxicants in the river.

Under normoxic conditions, the Mo_2 for each species of snail were not influenced by exposure to 24 hr of pesticide endosulfan . In addition to, Mo_2 was not influenced by the acetone employed. A same impact (no Mo_2 was observed) was registered among else aquatic animals after subjected to toxic compound (Shivakumar & David, 2004; Vutukuru 2005; Anita Susan *et al.*, 2010; Chebbi & David, 2010).

All species of snails were able to maintain Mo_2 independent of Po_2 , through a broad scope of oxygen tenseness. The critical values

of P_{O_2} (Pc) were analogous among snails species and were permanently increased when snails were subjected to 24 hr LC_{50} for pesticide (endosulfan), suggesting that their capability to preserve independence of respiratory through hypoxia decreased after subjected to endosulfan.

In general, the aquatic animals respiratory responses to toxic compound are extremely changeable. The low or high response for oxygen consuming can be imputed not only to variation in species but likewise for concentration of toxic substance (Chebbi & David, 2010). Roberts (1972) reported that pesticide endosulfan reduces the oxygen consumption rate in *Pecten maximus* and *Mytilus edulis* (bivalves). WHO (1984) documented an high respiration rate of the gill in *P. clarkia* (crayfish) after subject to pesticide endosulfan (96 hr LC_{50}) at 22 °C, while lindane pesticide generated a high respiratory rate only at high concentrations. The investigation of Sharp *et al.* (1979) showed that the oxygen consumption for *Crancon franciscorum* lowered with increased kelthane pesticide concentration. Whereas, Anderson (1971) reported that low grades of DDT pesticide led to reduce in the standard metabolic of salmon, (*Salmon salar*) while high concentrations of DDT (30-75) μ L⁻¹ enhanced the rate of fish standard metabolic.

Conclusion

The 24 hr LC_{50} indicated that the *M. tuberculata* was more resistant, while *R. auricularia* and *T. jordani* were more sensitive to endosulfan. No 24hr LC_{50} effect of endosulfan was observed on MO_2 . This can be attributed to the truth that the pesticide (endosulfan) concentrations utilized weren't sufficient for simulate the rate of respiration in different species of snails. More

acquaintance is required to determine the dose-impact relations of toxic substances on snails respiration.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: all applicable national and international guidelines for the care and use of animals were followed.

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