EFFECT OF DIMETOATE INSECTICIDE TOXICITY ON THE GILLS OF BIVALVE PITAR RUDIS AS BIOINDICATOR (HISTOLOGICAL STUDY)

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Abstract. Among the various types of pesticides that contaminate surface water, insecticides play an important role in the pollution of aquatic ecosystems as documented by the accumulated data. Their detriment processes affect several taxa including macroinvertebrates, fish, amphibians, birds and wildlife. Dimethoate is an organophosphorus insecticide medially toxic. It controls aphids, thrips, phanthoppers and whiteflies insects on apples, corn, cotton, lemons, oranges, melons and vegetable etc. Dimethoate inhibits acetylcholinesterase enzyme. Acetylcholine normally passes from presynaptic to postsynaptic membranes of a synapse. This passage is facilitated by the enzyme acetylcholinesterase, dimethoate renders the enzyme inactive and hence Acetylcholine is prevented from passing the cleft, thus accumulating at the presynaptic membrane. The present study is intended to follow up the toxicity of Dimethoate insecticide by using gills of Pitar rudis as a bioindicator. Results showed that LC50 of dimethoate on Pitar rudis is (6.6 ppm). Behavioral changes in bivalve were observed in the clams closed their valves tightly over longer periods and open them slightly for short time, extended the foot and siphons from time to time, Mucous secretion or a layer above the water surface when exposed to dimethoate. Histological changes were observed in the gills that the gills were broken-down at the base of water tubes, damage in the filament, and rupture of the tissues, fusion of the lamellae, broken at the base of the filament and increased of the nuclei.

Keywords: dimethoate, gills, Pitar rudis, histological changes, behavioral changes

Introduction

Pesticides are important production inputs for many agricultural commodities, and decisions concerning their use necessitate, balancing tradeoffs between their economic benefits and protection of environment and human health.

Pesticides are widely used in agriculture for the control of pests, weeds and ectoparasites on livestock all over the world. More, than 10,000 commercial formulations of the approximately 450 different toxic compounds are currently being in use. Insecticides are the most commonly used among pesticides.

It forms 49% of pesticide which are sold in the world markets in the present time [AL–ADEL and ABED, 1979–(in Arabic)]. In Yemen different kinds of insecticides are smuggled in large quantities into country [BA–ANGOOD, 1999—(in Arabic)]. Dimethoate was among insecticides used to control insects on Qat trees in Yemen, although its use banned [THABET, 2006]. The mollusca bivalve’s Pitar rudis concentrate a wide variety of man waste products and are excellent indicators for industrial and domestic pollution [RUIVO, 1972]. The present work is intended to study toxicity of the organophosphorous insecticide dimethoate by observing the behavioral changes of Pitar rudis and the histological changes of gill.

Dimethoate is one of a class insecticide referred to organophosphates these chemicals act by interfering with the activities of cholinesterase an enzyme that is essential for proper working of the nervous systems of both humans and insects [EXTOXNET, 1993] dimethoate is not persistent in environment its toxicity for aquatic organisms and birds has been reported to be moderate to high, it is very toxic for honeybees.

Khan [KHAN 1977] mentioned that some pesticides are extremely toxic to fish at very low concentration and to aquatic invertebrates at even lower concentration.
Toxicity may be confined to a small organism thus be highly specific, or at opposite extreme, affect almost all forms of plant and animal life in water.

The dimethoate is highly toxic to fish and to aquatic invertebrates.

The 96 h LC50 for dimethoate in rainbow trout is 6.2 mg/L. The LC50 in mosquito fish is 40 to 60 mg/L. The 48 h LC50 in Daphnia magna, a small freshwater crustacean is 2.5 mg/L.

Chronic Toxicity is long-term effect that may be related to changes in appetite, growth, metabolism, reproduction, even death and mutations [KOPPERDAHL, 1976; DEKRUIZF and DEZWARD 1988] defined chronic toxicity as exposure of a living organism or a population to chemical or physical factor frequently continuing for 21/28 days or more when concentration is lowered to small level, it is termed subchronic toxicity.

Materials and methods

Water quality

The seawater was brought from Abayn coast of Aden to avoid contaminant as much as possible. It was kept for settlement, filtered through glass wool and stored in closed plastic tanks.

The desired salinity was (36±1‰) prepared daily and checked by hand refractometer and the water was aerated for 24 hours before use.

Water quality parameters are mentioned in Table (1):

<table>
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<th>Physical and Chemical parameters of water used in experiments.</th>
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<td>Parameters</td>
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Bivalve (Experimental Organisms):

The phylum mollusca are among most conspicuous invertebrate animals and include such familiar forms as clams, oysters, squids, octopods, and snails.

This phylum is one of few invertebrate groups that have attained any popularity with laymen and amateur collectors [BARNES, 1974]. Bivalve mollusca are suitable test candidates to study pollutants effects, as they possess several characters to make them attractive as research models.

Cunningham [CUNNINGHAM 1979] cited following characters of bivalves:

They inhabit estuarine and coastal marine areas which are most susceptible to pollution. Bivalves exhibit a relatively long life span. The ease of collection and abundance in shore area has made them readily available.

The bivalve’s clams *Pitar rudis* was selected as experimental organisms. Bivalve (clam) *Pitar rudis* of 30–32 mg body weight and 42–46 mm length, 33–35 mm width, 27–30 mm height were use.

Chemical: Dimethoate insecticide (40%) was obtained from General Directorate of Plant Protection, Ministry of Agriculture and Irrigation, Sana’a.

Experimental aquaria: Glass aquaria of 4 liter capacity were cleaned daily. Salinity, temperature and pH were maintained at condition similar to the natural habitat of the clams.

Methods–as followed

Experimental Animals:

The selection of experimental organism for 96 h toxicity test depends upon many criteria.

Based on previous experience of laboratory personnel conducting bioassay and laboratory facilities available, experimental organisms are to be selected. The selected species should be indigenous to area of impact [MOHAPTRA and RENGARAJAN, 1995].

All clam were acclimated in glass aquaria (60 X 25X 20) cm for three days containing sea water (after 24 h of aerated sea water in big aquaria by air-richen), using also glass aquaria (40 X 25 X 20) cm of approximately 4 litter capacity for bioassay tests. For acute toxicity 60 clams were divided into six experimental groups in addition to control group, all consisting of ten clams.

Dimethoate insecticide:

Stock solution of dimethoate insecticide was prepared by using 0.1 mL of dimethoate (40 %) in 99.9 mL Aceton
(CH₃COOH)₃ in 100 mL volumetric flask, then were different concentrations of dimethoate prepared (3.0, 5.0, 7.0, 9.0, 11.0, 13.0) ppm by adding 15, 25, 35, 45, 55, 65 mL of dimethoate (400 ppm) to two litters of sea water in aquaria.

**Estimation of LC₅₀ of dimethoate to *Pitar rudis***:

Renewal bioassay tests, which are static with a change of water and chemical test every 24 h, are required most often with macroinvertebrate and fish especially when test material is volatile or relatively unstable [APHA 1985].

Ten clams of weighing 30–32 mg body weight, were transferred to each aquarium in addition to control, different concentrations of dimethoate (3.0, 5.0, 7.0, 9.0, 11.0, 13.0) ppm used, exposure period of test was 96 h for calculated median lethal concentration LC₅₀. The experiment repeated for 3 times.

**Chronic exposure studies**:

Sublethal toxicity tests were conducted to determine parameters including survival, behavioral, histological changes. Exposure period of test were 21 days using glass aquaria of 4 L capacity containing aerated seawater. Sublethal concentration were used in different aquaria in addition to control, eight clams were used for each aquaria. Seawater of aquaria were changed daily also clam feed (feed with plankton collected by mass culture made by cultured planktons in water by same site of collection).

**Histological Method**:

Method: The histological slide preparations steps are demonstrated, according to Bancroft method [BANCROFT and STEVENS, 1982], as follows: dissection, fixation, dehydration, clearing, infiltration, sectioning, attaching sections to slides using Mayer's albumen, dewaxing, hydration, staining, differentiation, dehydration and clearing, mounting and examination (slides were examined by light microscope. according to Bancroft method [BANCROFT and STEVENS, 1982]).

**Results**

Instructions for creation of entities that compose the database [Biofuels] were written in TransactSQL (T–SQL for short) specific language for programs of the Microsoft SQL Server suite.

**Acute Toxicity testing**:

This part of work includes estimation of LC₅₀ in order to find start line of the experiment. Two methods were employed in this respect, graphical and probability methods.

**Estimation of LC₅₀ of Dimethoate to *Pitar rudis***:

Percentage mortality of *Pitar rudis* in different concentration of Dimethoate insecticide at 96 h exposure is mentioned in Table 2.

**Estimation of LC₅₀ of Dimethoate to *Pitar rudis***:

Percentage mortality of *Pitar rudis* in different concentration of dimethoate insecticide at 96 h exposure are shown in Figure 1, median lethal concentration was 6.6 ppm.

*Figure 1*. Effect of Acute toxicity of Dimethoate (96 h) on *Pitar rudis*
The slope function has been included with LC_{50} values in Table 2 and represents the factor by which a dose must be multiplied or divided to produce a standard deviations change in response.

The larger the slope function larger is the standard deviations in test results and produce rather wide 95% confidence limits.

**Behavioral Changes:**

The clam closed their valves at the beginning of the bioassay tests with dimethoate solution for nearly one to two hours after which they opened their valves gradually to explore the environment for a period of 5–10 minutes and then closed their valves again.

When the clams exposed to higher concentration of the dimethoate closed their valves tightly over longer periods and open them slightly for a short time approximately of 4–7 min.

The clams exposed to lower dimethoate concentration opened their valves intermittently and extended the foot and siphon from time to time.

Mucous secretion appeared in second 24 h onward and in case of clam, *Pitar rudis* exposed to higher dimethoate concentration a copious amount of mucus was observed which sometimes formed a layer above water surface and mucus is secreted by clams to coat the gills as a protection against chemical but continuous exposure led to hyper secretion of mucus which clogs the mantle cavity of the clam.

At end of the exposure period, the clams exposed to higher dimethoate concentration opened their valves widely extending their feet with sluggish movement and whenever touched or disturbed with a glass rod, they were very slow in drawing the foot and siphon back and in some cases they closed their valves while part of the foot or siphon still extended outside the shell.

**Histological changes:**

**Acute toxicity:**

a) Histological Structure of gills of *Pitar rudis*:

*Pitar rudis*, like any bivalve mollusk, has a pair of branchiae which lies suspended from dorsolateral sides of the pedal mass, one on either side of body.

Each branchium is composed of two demibranchs (inner and outer demibranchs).

The two surfaces of the demibranchs are sculptured into vertical folds called the plicae (PLC).

The demibranchs are hollow boat shaped structures bounded by two lateral walls called the lamellae (L).

The two lamellae of a demibranch are connected by a number of interlamellae junctions (LJ) enclosing spaces called the water spaces or tubes (WT) between them.

The demibranch is made up of numerous filaments arranged in a single row along the lamellae.

Each filament is a minute "U" shaped tube (Figure 2).

Lateral gill cilia create the water current and frontal cilia remove sediment trapped on the gill [BARNES, 1974].

![Figure 2](image2.png)

Figure 2. Photo microscope: (2) Gill of control *Pitar rudis* showing: L, lamella (arrow); W.T, water tubes (star); LJ, interlamellar junctions (circle); Plc (arrow) (H&E–400X).

b) Histological changes of gills of *Pitar rudis*:

The histological changes observed in clams exposed to 5 ppm of dimethoate for 96 h showing no effect (Figure 3).

![Figure 3](image3.png)

Figure 3. Photo microscope: (3) Gill of *Pitar rudis* exposed 5 ppm of dimethoate for 96 h showing: no effect (H&E–400X).
When the clams exposed to 7 ppm of dimethoate for 96 h are showing breakdown at the base of water tubes (Figure 4).

In clams exposed to 9 ppm of dimethoate for 96 h histological changes are very different compared with control; showing break up of lamella (Figure 5 and 6). In (Figure 7) damage in filament.

When clam s exposed to 11 ppm (Figure 8) of the dimethoate for 96 h histological changes are very different compared with control; showing break down of filament.

In (Figure 9) a damage of filament.

**Chronic Toxicity tests:**

There are many histological changes in the gills and digestive gland of *Pitar rudis* to 3 ppm, 5 ppm, 7 ppm concentration of dimethoate insecticide for 21 days (three weeks).

a. Histological changes of gills of *Pitar rudis*
The histological changes were observed in clams exposed to 3 ppm of dimethoate in last week as compared with control showing broken inside plates (arrows), broken at the base of filament (star) (Figure 10). The 2nd week showing fusion of filaments (arrow) (Figure 11).

The changes became more obvious for three weeks showing rupture of the tissues (arrow) (Figure 12).

In clams exposed to 5 ppm of dimethoate changes became more obvious as compared with 3 ppm concentration of dimethoate increased.

The histological changes were observed in clams exposed to 5 ppm of dimethoate in one week showing babbles and fusion of lamellae (arrow) (Figure 13). The 2nd week showing separation of nucleus (arrows) (Figure 14). The changes became more obvious of the 3rd week showing increased of the nucleus (arrow) (Figure 15).

The changes became more obvious as concentration of dimethoate insecticide increased. The histological changes are very different compared with 5 ppm concentration. In clams exposed to 7 ppm of dimethoate for last week showing rupture of filaments (stars) (Figure 16).
The two–week showing separation of nucleus (arrow) (Figure 17); Showed break down of the filaments (arrow) (Figure 18). These changes became more obvious as concentration of dimethoate increased and exposure time prolonged.

At a concentration of 7 ppm (Figure 19) for three weeks, the gills showed separation of nucleus (arrow).

**Discussion**

The median lethal concentration of dimethoate to *Pitar rudis* were found to be 6.6 ppm, while PAN (2005) reported that the LC$_{50}$ of dimethoate to *Bulinus truncatatus* is 2.90 mg/L.

Dikshith [DIKSHITH and RAIZADA, 1981] mentioned that LC$_{50}$ of dimethoate to *Channa punctatus* were 20.5 mg/L While Dietrich [DIETRICH et al., 1996] pointed that 96 h toxicity of four organophosphorus insecticides (thiometon, disulfoton, dimethoate, malathion) in fresh water bivalve mollusca *Dreissenes polymorpha* LC$_{50}$ was 6–26 mg/L respectively.

Verma [VERMA et al., 1982] mentioned that LC$_{50}$ of dimethoate to *Saccobrachus fassilis* were 4.57mg/L.

Extoxnet [EXTOXNET 1993] reported the dimethoate is highly toxic to fish and aquatic invertebrates.

Difference of the results is due to different of environmental conditions and in some cases to variation of the pesticides.

The effects of exposure to dimethoate insecticide were on burrowing behaviour, valves activities and siphons and food reactions of the clam.

When the *Pitar rudis* exposed to sublethal concentrations of dimethoate, behavioral changes of clams exposed to higher concentration of dimethoate closed their valves tightly over longer time (45–60 min) and open them slightly for a short time approximately 4–7 min, and these exposed to lower concentration of dimethoate open their valves intermittently and extended foot and siphon from time to time, and mucous secretion form a layer above water surface when exposed to pesticide.

Verma [VERMA et al., 1982] reported that behavioral effect of sublethal concentration of dimethoate to fish *Salmo gairdneri* are manifested in erratic...
swimming ability and move to surface swimming slowly and sometimes backwards.

Edward [EDWARD et al., 1992] pointed that effects of pyrethroid insecticide esfenvalerate to fish lowest observable effect concentration for survival in a 90–d continuous exposure of esfenvalerate was 0.025 mg/L. Behavioural responses including gross body tremors, were highly sensitive indicators of toxicity among pulse exposed fish, with symptoms appearing within 4 h of exposure to concentrations as low as 0.025 mg/L. Similar behavioural responses were observed after continuous exposure to 0.025mg/L esfenvalerate.

Histological examination of the gills *Pitar rudis* exposed to dimethoate showed the break down at base of water tubes, a damage in the filament, rupture of the tissues, fusion of lamellae, broken at the base of filament, increased of nucleus.

Mallatt [MALLATT, 1985], reported that the histological study of the gill shows atypical structural organization of the lamella in the untreated bivalve. However, Bivalve exposed to dimethoate shows several histological alterations namely lamellar epithelium lifting epithelium proliferation, lamellar axis vasodilatation edema in the filament, fusion of lamellae and lamellar aneurisms.

While [WHILE, DAS and MUKHERJEE, 2000] mentioned that effects of sublethal concentration of hexachloro-cyclohexane to *Labeo rohita* histopathology changes of gills mild congestion of blood vessels were seen in primary lamellae at 0.35 ppm exposure level, where a fusion of primary lamellae and marked hyperplasia of branchial arch was evident at 1.73 ppm concentration.

While [WHILE DUTTA et al., 1993] pointed that gills of *Lepomis macrochirus* exhibited severe hyperplasia when exposed to concentration of 60mg/L diazinon which was also noticed in our study as sloughing in epithelial cells.

**Conclusion**

Dimethoate is toxic to bivalve as indicated by acute, chronic toxicity study as well as behavior and histological changes. The median lethal concentration LC₅₀ of dimethoate to *Pitar rudis* is (6.6 ppm). Sub chronic effect of bivalve exposure to dimethoate for 21 days caused histological changes in the gills.

**Recommendations**

Recommended the General Directorate of Plant Protection, the Ministry of Agriculture and Irrigation—Sana’a, Republic of Yemen, are to minimizing use of pesticides especially organophosphorous insecticides due to their toxicity.

Substitution of organophosphorous insecticides by natural biological methods, and is very important to bear in mind the evaluation impact of dimethoate insecticides on environment and health.

Also a possible option would be and prohibiting import and use of dimethoate insecticide.

**References**

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Dreissena polymorpha P. University of Zurich. 1996.

The author(s) of this paper have published a study investigating the effects of pesticides on fish. The study involved exposing Lepomis macrochirus to various pesticides and observing their responses. The authors concluded that certain pesticides had significant effects on fish, particularly on their gills. The study highlights the importance of understanding pesticide effects on aquatic life for environmental protection.

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