Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

**E-ISSN: 2320-7078**
**P-ISSN: 2349-6800**
JEZS 2018; 6(1): 103-107

Received: 12-11-2017
Accepted: 17-12-2017

**Subburaj A**
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

**Jawahar P**
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

**Jayakumar N**
Department of Fisheries Resource Management, Fisheries College and Research Institute, Tamil Nadu Fisheries University, Ponneri, Tiruvallur District, Tamil Nadu, India

**Srinivasan A**
Tamil Nadu Fisheries University, Nagapattinam, Tamil Nadu, India

**Ahilan B**
Fisheries College and Research Institute, Tamil Nadu Fisheries University, Ponneri, Tiruvallur District, Tamil Nadu, India

**Correspondence**
**Subburaj A**
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

# Acute toxicity bioassay of Malathion (EC 50%) on the fish, *Oreochromis mossambicus* (Tilapia) and associated histological alterations in gills

**Subburaj A, Jawahar P, Jayakumar N, Srinivasan A and Ahilan B**

**Abstract**
In the present study, the freshwater fish, *Oreochromis mossambicus* were exposed to five different acute concentrations of Malathion like 0.626, 1.252, 2.503, 5.006 and 10.012 ppb for 96 hrs. The 96 h $LC_{50}$ of Malathion for *O. mossambicus* was determined to be 0.5925±0.0625 ppb MAL/l. In addition, the study aimed to investigate the histopathological alterations of acute concentrations of Malathion in the gills. The most common histopathological changes in the gills of fish exposed to Malathion were characterized by thickening of secondary lamellae, hemorrhage at primary lamellae, epithelial lining in the tips, lamellar aneurysm, lifting up of epithelium, deformation of the cartilage core, erosion of secondary lamellae, cartilage tissue hypertrophy, shortening of the secondary lamellae, blood congestion in the secondary lamellae and curling of secondary lamellae. Ultimately, the study revealed that the degree of distortion of the gill was in proportion to the duration of exposure and concentration *i.e.*, dose and time dependent.

**Keywords:** Malathion, *Oreochromis mossambicus*, acute toxicity, gill, histology

## Introduction
Pollution problem is now encountered all over the world, with an increase in industrialization, urbanization, surplus use of agrochemicals such as insecticides, pesticides, herbicides, fungicides, etc. to improve yield of agricultural products. Among the different types of pesticides, organophosphates have become one of the widely used class of pesticides. The effects of exposure of aquatic ecosystem to these pesticides are difficult to assess because of their short persistent in water column due to low solubility and rapid degradation. Hence monitoring of these insecticides is important [1]. Malathion is one of the most effective organophosphorus insecticides used for the control of pest with relatively low human toxicity. Many reports have indicated that Malathion, even at low concentrations, harms fish. Alteration of growth parameters, haematological properties, swimming ability, and the depletion of some biochemical parameters (glycogen, cholesterol and total protein), have been reported after Malathion exposure in fish [2].
The toxicity studies are highly useful for sensitive species of an ecosystem that can be used to monitor in determining the indicator species, for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration $LC_{50}$ and or median tolerance. Histopathological assessment has been increasingly recognized as an important tool for the assessment of the impact of environmental pollutants on aquatic animals [3-4]. The gill of fish is the main site of gas exchange, ion regulation and nitrogen waste excretion. When fish are exposed to environmental pollutants, these vital functions are deleteriously affected and the functional impairment of the gill can significantly damage the health of fish [5]. The present work has therefore been undertaken to assess the acute toxic effects of Malathion on the histology of gill of Tilapia, *Oreochromis mossambicus*.

## Materials and Methods
The freshwater fish, *Oreochromis mossambicus* with a length of 10.57±1.69 cm and weight of 17.16±6.45gm were collected from the local fishermen of Pazhayakayal fishing village of Thoothukudi District, Tamil Nadu, India. They were acclimatized to laboratory condition for 10-15 days in FRP tanks of 500 litre capacity and were fed *ad libitum* with floating fish feed (Premium). The commercial grade of Malathion solution was prepared with distilled water for

acute toxicity studies. The test organisms were randomly selected and stocked in plastic tubs filled with five different concentrations of Malathion solution, obtained through range finding tests, *i.e.*, 0.626, 1.252, 2.503, 5.006 and 10.012 ppb/ MAL with ten fishes per concentration. A plastic tub having diluent water without Malathion served as control. Care was taken to maintain a water level of 40 liter in each plastic tub including control tank. The experiment was conducted in duplicate. Static bioassay was carried out for a period of 96 h following standard methods [6]. The experimental concentrations were renewed after every 24 h during the period of bioassay. Mortality was noted at 24, 48, 72 and 96 h after exposure to MAL and data were subjected to Probit analysis [7] for the calculation of 24, 48, 72, 96 h $LC_{50}$ value.

After conducting bioassay for 96 h, fishes were collected from the first two concentrations and also from the control group as no fish were alive at the end of the bioassay in the last three concentrations. Then fishes were sacrificed and gill tissues were dissected out and cut into small pieces of 3 mm thickness and fixed in bouins fixatives for 24 h. Gill tissues were decalcified in Formic acid - Sodium Citrate solution for 3 h. Fixed tissues were rinsed in tap water, dehydrated through a graded series of ethanol, infiltrated with xylene and then embedded in paraffin. Five-micron thick sections were prepared using the microtome from the tissue blocks and taken up on glass slides. The sections were deparaffinized in xylene, rehydrated through decreasing concentrations of ethanol, stained with hematoxylin and eosin [8], and then examined by light microscopy.

**Results and Discussion**

The fishes were exposed to five different concentrations of Malathion like 0.626, 1.252, 2.503, 5.006 and 10.012 ppb/MAL for a period of 96 h. The results revealed a different mortality rate of fishes which ranged from 0 to 100 % showing that the mortality rate was totally dose and time dependent (Fig.1). The mean $LC_{50}$, 95% confidence limits, slope, $r^2$ and chi-square values of MAL for *Oreochromis mossambicus* are presented in Table 1. The 24, 48, 72 and 96 h $LC_{50}$ values of MAL were 4.0985, 2.182, 0.969 and 0.5925 ppb MAL/l respectively (Fig.2). A direct relationship between exposure duration and concentration was clearly evident from the present bioassay test.

There are many studies in concern with the toxicity of MAL on fish. Even at very low and lethal toxic concentrations, Malathion is known to harm different species of fishes as reported by various scientists. $LC_{50}$ values were estimated to range from 0.25 to 15 ppm [9], 0.091 to 22.09 ppm [10] and 0.06 to 7620 µg/l [11] depending upon the species and concentrations of Malathion. The $LC_{50}$ values of Malathion for different fish species reported by various investigators are as below:

**Table.** Median lethal concentrations of Malathion reported in the literature for different fish species

| Sl. No. | Species | Time | $LC_{50}$ values | References |
|---|---|---|---|---|
| 1. | *Ameiurus melas* | 96 h | 11.8 ppm | Martinez and Leyhe [12] |
| 2. | *Brown Trout* | 96 h | 101 ppb | Durkin [13] |
| 3. | *Carassius auratus* | 96 h | 4.71 ppm | Naserabad [14] |
| 4. | *Channa punctatus* | 96 h | 6.65 ppm | Pandey [15] |
| 5. | Cichlids | 96 h | 4.6 ppm | Shao-Nan and De-Fang [9] |
| 7. | *Clarias batrachus* | 96 h | 0.25 ppm | Wasu [16] |
| 8. | *Clarias gariepinus* | 96 h | 8.22 ppm | Ahmad [17] |
| 9. | *Cyprinus carpio* | 96 h | 15.24 ppm | Sharmin [18] |
| 10 | *Esomus danricus* | 96 h | 17.9 µg/l | Das and Gupta [19] |
| 11. | *Gambusia affinis* | 96 h | 0.7 ppb | USEPA[20] |
| 12. | *Heteropneustes fossilis* | 96 h | 10.7 ppm | Begum and Mithra [21] |
| 13. | *Labeo rohita* | 96 h | 5 µg/l | Ullah [22] |
| 14. | *Ophiocephalus punctatus* | 96 h | 16 µl/l | Pugazhvendan [23] |
| 15. | *Oreochromis niloticus* | 96 h | 1.06 ppm | Al-Ghanim [24] |
| 16. | *Xiphophorus maculatus* | 96 h | 12.05 ppm | Sadeghi and Imanpoor [25] |

However, in the present investigation, 96 h $LC_{50}$ value (0.5925 ppb) of Malathion for *O. mossambicus* was estimated to be lower than the studies mentioned in the above table. This might be due to the high toxic potential of the commercial grade of Malathion and variation in size of the fish used. The difference in the toxic potential of the pesticides may be attributed mainly to the susceptibility of the test animals and factors like fish size and weight, and environmental factors, such as temperature, pH, total alkalinity and dissolved oxygen [15].

Histopathological biomarkers are closely related to the other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. Even a slight damage to the tissue may result in a stress to the fish. Respiratory distress is one of the early symptoms of pesticide poisoning. Gills are generally considered as good organ to indicate water quality [26]. They are the primary route for the entry of pesticide. They are primary respiratory organs and all metabolic pathways depend upon the efficiency of the gill for their energy and damage to this organ cause a chain of destructive events, which ultimately lead to respiratory distress [27].

The histological examination of sections of gill of control *O. mossambicus* showed normal cartilaginous core, secondary lamellae, blood vessels, primary lamellar epithelium, primary lamellae, respiratory epithelium and inter-lamellar region (Fig. 3). The histopathological investigation of gill of *O. mossambicus* exposed to acute MAL concentrations of 0.626 and 1.252 ppb showed thickening of secondary lamellae, hemorrhage at primary lamellae, epithelial lining in the tips, lamellar aneurysm, lifting up of epithelium, deformation of the cartilage core, erosion of secondary lamellae, cartilage tissue hypertrophy, shortening of the secondary lamellae, blood congestion in the secondary lamellae and curling of secondary lamellae (Fig. 4 & 5). The histopathological observations made in the present investigations are in conformity to the studies done in gill of different species of fishes exposed to various kinds of pesticides.

Mallatt [28] reported that branchial changes were common in freshwater fishes rather than in seawater fish. The exposure of the fish Sepat Siam (*Trichogaster pectoralis*) to lethal doses of Malathion caused numerous mucus cells along the

epithelium of gill filaments [29]. Rosety *et al*. [30] observed histopathological alterations in gills of gilthead seabream (*Sparus aurata*) after acute exposure to Malathion. The changes included secondary lamellae fusion, clubbing, hyperplasia, thickening of the basal membrane in the secondary lamellae, detachment of the epithelial layer triggered by strong edema and shrinkage of lamellar cells. Malathion induced histological modifications in gills of *Carassius auratus gibelio* [31]. These alterations included epithelial ruptures, secondary lamellae fusion, vascular congestion and hyperplasia of branchial epithelium in the gills. Pugazhvendan *et al*. [23] observed severe damage, noticeable edema and active secretion of mucous, destructed epithelial cells, curled lamellae which finally led to congestion and haemorrhage of gill structure of *Ophiocephalus punctatus* exposed to Malathion. Parikh *et al*. [32] investigated the toxic effect of dimethoate on the gill of *Oreochromis mossambicus* and observed changes like clubbing of secondary lamellae (telangiectasis), enlargement of primary lamellae, and loss of secondary lamellae at high dose. In common carp exposed to Malathion, the morphological changes, such as telangiectasia, blood lamellar congestion, hypertrophy of filaments, lamellar fusion were observed in the gill structure [33].

## Conclusion

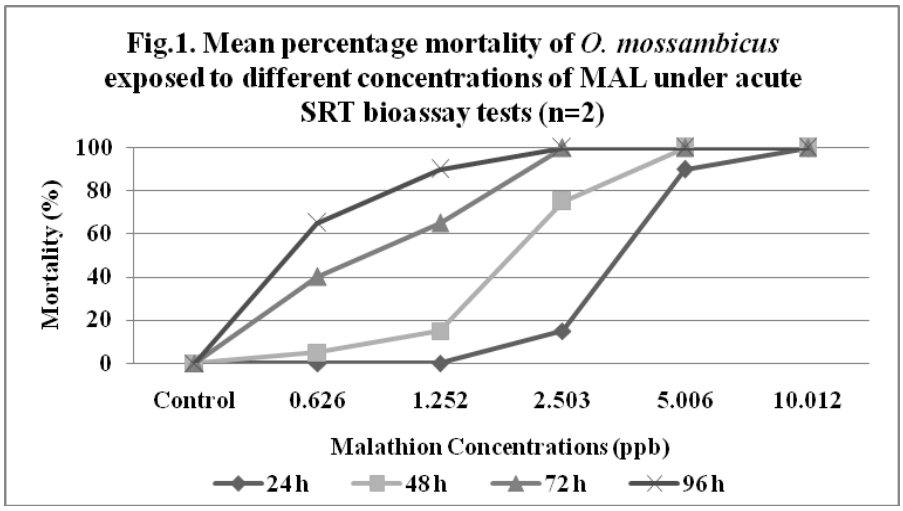
The present study demonstrated that Malathion is toxic to Tilapia, *Oreochromis mossambicus* and the histological observations showed that exposure to the toxic concentration of Malathion caused the alterations in the gill tissues of *O. mossambicus* to a greater extent. These degenerative changes are expected to adversely affect the physiological process of respiration and cause many abnormal effects in the fishes in due course.

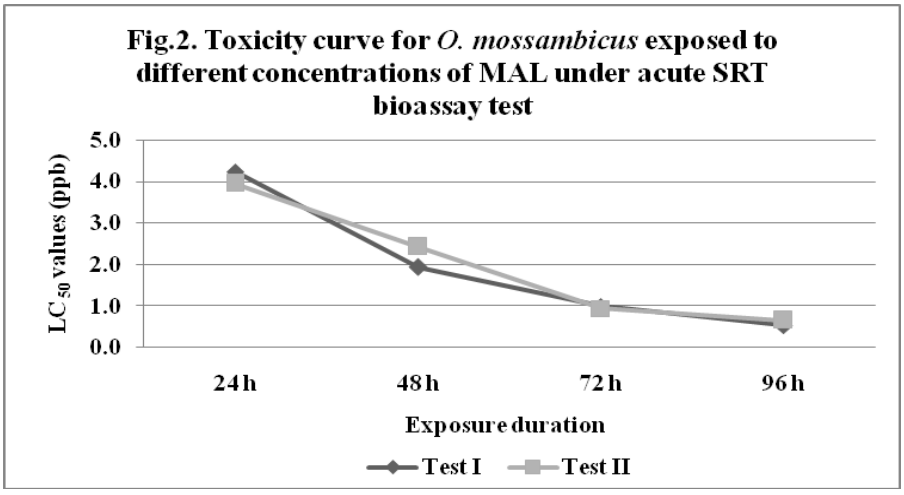
## Acknowledgement

The authors wish to thank the Dean, Fisheries College and Research Institute, Thoothukudi for his guidance and moral support. In addition, we are grateful to the authority of the Tamil Nadu Fisheries University for having provided facilities to conduct the research.

**Table 1:** Mean $LC_{50}$, slope, regression coefficient ($R^2$) and Chi-square values for *O. mossambicus* exposed to acute concentrations of MAL

| Exposure Period | Mean $LC_{50}$ value (n=2) | 95% Confidence limits | Slope value | $R^2$ value | Chi-square value |
|---|---|---|---|---|---|
| 24 h | 4.0985 ± 0.1445 | 2.764 - 4.328 | 7.8528 ± 0.6757 | 0.777 | 0.0095 ± 0.0085 |
| 48 h | 2.182 ± 0.248 | 1.156 - 2.689 | 5.2958 ± 1.1196 | 0.897 | 0.9005 ± 0.7995 |
| 72 h | 0.969 ± 0.031 | 0.384 - 1.099 | 3.6817 ± 0.1265 | 0.860 | 1.21 ± 0.447 |
| 96 h | 0.5925 ± 0.0625 | 0.004 - 0.797 | 3.511 ± 0.3302 | 0.690 | 0.1425 ± 0.0445 |
| *Values are given as Mean ± Standard Error of Mean (SEM) | | | | | |



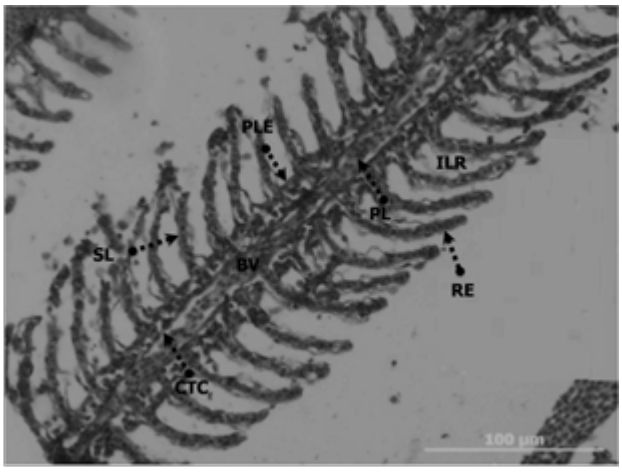



**Fig 3:** Photomicrograph of gill control. CTC - Cartilaginous Core, SL – Secondary Lamellae, BV - Blood Vessel, PLE – Primary Lamellar Epithelium, PL - Primary Lamellae, RE - Respiratory Epithelium, ILR – Inter-Lamellar Region (5µm thick; H&E staining; 200X)

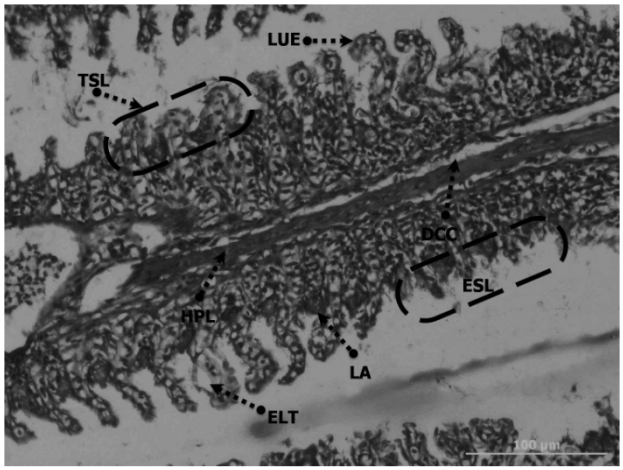



**Fig 4:** Photomicrograph of gill of fish exposed to MAL at 0.626 ppb after 96 h. TSL-Thickening of Secondary Lamellae, HPL - Hemorrhage at Primary Lamellae, ELT - Epithelial Lining in the Tips, LA - Lamellar Aneurysm, LUE - Lifting Up of Epithelium, DCC - Deformation of the Cartilage Core, ESL - Erosion of Secondary Lamellae (5µm thick; H&E staining; 200X)

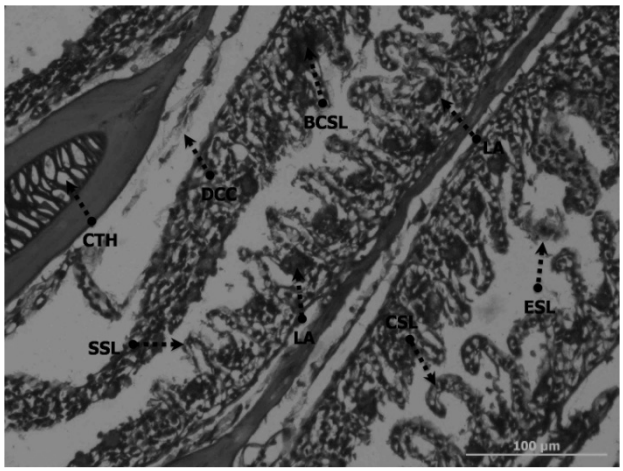



**Fig 5:** Photomicrograph of gill of fish exposed to MAL at 1.252 ppb after 96 h. CTH - Cartilage Tissue Hypertrophy, SSL - Shortening of the Secondary Lamellae, DCC - Deformation of the Cartilage Core, LA - Lamellar Aneurysm, BCSL - Blood Congestion in the Secondary Lamellae, CSL - Curling of Secondary Lamellae, ESL - Erosion of Secondary Lamellae (5µm thick; H&E staining; 200X)

## References


1. Chebbi SG, David M. Respiratory responses and behavioural anomelies of the carp *Cyprinus carpio* under qinalthos intoxication in sublethal doses. Science Asia. 2010; 36:12-17.
2. Huculeci R, Dinu D, Staicu AC, Munteanu MC, Costache M, Dinischiotu A. Malathion-induced alteration of the antioxidant defence system in kidney, gill, and intestine of *Carassius auratus gibelio*. Environmental Toxicology. 2009; 24:523-530.
3. Chourpagar AR, Kulkarani GK. Effect of mercuric chloride on gill structure of a freshwater female crab, *Barytelphusa cunicularis* (Westwood). Journal of Global Biosciences. 2014; 3(2):423-427.
4. Paruruckumani PS, Maharajan A, Ganapiriya V, Narayanaswamy Y, Jeyasekar RR. Surface ultrastructural changes in the gill and liver tissue of asian sea bass *Lates calcarifer* (Bloch) exposed to copper. Biological Trace Element Research. 2015; 168(2):500-507.
5. Alazemi BM, Lewis JW, Andrews EB. Gill damage in the fresh water fish *Gnathonemus ptersii* (Family: Mormyridae) exposed to selected pollutants: an ultra-structural study. Environmental Toxicology. 1996; 17:225-238.
6. APHA. Standard methods for the examination of water and waste water, Edn. 20. Washington, D.C, 1981.
7. Finney DJ. Probit analysis, Edn. 3. Cambridge University Press, New York, 1971.
8. Humason GL. Animal tissue techniques. Edn. 3. San Francisco: W.H. Freeman and Company, 1972.
9. Shao-Nan L, De-Fang F. Correlation between biochemical parameters and susceptibility of freshwater fish to malathion. Journal of Toxicology and Environmental Health. 1996; 48:413-418.
10. Vittozi OL, De-Angelis G. A critic review of comparative acute toxicity of data on fresh water fish. Aquatic Toxicology. 1991; 19:167-204.
11. Newhart KL. Environmental fate of malathion. California Environmental protection Agency. 2006, 20.
12. Martinez J, Leyhe J. Malathion analysis of risks to endangered and threatened Salmon and Steelhead. Office of pesticide programme, Environmental field branch, United States Environmental Protection Agency. 2004; 108.
13. Durkin PR. Malathion; Human Health and ecological risk assessment. Final report submitted to Paul Mistretta, PCR, USDA/Forest Service, Suthern region, Atlanta Georgia. SERA TR-052-02-02c. 2008, 325.
14. Naserabad SS, Mirvaghefi A, Gerami MH, Farsani HG. Acute Toxicity and Behavioral Changes of the Gold Fish (*Carassius auratus*) Exposed to Malathion and Hinosan. Iranian Journal of Toxicology. 2015; 8(27):1203-1208.
15. Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety. 2005; 61(1):114-120.
16. Wasu YH, Gadhikar YA, Ade PP. Sublethal and Chronic Effect of Carbaryl and Malathion on *Clarius batrachus* (Linn.).Journal of Applied Sciences and Environmental Management. 2009; 13(2):23-26.
17. Ahmad Z. Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. African Journal of Biotechnology. 2012; 11(34):8578-8585.

18. Sharmin S, Shahjahan M, Hossain MA, Haque MA, Rashid H. Histopathological Changes in Liver and Kidney of Common Carp Exposed to Sub-lethal Doses of Malathion. Pakistan Journal of Zoology. 2015; 47(5):1495-1498.
19. Das S, Gupta A. Histopathological changes in the intestine of indianflying barb (*Esomus danricus*) exposed to organophosphate pesticide, malathion (EC 50). Global Journal of Biology, Agriculture and Health Sciences. 2013; 2(2):90-93
20. USEPA. (United States Environmental Protection Agency). Registration eligibility decision for malathion: Case No. 0248. Office of prevention, pesticides, and toxic substances. Office of pesticide programs, United States Environmental Protection Agency. 2006, 196.
21. Begum BH, Mithra D. Effects of an Organophosphate Pesticide, Malathion (50% E.C) on the Liver of air breathing fish, *Heteropneustes fossilis*. International Research Journal of Environment Sciences. 2015; 4(9):21-24.
22. Ullah S, Begum M, Dhama K, Ahmad S, Hassan S, Alam I. Malathion Induced DNA Damage in Freshwater Fish, *Labeo rohita* (Hamilton, 1822) Using Alkaline Single Cell Gel Electrophoresis. Asian Journal of Animal and Veterinary Advances. 2016; 11(2):98-105.
23. Pugazhvendan SR, Narendiran NJ, Kumaran RG, Kumaran S, Alagappan KM. Effect of Malathion Toxicity in the Freshwater Fish *Ophiocephalus punctatus* - A Histological and Histochemical Study. World Journal of Fish and Marine Sciences. 2009; 1(3):218-224.
24. Al-Ghanim KA. Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of *Oreochromis niloticus*. Scientific Research and Essays. 2012; 7(16):1674-1680.
25. Sadeghi A, Imanpoor MR. Investigation of $LC_{50}$, NOEC, and LOEC of oxadiazon, deltamethrin, and malathion on platy fish (*Xiphophorus maculatus*). Iranian Journal of Toxicology. 2015; 9(28):1271-1276.
26. Rankin JC, Stagg RM, Bolis L. Effects of pollutants on gills. In-Gills. Cambridge University Press. 1982, 207-220.
27. Magare SR, Patil HT. Effects of pesticides on oxygen consumption, red blood cells count and metabolites of a fish, *Puntius ticto*. Environment and Ecology. 2000; 18(5):891-894.
28. Mallatt J. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Fisheries and Aquatic Sciences. 1985; 42:630-648.
29. Mohsin AKM, Lui OS, Ambak MA. Effect of Malathion on Sepat Siam and Tilapia. Pertanika. 1984; 7(1):57-60.
30. Rosety M, Rosety-Rodriguez M, Ordonez FJ, Rosety I. Time course variations of antioxidant enzyme activities and histopathology of gilthead seabream gills exposed to malathion. Histology and Histopathology. 2005; 20:1017-1020.
31. Cristina SA, Cristina MM, Anca D. Malathion induced histological modifications in gills and kidney of *Carassius auratus gibelio*. Lucraristiintifice Zootehniesi biotehnologii. 2008; 41(1):448-453.
32. Parikh PH, Rangrez A, Adhikari-Bagchi R, Desai BN. Effect of dimethoate on some histoarchitecture of freshwater fish *Oreocromis mossambicus* (peters, 1852). The Bioscan. 2010; 5(1):55-58.
33. Sharmin S, Salam MA, Haque F, Islam MS, Shahjahan M. Changes in hematological parameters and gill morphology in common carp exposed to sub-lethal concentrations of Malathion. Asian Journal of Medical and Biological Research. 2016; 2(3):370-378.