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Effect of Malathion Toxicity in the Freshwater Fish *Ophiocephalus punctatus*-A Histological and Histochemical Study

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Abstract: The present study deals with the effect of some water pollutant; malathion as an insecticide on histological and histochemical study in *ophicoephalus punctatus*. The exposure of fish to the examined pollutants induced a disappearance of some fractions and consequently changes of relative mobility and area that indicate damage. *Ophicoephalus punctatus* was exposed for 7 days to Malathion at a concentration of 10, 12, 14,16,18,20 µ1/1 and 100ppm under laboratory conditions. The fish showed severe histological changes in brain, liver, ovary and tissues. The degenerative changes included hypertrophy of cells and their nuclei, liver on the whole showed distance appearance. Pycnosis, Vaculation, Necrosis, fragmentation of ova were recorded in an increasing order towards the higher tested doses.

Key words: *Ophicoephalus punctatus* % Malathion % Histochemical study % Histoogical study

INTRODUCTION

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors [1]. Among these pesticides, organophosphorus compounds (Ops) are commonly used as insecticides [2]. Malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), a commonly used organophosphate, is applied for mosquito control at concentrations designed to achieve a surface exposure of 5 lg/cm2 [3] and is applied to combat agricultural pests at a rate 10 times higher, resulting in an exposure rate of 50 lg/cm2 [4]. Malathion is not considered a persistent pesticide (log Kow = 2.89, halflife = 1-10 days) [4]; however, the annual application of 17 million pounds of malathion in the United States [4]. This is used for agricultural and nonagricultural purposes. Once malathion is introduced into the environment, usually from spraying on crops or in wide urban or residential areas, droplets of malathion in the air fall on soil, plants, water or man-made surfaces. While most of the malathion will stay in the areas where it is applied, some can move to areas away from where it was applied by rain, fog and wind. In water, malathion breaks down quickly by the action of water and bacteria in the water. Malathion is broken down in air by reacting with other chemicals formed naturally in the air by sunlight to form a more toxic product called malaoxon. If malathion is present on dry soil or on man-made surfaces such as sidewalks, pavements, or playground equipment, it usually does not break down as fast as it would in moist soil. Once malathion is introduced into the environment, it may cause serious intimidation to the aquatic organisms and is notorious to cause severe metabolic disturbances in non-target species like fish and fresh-water mussels [5]. The acute toxicity of malathion and its active primary metabolic product malaoxon results from impediment of acetylcholine degradation at the neuromuscular junction through irreversible inhibition of acetylcholinesterase [6]. In the present investigation an attempt has been made to observe the possible biochemical changes in muscle tissue and histological changes in the vital organs like Brain, gill, Liver and Ovary of the teleost fish *Ophiocephalus punctatus* exposed to sub lethal

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concentration of malathion for 7 days. So for not much work has been done for the effects of Malathion on fresh water fish *Ophiocephalus punctatus*. Hence, the present investigation aimed to evaluate the effect of sublethal concentration of Malathion on biochemical and histopathological changes of fresh water fish *Ophiocephalus punctatus*.

MATERIALS AND METHODS

Fish: *Ophiochealaus punctatus* fingerlings (20 ± 1.5 g, 10-12 cm) were collected from the lake of Poondi at Thiruvallur District, Tamil Nadu, India and acclimated to laboratory conditions for a period of 15 days in large cement tank 1000 liter previously washed with potassium permanganate and water was sterilized with UV to prevent microbial infection under aeration. Temperature: 26 ± 2 °C, pH: 7.0 ± 0.2: were maintained.

Toxicity Assay: Ten fishes were kept in 50l glass tank of dechlorinated tap water. The fishes were treated with concentration of Malathion to observe the LC50 and the biochemical as alas histological changes. Each group was exposed to different concentration like 10, 12, 14, 16, 18 and 20 µ1/l for 4 days (i.e) 24, 48, 72 and 96 hours. Control fishes were maintained pesticide free dechlorinated tap water in separate tank during this experiment. Mortality was recorded every 12 hrs during this period.

Biochemical Assay: Fishes were treated to sub-lethal concentration of (10, 12 and 14 µ1/l for malathion). After exposed the completion of treatment they were dissected and liver and muscle tissues for biochemical analysis. The protein was analyzed by adopting the method of Lowry *et al.* [7] and the histological changes of the tissue sample like Brain, gill, liver and ovary were removed both experimental and control group at 1st to 7th day. The tissue were fixed in 10% formalin and processed for paraffin embedding. Sections were cut and stained with hematoxylin and eosin [8].

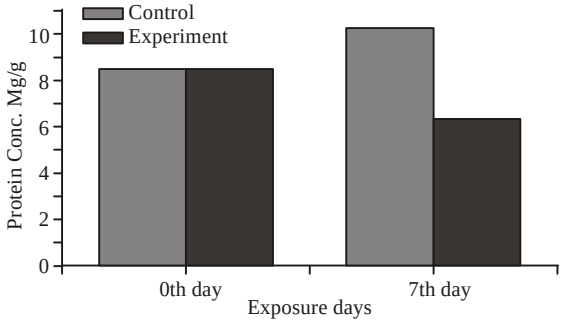
Statistical Method: Data was interpreted by using the statistical method described by Vittal [9].

RESULTS

Toxicity Assay: Lethal concentration of malathion for the freshwater fish, *Ophiocephalus Punctatus* was found to be 16µl/L. The upper and lower 90% confidence limits were found to be 20 µl/L and 12 µl/L respectively (Table 1).

Biochemical Assay: The biochemical analysis (Protein) of control and experimental group of fishes of *Ophiocephalus punctatus* are shown (Graph. 1). From the above results the protein content in the muscle of the control fishes showed highest activity at 7th day (10.25 ± 0.4mg/g)whereas the lower value was observed at 0th day i.e. (8.5 ± 0.4mg/g) in the control group. But 7th day of experiment the fishes showed very lowest value of muscle protein i.e., (6.25 ± 0.1mg/g).

The histology of control fish brain showed (Fig. 1a) normal configuration the neural cells were uniformly arranged. The brain showed many neural bundles in between the neural cells many glial cells were seen. The experimental fish brain showed (Fig. 1b) severe damage in the brain cells. The neural cells were disintegrated. The broke down of neural bundles were seen. The histology of gill in control fish (Fig. 2a) structure of the gill bearing four Paris of gill lamellae on both the sides are supported by bony structure and primary lamellae.



Graph 1: Protein changes in the liver of *Ophiocephalus punctatus*

Table 1: Mortality rate of fresh water fish *Ophiocephalus punctatus* at different concentration of Malathion at 96 hrs. exposure of Malathion

Sl.No.	Concentration of Malathion µ1/l	No. of fishes exposed	No. of fishes dead	Percentage of Mortality (%)
1	10	10	0	0%
2	12	10	1	10%
3	14	10	3	30%
4	16	10	5	50%
5	18	10	8	80%
6	20	10	9	90%

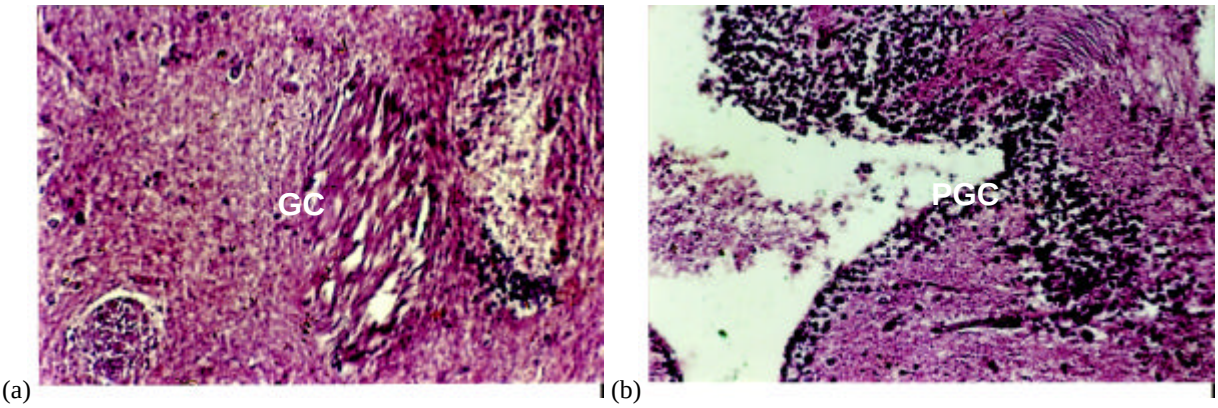


Fig. 1a: Showing normal fish brain, HE, X 400, NC-Neuroncells, GC-Glial cells,NB -Neural bundles
Fig. 1b: Showing experimental fish brain, HE, X 400, BNB-Broke down of neural bundles,PGC-Proliferation of glial cells

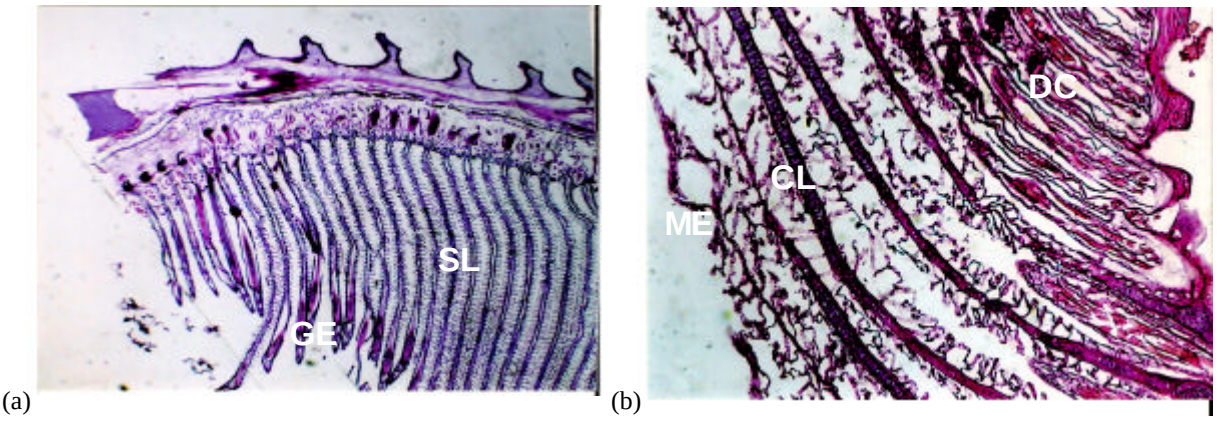


Fig. 2a: Showing normal fish gill, HE, X 50, PL-Primary lamellae,SL-Secondary lamellae,GE-Gill ephithelium
Fig. 2b: Showing experimental fish gill, HE,X50, CL-Curled lamellae,DC-Damaged cells, ME-Marked edema, V-Vacuolation

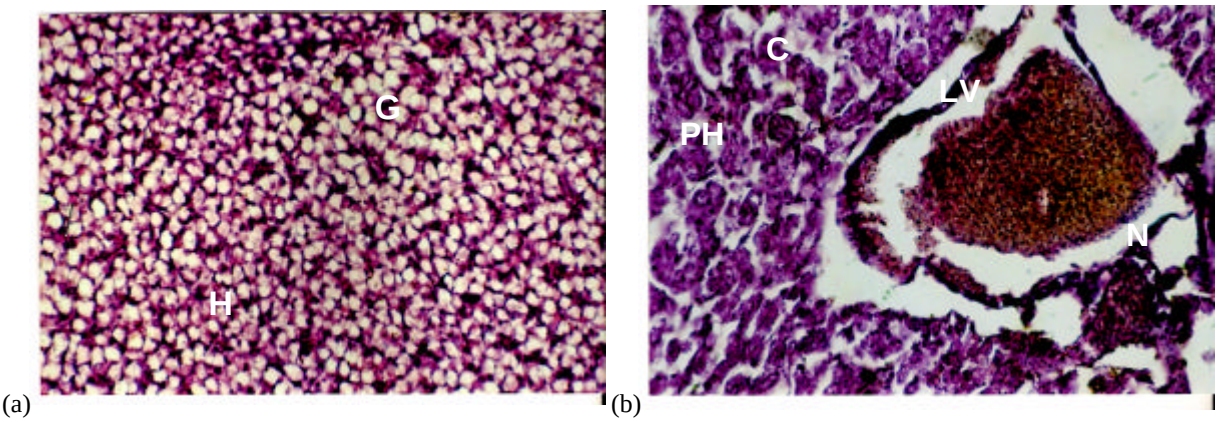
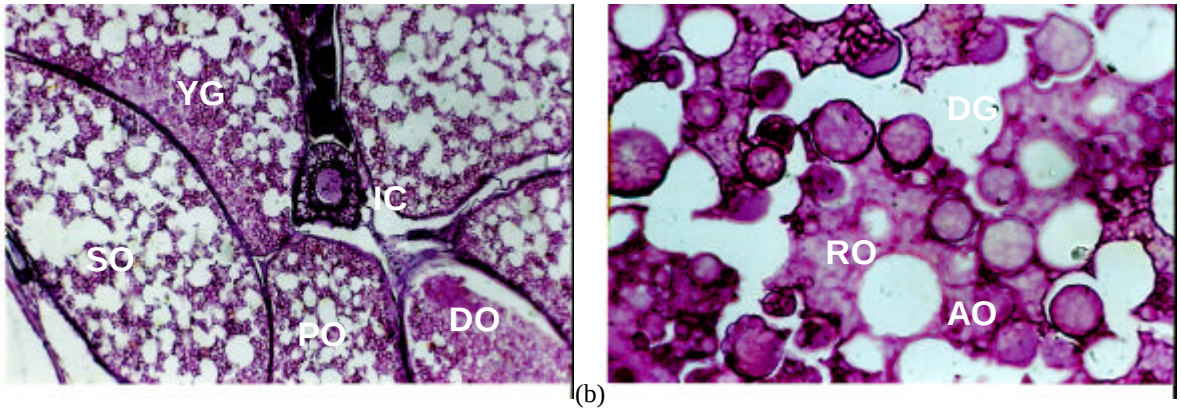


Fig. 3a: Showing normal fish liver, HE, X 400, G-Granulation, H-Hepatocyetes
Fig. 3b: Showing experimental fish liver, HE, X 400, LV-Large vacuoles, N-Necrosis, PH-Proliferation of Hepatic cells, C-Cirrhocis



(a) (b)

Fig. 4a: Showing normal fish ovary, HE, X 50, DO-Developing oogonia, PO-Primary oocyte, SO-Secondary oocyte, YG-Yolk granules, IC-Interstitial cells

Fig. 4b: Showing experimental fish liver, HE, X 400, DG-Degranulation, RO-Reduced oocytes, AO-Aretic oocytes

The secondary lamellae showed numerous channels of blood capillaries, each separated by single layered pillar cells when observed in vertical section. The laminar epithelium was thick followed by basement membrane below which the pillar cells enclosed blood spaces, large number of mucous cells were observed of the epithelial gill racker, where as lamellae had comparatively small and less number of mucous cells. The experimental fish gill given in (Fig. 2b). The gill showed severe damage, marked edema and active secretion of mucous, increased in size but decreased in number and most of them were either vacuolated or almost empty. The secondary lamellae are also showed destruction of either epithelial cells or few lamellae were curled, that leads to congestion and haemorrhage of gills. After 7 days of experiment the gill became lost its colour. The histology of control fish liver *Ophiocephalus punctatus* was given in the (Fig 3a). The control liver showed normal exo-structure of hepatic cells, the connective tissue of liver expressed normal condition, the hepatic mass normal granulation were observed, in the pancreatic tissue no changes were noticed. The histopathology of experimental fish liver *Ophiocephalus punctatus* was given in (Fig 3b). In the experimental fish liver showed proliferation of ducted cells and small spaces were appeared in between hepatic cells. The liver cells showed severe damage and marked proliferation. The liver tissue was converted into degenerative mass and the cells were showed scattered in nature. The pancreatic tissue was broken and large vacuoles were seen. Many places in the liver necrosis were observed. The histology of control fish ovary was given in (Fig 4a). The control fish ovary showed normal exo-structure. The oogenesis of the was in the late perinucleolar stage and in the beginning of the oil drop stage, very few oocytes were in the chromatin-nucleolar stage. In the oocytes the multiple nucleoli became located around the periphery of the nucleus had a small juxta nuclear mass of yolk nucleus and exhibited the initiation of the vitollogenesis by formation of cytoplasmic oil drop. In experimental fish ovary was given in (Fig 4b). In experimental fish the ovary showed inhibition of oocyte development. The break down of germinal vesicles were observed in the ovary. The yolk granules were disappeared. Many disturbed oogonia were observed in the experimental group fish ovary

DISCUSSION

It is evident from the results that the malathion can be rated as moderately toxic to fish. In the present study, the control fish behaved in natural manner i.e. they were active with their well coordinated movements. They were alert at the slightest disturbance, but in the toxic environment, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation [10]. The constituents of the cell membrane, proteins have a major role in the interactions between intra and extra cellular media. As enzyme, proteins participate in the in the intricately balanced sub cellular functions.

When compared to experimental group of fishes which are administered with malthion showed same protein level at 0^{th} day (8.5 ± 0.4mg/g) in the muscle. Kumar and Asari, [11] concluded that the marked

inhibition DNA, RNA, Protein, Acid and Alkaline phosphatase in the liver of four month old Zebra Fish after 7th day of exposure of different concentration of malathion (0.5,0.7,0.9 and 1.1 mg/l). Malathion is esterase inhibitor neurotoxicants, with acute cholinergic effect preceded by inhibition of acetylcholinesterase [12]. Accumulation of acetylchlinesterase which causes twitching of muscle leading to tetanus and eventual paralysis of the muscle. Paralysis of respiratory muscle may lead to death.. In sublethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicate reduced amount of dietary protein consumed by the fish at pesticide stress, which was immediately utilized and was not stored in the body weight [13].

A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. The histopathological changes in the Brian, Gill, Liver and Ovary of *Ophiocephalus punctatus* in the control and experimental group of fishes have been analyzed. In this present investigation the histology of the experimental brain cells were lost their differentiation and scatterly arranges severe necrosis were seen in the brain of experimental group of fishes exposed to malathion pesticide.

In this Present finding results are supported by the work of Dutta *et al.,* [14]. They studied the effects of sub lethal concentration of malathion in the gills of Heteropneustes fossils by scanning electron microscopy. They found out enlarged mucous gland opening on the gill arch, the lamellar surface had many crevices, elevated depression and broken of micro ridges in the gill arch after 72 hours of exposure of malathion (6mg/l). The experimental liver results showed are in agreement with those of Saxena, *et al.,* [15]. These authors found out malathion is considered is more toxic than carboxyl in inhibiting the de-nova synthesis of lipid and protein in the liver, of *Ophiocephalus punctatus.* The malathion exposure fishes ovary are severe metabolic changes were observed based on the result. Inbaraj and Hider, [16]. These authors included that the loss of stage II and stage III oocytes in *Ophiocephalus punctatus* treated with insecticide malathion. Malathion known to effect the nervous system by inhibiting acetyl cholinesterase (ACHE), the enzyme that modulated the amount of the neurotransmitter, acetylcholine [17]. They are several metabolic routes by which an organism can detoxify organophosphorus insecticide. In addition, the physiological condition of the organism during toxic impact must be consider to understand the influence of pesticide. In the present study malathion was chosen to evaluate the influence on the muscle protein of the fish *Ophiocephalus punctatus* at sub lethal concentration of malathion exposure have been analyzed and discussed. The gradual decrease of protein from various days of exposure may be due the influence of exogenous factors like toxic environment, Sapna Srivastava, *et al.* [18]; Khalaf Allah, [19] reported that the decreased level of protein, globulin and serum enzyme activity in vaccinated *Tilapia nilotica* exposed to sub lethal concentration malathion pesticide.

In the present study the low, level of protein content estimated in the malathion treated fishes. This may be due to the pollution stress posted to the fishes, mobilization protein from muscle to blood, to compensate to certain acidosis caused by the lactate accumulation [20] whereas Sahib, *et al.* [21] found out elevated level of protein in muscle, gills and liver tissues of fresh water fish *Tilapia mossambica* under malathion exposure. Severe damage was found after 48 and 72 hours of exposure. After a 24 hours of exposure to a 6mg/L malathion showed more severe damage in the gills. The microridged epithelial cells of the gill arch became perforated and the control portion of the filament appeared elevated. Numerous mucous gland openings were visible. After 48 and 72 hours of exposures, the damage of gill and structural changes were more pronounced. When compared with the 4mg/L exposure. Enlarged mucous gland opening were formed on the gill arch. The lamellar surface had many crevices, elevation and depression. Broken micro ridges in the gill arch surface3 were visible at 72 hours of exposure [14]. Gills of the fishes are highly susceptible to water soluble toxicant when immersed in it. In the lethal concentration of 24 hours exposure, mucous secretion of gill surface forming thick coat over it. The changes observed in the present study clearly indicate the direct effect of malathion on the respiratory organs of fish which may be result in gradual failure. [22]. Kabeer Ahamed Sahib, *et al.* [23] found out sub lethal concentration of malathion inhibiting acetyl cholinesterase activity of brain, muscle, gill and liver tissues of the fish, *Tilapia mossambica*. The liver is the largest gland of the body connected with several function. It has no direct contact with the environmental pollutants dissolved in water but due to its contact with blood, its indirectly affected. The malathion and dieldrin enhances the activation of aromatic

amines in liver of fish *Sparus aurata* exposed to different environmental pollutants. [24]. In this study the liver tissues were converted into sponge mass and the cells were showed scattered in nature, necrosis of hepatic cells. Pancreatic tissue was broken and large vacuoles were seen. This might be due to the effect of organophosphate insecticide malathion exposure. The ovaries are pair of compact bodies which remain in their original foetal position in the abdominal cavity attached to the dorsal body wall by a fold of peritoneum. The germ cells became associated with small epithelial cells and more into the cortex. The associated epithelial cells multiply and surround the germ cell which is now termed the oocyte. The developing oocytes are nourished by sertolic cells or lydig cells. It has not directly contact with the pollutants dissolved in water but due to its contact with blood it is indirectly affected by pesticides and insecticides.

The present investigation evidenced that malathion is toxic and had profound impact on behaviour and respiration in *Ophiochealaus punctatus* in both lethal and sublethal concentrations. Thus it has led to the altered fish respiratory physiology. Therefore, dysfunction of behaviour and respiration can serve as an key of malathion toxicity.

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