Eco. Env. & Cons. 28 (3) : 2022; pp. (1647-1659)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i03.076

Fish Gills as a Tool for the Assessment of Sublethal Effects of Monocrotophos on a Freshwater Fish, *Etroplus maculatus*

[1]*B.T. Sulekha and [2]T.V. Anna Mercy

[1]Department of Zoology, Sree Narayana College, University of Kerala, 691001, Kollam, Kerala, India
[2]Department of Aquaculture, Sacred Heart College, Thevera, Ernakulam 682 013, India

(Received 25 May, 2022; Accepted 31 July, 2022)

ABSTRACT

Agricultural pesticides are nowadays being extensively used for the control of a wide variety of agricultural pests. The world over, human health is at risk. While on one hand people are dying due to scarcity of food, on the other they are slowly being poisoned by the chemicals used for growing food. Other than targeted pests, pesticides affect a wide range of non-target organisms, such as invertebrates and fin fish inhabiting aquatic environment. The present laboratory study is aimed at assessing the sublethal effects of monocrotophos on the gills of *Etroplus maculatus*, a fresh water food fish as well as an indigenous ornamental fish of the paddy fields of Kuttanad, the rice bowl of Kerala. Sublethal exposure was done in a static system and the target organ undergoes histological Hematoxylin-eosin staining procedures. The pathological changes were obviously dose–dependent and maximum severity was noticed in the gills of fishes exposed to the highest sublethal concentrations. The overall changes observed due to the exposure to different concentrations of monocrotophos are hypertrophy, hyperplasia, fusion of secondary lamellae, oedematous separation of respiratory epithelium, lamellar telangiectasis, stasis, filamental blood vessel enlargement and necrosis. Based on the histological lesions observed the index values were calculated. On the basis of the index values, No Observable Effect Concentration (NOEC), Least Observable Effect Concentration (LOEC) and Maximum Allowable Toxicant Concentrations (MATC) of monocrotophos to *E. maculates* were calculated. The concentration which showed index value less than 10 is taken as NOEC and between 10-20 is taken as LOEC. MATC of monocrotophos to *E. maculates* is estimated as 0.4243 ppm. Based on the MATC the calculated application factor (AF) was 0.1263. These NOEC, LOEC, MATC and AF so obtained for this species can also be used to assess tentative water quality status for the pollutants in the natural environment, and the AF derived from the laboratory studies can be directly applied to the filed situations, as they are the representative sample of Kuttanad. Histopathology is an important biomonitoring tool for the farmers in deciding the concentrations of pesticides before its application into the paddy fields.

Key words : *Pesticide, Monocrotophos, Etroplus maculatus, Gills, Histopathology, MATC, AF*

Introduction

The agricultural chemicals have been found in the aquatic environment either as a result of direct contamination or applied directly to water to kill the undesirable organisms in order to stock most desirable fish species or plant crops such as water nut and lotus. In sublethal concentrations, living organ-

[1]*Associate Professor, [2]Director

isms survive for more hours with some metabolic alterations, but in acute toxicity, organisms cannot survive for longer periods. Insecticides, thus exerts an imbalance of the self-sustaining ecosystem and impart a great deal of stress on body growth, fertility and population size of fish and other organisms living therein.

The number of chemicals used as pesticides are increasing year after year, thereby causing fatal effects on fish fry and fingerlings, changes in the spawning as well as feeding grounds, restricting migration of fish, reducing disease resistance and deteriorating the quality of fish produce. Thus, pesticides are becoming potential dangers for the rejuvenation of fish wealth of the country.

The seasonal utilization of paddy fields for fish culture is quite common in Kerala and West Bengal. In recent years, with the advent of high yielding varieties of paddy, the use of pesticides has become very popular. Therefore, an assessment of environmental hazards due to toxic substances is an important challenge to toxicologists and ecotoxicologists.

Kuttanad, the rice bowl of Kerala, is a region where there is overdose application of pesticide during the punja cultivation periods. Dimecron, Monocrotophos, Henosan, Thymet, Fernoxan and Nuvacron are the major components of the pesticides being used in Kuttanad. In such a degraded aquatic environment, particularly where pollutants occur at chronic sublethal concentrations, changes in the structure and functions of aquatic organisms occur more frequently than their mass mortality.

Histological changes appear as a medium term response to sublethal stressors and histology provides a rapid method to detect the effects of irritants, especially chronic ones in various tissues and organs (John *et al.* 1990). With a thorough prior knowledge of normal anatomy, the investigator can use histological analysis to detect alterations in tissues and organs caused by exposure to toxicants. When concentration of a toxicant is sufficient to result only in cellular injury and not death, sublethal (adaptive) changes can be observed in affected cells. On the other hand, death of cells followed by a series of cellular reactions occurs without death of the organism.

The exposure of fish to chemical contaminants is likely to induce a number of lesions in body organs like gills, liver and kidney (Sulekha and Mercy, 2009; 2021a; 2021b; 2022). These organs are suitable for histological examination in order to determine the effect of extent of pollution (Hinton, 1993). Gills exhibit large surfaces, which are subjected to direct and permanent contact with potential irritants.

In the above background, it is felt that a study on the pesticide, monocrotophos, a widely used pesticide in Kuttanad, induced histopathological changes in the gills of *Etroplus maculatus*, a true denizen of Kuttanad, would be helpful in bringing out the lethal effect caused to fish wealth due to ubiquitous application of pesticides and thenceforth establishing the necessity for a judicious use of pesticides in agriculture in future. *E. maculatus* is considered as a food fish as well as an indigenous ornamental fish of Kerala.

Materials and Methods

The experiments on the sublethal toxicity of monocrotophos, an organophosphate pesticide, on the juveniles of *Etroplus maculatus* were conducted for 30 days during the period of investigation. The experiments were conducted in wet lab which has concrete floor with gentle slope, having proper drainage to remove pesticide contaminated water to minimize the hazards. There were provisions for water supply, lighting and adequate ventilation in the shed. The experiment was done in cement cisterns of 35 litres capacity.

Juveniles of *E. maculatus* were collected from pollution free ponds from the natural habitat. The average size of *E. maculatus* with 4.75 ± 0.9cm in total length and 3.30 ± 0.8mg in weight were used for monocrotophos exposure. Monocrotophos is a water soluble organophosphate and is a broad-spectrum systemic and contact insecticide-cum-acaricide with long term residual action. This is effective against sucking, chewing and mining insects on paddy, maize, barley, etc.

Based on the LC_{50} value (3.36ppm) obtained (Mercy *et al*. 2000b), five nominal concentrations of the pesticide were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on (Konar, 1969; Sprague, 1973). The concentrations of pesticide used for each sublethal exposure were 0.0ppm, 0.1ppm, 0.3ppm, 0.6ppm 1.0ppm and 1.5ppmof monocrotophos. Sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 hr to maintain the desired pesticide concentration. A control free of pesticide was also maintained in the experiment. All the treat-

ments and the controls were made in triplicates. Ten healthy fishes, chosen at random from the acclimated stock were reared in 32 litres of water in seasoned cement cisterns. Well water was used for the experiment and fresh water was filtered using nylon bolting cloth and aerated to saturation prior to use. The fishes were fed once a day on fresh clam meat *ad libitum*.

Water quality parameters in the experimental tanks were measured by the following methods. Modified standard Wrinkler's method (Strictland and Parsons, 1972) was used for measuring the dissolved oxygen. pH was tested using universal pH indicator solution method. Temperature tested using thermometer with an accuracy of 0.1°C. After 30 days of the experiment five specimens from each of the treated as well as the control group were sacrificed and gills, the target organ was dissected out and fixed immediately in Bouin's fluid. This fixed organ was washed, dehydrated, cleared and embedded in paraffin wax. Serial sections of the organ were taken at 3 to 5µm thickness and stained with Hematoxylin-eosin staining procedures (Stevens, 1982). Detailed histological observations were carried out with the help of a binocular microscope. Based on the histological lesions observed in the gills of these fishes the index values were calculated. The method followed for the analysis is that of (Poleksic and MItrovic-Tutundzic, 1994) who have classified the gill lesions based on two criteria

First criterion

The first criterion classified gill lesions, based on the type and location of the damaged gill tissue, into five main groups.

(a) hypertrophy and hyperplasia of gill epithelia and related changes

(b) changes in the mucous and/or chloride cells

(c) gill parasites

(d) blood vessel changes

(e) terminal stages

Second criterion

The second criterion - of severity, is based on the scope for repair of the lesions, i.e. the possibility of restoration of normal morphological structure with an improvement in the environmental conditions, or the cessation of pollution.

With regard to this second criterion, gill lesions are classified into three progressive stages:

(a) for the first stage 10^0

(b) for the second stage 10^1

(c) for the third stage 10^2

Of the total of 26 types of gill change, 19 belong to the first stage, 5 to the second and 2 to the third (Poleksic and Mitrovic-Tutundzic, 1994).

$$I = \sum_{i=1}^{19} a_i + 10 \sum_{i=1}^{5} b_i + 10^2 \sum_{i=1}^{2} c_i$$

The sum of the number of lesion types within each of the three stages multiplied by the stage index as above represents the numerical value of the degree of damage in a single fish gill, i.e.

Where I is the degree of changes in a single fish gill

a = first stage alterations

b = second stage alterations

c = third stage alterations

The method of calculating a value of I marks it possible to compare the degree of tissue change in a large number of fish from different situation of pollution, and to correlate the intensity of pollution with the intensity of the changes found.

Application of the mathematical equation to derive categories of gill damage

Effects of I values are denoted as follows

***I* values**	**Effects**
0-10	Functionally normal gills
11-20	Slightly to moderately damaged gills
21-50	Moderately to heavily damaged gills
>100	Irreparably damaged gills.

Based on these I values, obtained for the gills in each experiment No Observable Effect Concentration (NOEC - it is the concentration at which there is no significant change in the histology from that of the control) and Least Observable Effect Concentration (LOEC - in which there are significant changes in the histological structure from that of the control) is determined. Concentrations which showed I values less than 10 (functionally normal) were taken as NOEC and concentrations that gave I values between 10 and 20 were taken as LOEC.

Based on these NOEC and LOEC values MATC - the concentration in which the animal can tolerate for survival and reproduction without much effect on its metabolic activities - is calculated using the formula

$$MATC = (NOEC \times LOEC)^{1/2}$$

From this MATC value, application factor was calculated using the formula (Mount and Stephan, 1967).

Table 1 The intensity of alterations in each *E.maculatus* treated with monocrotophos

Concentrations (ppm)	C					0.1					0.3					0.6					1.0					1.5				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
GILL ALTERATIONS **FIRST STAGE**																														
1. Hypertrophy of respiratory epithelium	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. Irregular hyperplasia of epithelial cells	+	+	-	-	-	+	+	+	-	-	+ +	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. Lifting of respiratory epithelial cells	-	-	-	-	-	+	+	-	-	-	+ +	+	+	+	-	+ + +	+ +	+ +	+	+	+ + +	+ + +	+ +	+ +	+	+ + +	+ + +	+ + +	+ +	+ +
4. Thinning of respiratory epithelium	-	-	-	-	-	+	+	-	-	-	+ +	+	+	+	-	+ +	+ +	+	+	-	-	-	-	-	-	+ + +	+ +	+ +	+ +	+
5. Focal hyperplasia of epithelial cells	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. Hyperplasia from the base to approximately half the length of the secondary lamellae	-	-	-	-	-	-	-	-	-	-	+ +	+	+	-	-	+ +	+ +	+ +	+	-	+ + +	+ +	+	+	+	-	-	-	-	-
7. Fusion of tips of secondary lamellae	-	-	-	-	-	+	+	+	-	-	+ +	+	+	-	-	-	-	-	-	-	-	-	-	-	-	- + +	-	-	-	-
8. Fusion of primary lamellar tips	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
9. Fusion of several secondary lamellae	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10. Shortening of secondary lamellae	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+ + +	+ +	+	+	+	+ + +	+ + +	+ +	+ +	+ +	+	+ + +	+ + +	+ +	+ +
11. Empty mucuous cells or their disappearance	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+ +	+	-	-	-	+ +	+	+	+	-
12. Lamellar telangiectasis	-	-	--	-	-	-	-	-	-	-	+ +	+ +	+	-	-	+ +	+ +	+	+	-	+ + +	+ +	+	+	+	+ + +	+ + +	+ +	+ +	+
SECOND STAGE																														
1. Haemorrhage with rupture of epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+ + +	+	-	-	+ +	+ +	+ +	+	+	+ + +	+ + +	+ +	+ +	+ +
2. Stasis																														
THIRD STAGE																														
1. Scar tissue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
2. Necrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+ + +	+ +	+	+	+	+ + +	+ + +	+ +	+	+

$$AF = \frac{MATC}{48hr\ LC_{50}}$$

The Application Factor (AF), which is worked out for one species can be used for other related species also, provided their 48hr LC_{50} is known. From this the MATC for latter species can be conveniently found out. This will be an important tool for the farmers in deciding the concentrations of pesticides before its application into the paddy fields.

An evaluation of histopathological changes in the vital organs of fish exposed to different concentrations of pesticides would help in assessing the magnitude of pesticide induced pathogenesis in fish.

Results

Physico-chemical parameters

Weekly mean temperature, pH and DO values ranged from 28.0 to 28.4°C, 6.8 to 7.5 and 6.2 to 7.1 $mg.l^{-1}$ respectively.

Histopathological observations

Structure of the gill of Etroplus maculatus

In *E. maculatus* each holobranch consists of an anterior and posterior hemibranch. Each hemibranch in turn consists of a series of thin leaf-like gill filaments referred to as the primary lamella arranged alternately on either side of the interbranchial septum. They have a large number of longer secondary filaments. The gill filaments are lined externally with a thick stratified epithelium or the gill filament epithelium (Hughes *et al.* 1979). Mucous cells are scattered in the interlamellar epithelium. Chloride cells are few or absent because *E. maculatus* is reared in fresh water. The chloride cells are the characteristic of marine fishes (Zadunaisky, 1984). The lamellae, which are the actual sites of the gaseous exchange, are arranged on the two sides of the filaments. These lamellae are lined by an epithelium that is two squamous cell layers thick. Internal to the epithelium is the lamellar blood sinus lined and spanned by pillar cells of contractile function. The marginal blood channel lined by endothelium occurs at the apex of the lamella. The details of the number of fishes examined under the monocrotophos exposure and damages noticed in each fish are summarised in the Table 1.

Control: Hypertrophy (Fig.1-A) and hyperplasia (Fig.1-B) could be observed in a few lamellae. The index value calculated for the gills in control was 1.0 (Table 2).

0.1 ppm: Histological details of the gills of fishes exposed to this lowest concentration of monocrotophos varied much from that of the gill structure of control fishes. A few lamellae of all the fishes exhibited hypertrophy (Fig.1-C), hyperplasia (Fig.1-C) and oedematous separation of respiratory epithelium (Fig.1-D). Tips of several secondary lamellae were fused (Fig. 1-E). A few secondary lamellae exhibited shortening upto half of its length and certain others showed thinning of secondary lamellae (Fig. 1-F). Focal hyperplasia and distorted secondary lamellae could be rarely observed (Fig. 1-F). Certain lamellae exhibited empty mucous cells near the base of the secondary lamellae (Fig. 1-G). The index value calculated for these changes is 6.4 (Table 2).

Table 2. The index values of the gill alterations (following Poleksic and Mitrovic-Tutundizic, 1994)

***E. maculatus* treated with monocrotophos**	
Treatment (ppm)	Index value
0.0	1.0
0.1	6.4
0.3	8.2
0.6	11.2
1.0	105.2
1.5	148.0

Index values represents average of 5 fishes

0.3 ppm: The characteristic changes of the gill lamellae exposed to 0.3ppm consisted of hypertrophy (Fig.1-H), proliferation of lamellar and filamental epithelium giving an abnormally thickened appearance and telangiectasis (Fig.1-I). Fusion of tips of certain secondary lamellae (Fig.1-H, I) was rarely observed. Hyperplasia fused the secondary lamellae approximately upto half of its length in a few lamellae (Fig. 1-J) and certain other regions exhibited fusion of several secondary lamellae (Fig. 2-A 13). Oedematous separation of respiratory epithelium with the appearance of spaces from base to tip of the lamellae was quite common and conspicuous in all fishes (Fig. 1-J; Fig.2-A). Distortion of secondary lamellae, shortening of secondary lamellae (Fig.1-J) and telangiectasis (Fig.1-K) were frequently observed. Vacuolation or emptying of mucous cells at the base of the secondary lamellae (Fig.1-L) was also observed. In two fishes, distortion of both pri-

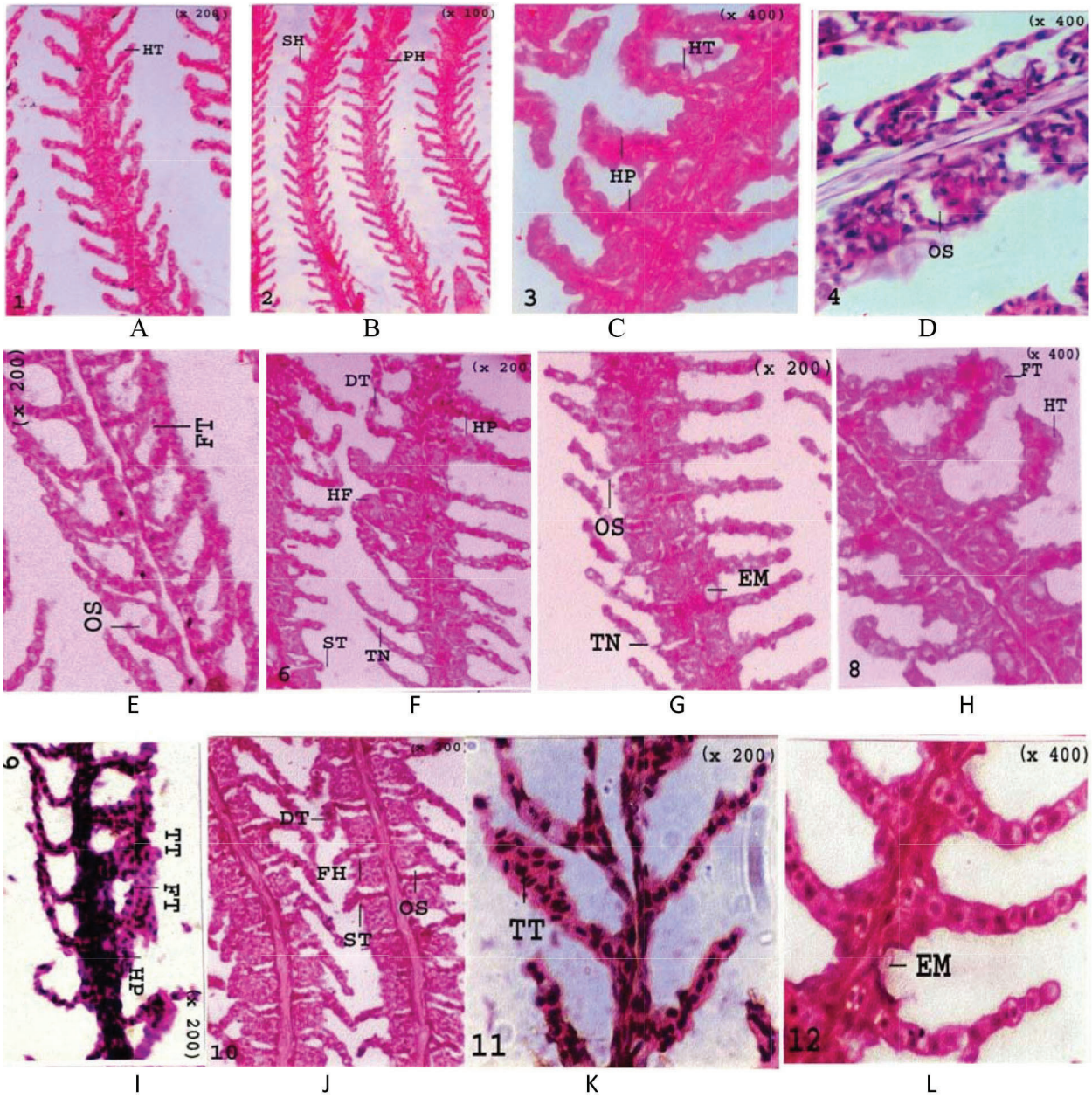

Fig. 1. Gills of *E. maculatus* treated with monocrotophos: [A] Control hypertrophy (HT). H+Ex200. **[B]** Control - secondary lamellar hyperplasia (SH) and primary lamellar hyperplasia (PH). H+Ex100. **[C]** 0.1 ppm - hypertrophy (HT) and hyperplasia (HP). H+Ex400. **[D]** 0.1 ppm- oedematous separation of respiratory epithelium (OS). H+Ex400. **[E]** 0.1 ppm - tips of several secondary lamellae fused (FT). H+Ex200. **[F]** 0.1 ppm- hyperplasia (HP), Focal hyperplasia (HF), shortening of secondary lamellae (ST), thinning of secondary lamellae (TN), and distortion of secondary lamellae (DT). H+Ex200. **[G]** 0.1 ppm - empty mucous cells (EM) oedematous separation of respiratory epithelium (OS) and thinning of secondary lamellae (TN). H+Ex200. **[H]** 0.3 ppm - hypertrophy (HT) and fusion of tip of secondary lamellae (FT). H+Ex400. **[I]** 0.3 ppm - hyperplasia (HP), telangiectasis (TT), and fusion of tips of several secondary lamellae (FT). H+Ex200. **[J]** 0.3 ppm - hyperplasia fused approximately half the length of secondary lamellae (FH), oedematous separation of respiratory epithelium (OS), distortion of secondary lamellae (DT) and shortening of secondary lamellae (ST). H+Ex200. **[K]** 0.3 ppm- telangiectasis (TT). H+Ex200. **[L]** 0.3 ppm - empty mucous cells (EM). H+Ex400.

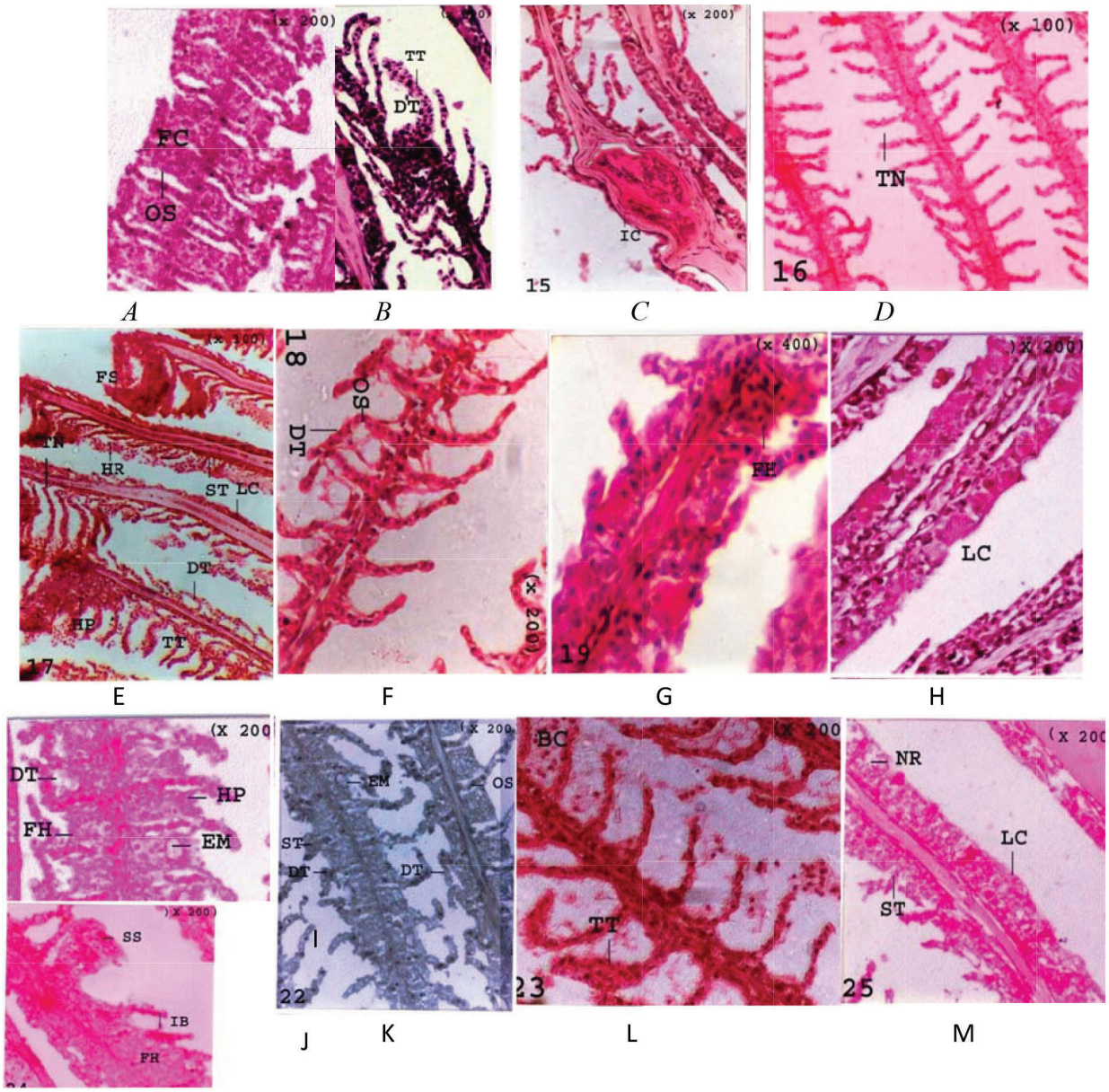

Fig. 2 Gills of *E. maculatus* treated with monocrotophos: [A] 0.3 ppm - complete fusion of secondary lamellae (FC), oedematous separation of respiratory epithelium (OS) and stasis (SS). H+Ex200. **[B]** 0.3 ppm- distortion of secondary lamellae (DT), telangiectasis (TT) and complete loss of secondary lamellae (LC). H+Ex200. **[C]** 0.3 ppm - irregularly shaped cartilage (IC). H+Ex200. **[D]** 0.3 ppm - thinning of secondary lamellae (TN). H+Ex100. **[E]** 0.6 ppm - hyperplasia (HP), fusion of secondary lamellae (FS), telangiectasis (TT), shortening of secondary lamellae (ST), complete cross of secondary lamellae (LC), haemorrhage (HR), thinning of secondary lamellae (TN), and distortion of secondary lamellae (DT). H+Ex100. **[F]** 0.6 ppm - oedematous separation of respiratory epithelium (OS) and distortion of secondary lamellae (DT). H+E x 200. **[G]** 0.6 ppm - hyperplasia approximately half the length of secondary lamellae (FH). H+Ex400. **[H]** 0.6 ppm - complete loss of secondary lamellae (LC). H+Ex200. **[I]** 1.0 ppm - hyperplasia (HP), fusion of secondary lamellae approximately half the length (FH), empty mucous cells (EM) and distortion of secondary lamellae (DT). H+Ex200. **[J]** 1.0 ppm - stasis (SS), fusion of secondary lamellae approximately half the length (FH) and infiltration of blood cells into the secondary lamellae (IB). H+Ex200. **[K]** 1.0 ppm - oedematous separation of respiratory epithelium (OS), shortening of secondary lamellae (ST), distortion of secondary lamellae (DT) and empty mucous cell (EM). H+Ex200. (Monochrome photograph). **[L]** 1.0 ppm - telangiectasis (TT) and blood cells (BC). H+Ex200. **[M]** 1.0 ppm - necrosis (NR), complete loss of secondary lamellae (LC) and shortening of secondary lamellae (ST). H+Ex200.

mary and secondary lamellae, or complete loss of secondary lamellae, destroyed the normal architecture of the gills (Fig.2-B). Broad and irregularly shaped cartilaginous region was noted in two lamellae of one fish (Fig.2-C). At these regions primary lamellae were devoid of secondary lamellae. Thinning of secondary lamellae was also rarely observed (Fig.2-D). The index value obtained for the damages in this concentration is 8.2 (Table 2).

0.6 ppm: Hyperplasia, fusion of secondary lamellae, telangiectasis, shortening of secondary lamellae (Fig.2-E), A series of secondary lamellar telangiectasis and they were fused to one another. Secondary lamellae of adjacent filaments were also fused in rare cases (Fig.2-E). Thinning of secondary lamellae due to the degeneration of cells and distortion of secondary lamellae could also be frequently noticed (Fig.2-E). Haemorrhage - a severe damage was noticed in a few fishes in this exposure (Fig.2-E). Oedematous separation of respiratory epithelium (Fig.2-F) were the changes noticed in gills of all the fishes exposed to this concentration. Hyperplasia fused the secondary lamellae approximately half of its length (Fig.2-G). In certain regions of the filaments, the secondary lamellae were completely lost (Fig.2-E and Fig.2-H). The index value calculated for this concentration is 11.2 (Table 2).

1.0 ppm: The gill lamellae of all the fishes exposed to this concentration showed hyperplasia and fusion of secondary lamellae (Fig.2-I), stasis (Fig.2-J), oedematous separation of respiratory epithelium (Fig.2-K), telangiectasis (Fig.2-L), and shortening of secondary lamellae (Fig.2-M). The primary lamellar epithelial hyperplasia fused the secondary lamellae approximately half of its length; empty mucous cells seen in the interlamellar spaces appeared as vacuoles (Fig.2-J). Infiltration of blood cells into the secondary lamellae and marginal blood vascular sinusoid resulted in stasis (Fig.2-J). Certain lamellae exhibited complete loss of secondary lamellae (Fig.2-M). Necrosis was observed in some fishes (Fig.2-M). The mucous cell hyperplasia (mucinous metaplasia) fused the tips of primary lamellae in many regions (Fig.3-A). The gills of most of the fishes in this concentration also exhibited haemorrhage (Fig.3-B). In the fused secondary lamellae near the margin, large number of blood cells could also be noticed, which is a unique observation in this exposure. The increase in the length of secondary lamellae also could be observed in this exposure (Fig.3-C). These lamellae as well as other lamellae distorted distinctly. The index value calculated for this concentration is 105.2 (Table 2).

1.5 ppm: The characteristic histological changes observed in the gills of all the fishes exposed to this concentration are as follows. Hyperplasia and the fusion of several secondary lamellae resulted in the obliteration of interlamellar spaces in all the fishes (Fig.3-D). In certain filaments the severe hyperplasia did not form the complete fusion of secondary lamellae because of the degeneration of these hyperplastic cells (Fig.3-D). Fusion of the tips of all the secondary lamellae of the same filament (Fig.3-E) and that of adjacent filaments in certain cases could be frequently observed (Fig.3-H). Empty mucous cells were seldom seen in the primary lamellar hyperplastic cells (Fig.3-E). Thinning of secondary lamellae, infiltration of blood cells into the secondary lamellae, and stasis could be observed in certain lamellae (Fig.3-F). A number of lamellae exhibited telangiectasis and distortion of secondary lamellae (PFig.3-G). A series of lamellae exhibited secondary lamellar hyperplasia that resulted in increasing the length of the lamellae (Fig.3-I). Appearance of fibrosis was also seen at the filamental region (Fig.3-I). Oedematous expansion of the respiratory lamellae with the appearance of wide space from the base of the lamellae and shortening of secondary lamellae were also very common and conspicuous changes in all the fishes (Fig.3-J). Complete loss of secondary lamellae and the degeneration of primary lamellar epithelium changed the normal architecture of the gills (Fig.3-K). Haemorrhage (Fig.3-J, Fig.3-L) and necrosis (Fig.3-K) were the two severe damages that were seen in the lamellae exposed to this concentration. Most of the gill lamellae were clumped together with the blood and mucous (Fig.3-L). The index value calculated is 148 (Table 2).

In all the sublethal concentrations studied, pathological changes in gills showed a dose-related degeneration. The no observable effect concentration (NOEC) in this study is taken as 0.3 ppm and the least observable effect concentration (LOEC) is taken as 0.6 ppm (based on the index values - Table 3). From this the maximum allowable toxicant concentration (MATC) calculated $(NOEC \times LOEC)^{1/2}$ is 0.4243. Based on the MATC the application factor (AF) is calculated

$AF = MATC / 48\ hr\ LC_{50} = 0.4243 / 3.36 = 0.1263$

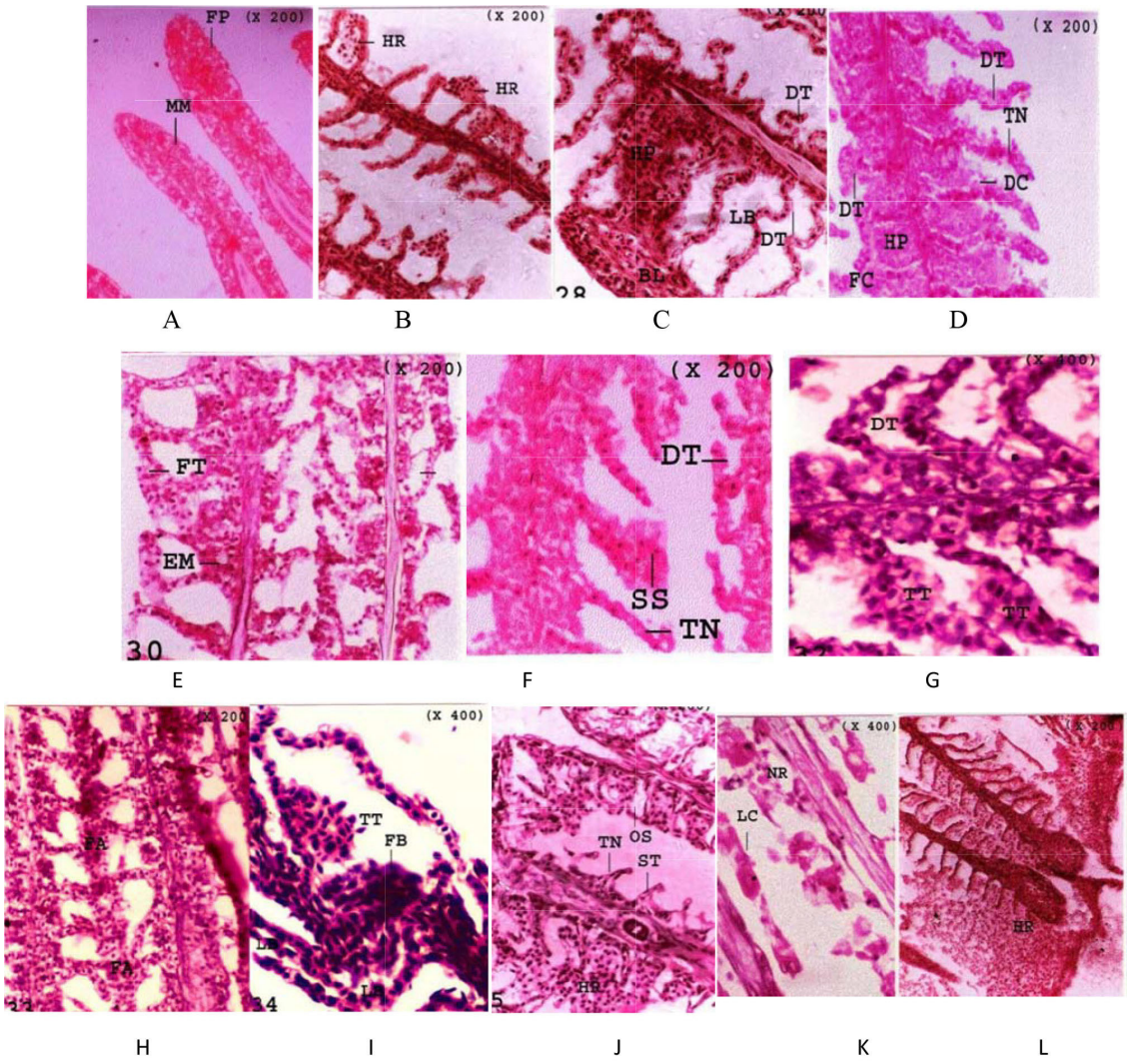

Fig. 3 Gills of *E. maculatus* treated with monocrotophos: [A] 1.0 ppm - fusion of tip of primary lamellae (FP) and mucinous metaplasia (MM). H+Ex200. **[B]** 1.0 ppm - haemorrhage (HR). H+Ex200. **[C]** 1.0 ppm increase of length of secondary lamellae (LB), blood channel (BL), hyperplasia (HP) and distorted secondary lamellae (DT). H+Ex200. **[D]** 1.5 ppm - hyperplasia resulted complete obliteration of inter lamellar space (HP), complete fusion of secondary lamellae (FC), degeneration of cells (DC), thinning of secondary lamellae (TN) and distortion of secondary lamellae (DT). H+Ex200. **[E]** 1.5 ppm - fusion of tips of secondary lamellae (FT), oedemation separation of secondary epithelium (OS), and empty mucous cells (EM). H+Ex200. **[F]** 1.5 ppm - stasis (SS), infiltration of blood cells into the secondary lamellae (IB), thinning of secondary lamellae (TN), distortion of secondary lamellae (DT), and shortening of secondary lamellae (ST). H+E x200. **[G]** 1.5 ppm - telangiectasis (TT) and distortion of secondary lamellae (DT). H+Ex400. **[H]** 1.5 ppm - fusion of tips of secondary lamellae of adjacent filament (FA). H+Ex200. **[I]** 1.5 ppm - increased length of lamellae (LB), tetangiectasis (TT) and Fibrosis (FB). H+Ex400. **[J]** 1.5 ppm - haemorrhage (HR), shortening of secondary lamellae (ST), oedematous separation of respiratory epithelium (OS) and thinning of secondary lamellae (TN). H+Ex200. **[K]** 1.5 ppm - complete loss of secondary lamellae (LC) and necrosis (NR). H+Ex400. **[L]** 1.5 ppm - haemorrhage (HR). H+Ex200.

Discussion

Etroplus maculatus exposed to various sublethal concentrations of monocrotophos exhibited almost comparable pathological lesions in the gill tissues. The pathological changes were obviously dose–dependent and maximum severity was noticed in the gills of fishes exposed to the highest sublethal concentrations. The overall changes observed due to the exposure to different concentrations of monocrotophos

are hypertrophy, hyperplasia, fusion of secondary lamellae, oedematous separation of respiratory epithelium, lamellar telangiectasis, stasis, filamental blood vessel enlargement and necrosis.

Hypertrophy of the respiratory epithelium was observed in the gills of some of the fishes. This increases the distance across which water born irritants must diffuse to reach the blood stream (Mallatt, 1985). In the present study, these changes found in lower concentrations and also in control fishes manifested the possibility that they are the first stage of defense response of the fishes as suggested by (Morgan and Tovell, 1973).

In the present study varying degrees of hyperplasia – mild to acute – could be observed in accordance with the increasing concentration of pesticides. It could be observed that as concentrations increased, hyperplasia also increased which resulted in the interlamellar to interfilamentar fusion as reported by (Zahran *et al.* 2018; Devi and Mishra, 2013; De Silva and Samayawardhena, 2002; Rao *et al.* 2005). According to Rao *et al.* 2006 lamellar fusion could be protective, so that it diminishes the area of vulnerable gill surface. As concentration increased the respiratory surface area showed a reduction which gradually affected the functioning of the gills.

Oedematous separation of respiratory epithelium due to the lifting was very mild in the lower concentrations but the intensity and frequency increased towards the exposure to higher concentrations. According to Ghasemzadeh *et al*. (2015) the oedematous separation of gill epithelium from the basement membrane is due to the increased capillary permeability or lowered efficiency of epithelial cells in maintaining normal water balance. Extensive oedema in the gill has been reported by several scientists (De Silva and Samayawardhena, 2002, Rao *et al.* 2005; Rao *et al.* 2006; Ghasemzadeh *et al*. 2015; Roberts, 1989; Mishra and Shukla, 2003).

Towards the higher concentration of exposure, the separation was severe. In the case of 0.5 and 1.0 ppm phosphamidon treated *E. maculatus*, it is seen that the separation was complete except at the base and tip of the secondary lamellae, which may be an escape mechanism from the problems of osmoregularity rather than a defense mechanism. According to Babu *et al.* (2009) the telangiectasis as the burst of pillar cells. In the present study telangiectasis occurred due to disruption of pillar cells and was observed in sublethal concentrations of 0.3, 0.6, 1.0 and 1.5ppm monocrotophos.

In the present study, stasis was observed only in higher concentrations (1.0 and 1.5ppm). This supports Mallatt (1985) view that the vascular damage, usually was found only in animals exposed to very high doses or to animals that were near death. Both these changes, telangiectasis and stasis, observed in the present study, can be considered as the branchial defense response to pesticides as an inflammatory reaction as suggested by Mallatt (1985), Maharajan, and Parurukmani (2012), Clark *et al.* (1997), Skidmore and Tovell, (1972), Abel, (1974), Abel and Skidmore, (1975), Abel (1976).

Mallatt (1985) and Sulekha and Mercy (2021a) emphasized that the rupture and bleeding (haemorrhage) are noticed only under the most highly toxic conditions or the animals that were near death. The present study showed haemorrhage in the gills of fishes exposed to 0.6, 1.0 and 1.5ppm monocrotophos (higher concentrations of exposure). According to Maharajan, and Parurukmani (2012), cells comprising branchial blood vessel seemed relatively resistant to irritant substances. But the occurrence of haemorrhage in the fishes exposed to higher concentrations explains that they were exposed to a concentration beyond the tolerance limit.

Histopathological changes in the gills of fishes exposed to higher concentrations were exhibited degeneration and necrosis of the branchial epithelium resulting in gradual degradation and sloughing of this layer (Maharajan, and Parurukmani, 2012; Sulekha and Mercy, 2021a). This is because of the reason that the necrosis occurs under most highly toxic conditions (Rao *et al. 2005*; Rao *et al.* 2006; Mishra and Shukla, 2003; Skidmore and Tovell, 1972). They reported that epithelial necrosis and rupture are the most dose-dependent of branchial lesion types, as they are clearly more often re-

Table 3. Maximum allowable toxicant concentration and its corresponding application factor calculated based on the histopathological analysis of *E. maculates* treated with monocrotophos

Fish species	Pesticides	NOEC (ppm)	LOEC (ppm)	MATC (ppm)	48 hr LC50 (ppm)	AF
E. maculatus	Monocrotophos	0.3	0.6	0.4243	3.36	0.1263

ported under lethal than under sublethal conditions as in the present study.

Similar to the observation of Zahran *et al.* (2018), Devi and Mishra (2013), Abel and Skidmore (1975) the empty mucous cells or vacuoalation denoted the discharge of mucous. It is a defense response to pesticides, probably by interacting with the toxic ions and then resulting in the formation of a film of coagulated mucous on the surface of the gill, or by providing a physical barrier for macromolecules (Abel, 1976). It is quite clear that, as concentration increased the mucous cells were found damaged and hence in the higher concentrations this type of defense mechanism could not be observed. Haemorrhage in the gills of fishes exposed to pollutants reported by several authors (Sulekha and Mercy, 2021a; De Silva and Samayawardhena, 2002; Rao *et al.* 2005; Rao *et al.* 2006; Roberts, 1989).

E. maculatus exposed to 1.5pm monocrotophos showed fibrosis or scar tissue in primary as well as secondary lamellae. According to (Morgan and Tovell, 1973), gill lamellar telangiectasis is primarily caused by the disruption of pillar cells, as a result, capillary distention occurs and blood accumulation may lead to further fibrosis. Finally, there occurs a proliferation of fibroblasts and scar tissue. In the present study, fibrosis is found only in the higher concentrations, which might be due to the chronic exposure to the pesticides.

Distortion of secondary lamellae was one of the frequently observed gill alterations in almost all the concentrations of the current study. This may be due to the weakening of pillar cells. Mallatt (1985) has noted distortion or curling of secondary lamellae. In fact, cellular involutive processes were common in the starved fish during the long-term assays. Shortening of secondary lamellae was also reported by Sulekha and Mercy (2021a), De Silva and Samayawardhena, (2002), Rao *et al.* (2005), Rao *et al.* (2006), Roberts (1989).

In the present study, the shortening of secondary lamellae was characteristic in all the concentrations of monocrotophos treated on *E. maculatus*. According to Rombough and Garside (1977), the shortening of secondary lamellae could be induced merely by starvation. In fact, cellular involutive processes were common in the starved fish during the long-term assays. Walters and Plumb (1980) found histopathological changes such as shortening and hypertrophy of secondary lamellae leading to inter-lamellar obliteration in the gills of fish undergoing food deficiency. In the present study in higher concentrations the food intake was less and this might have led to the starvation of fishes.

Thinning of secondary lamellae appeared in almost all the concentrations of monocrotophos. Thinning of secondary lamellae was reported previously by Poleksic and MItrovic-Tutundzic (1994). In the present study, this thinning may be caused by the degeneration of secondary lamellar epithelial cells. The cartilage deformation in the present study might indicate that this is a progressive lesion.

Maximum Allowable Toxicant Concentration (MATC)

In the present study, NOEC, LOEC and MATC values of monocrotophos of *E. maculates* is calculated based on the histopathological studies on the gills. The intensity of pathology is quantified using the index values suggested by Poleksic and MItrovic-Tutundzic (1994). According to them, the concentration which showed index value less than 10 is taken as NOEC and between 10-20 is taken as LOEC. MATC of monocrotophos to *E. maculates* is estimated as 0.4243 ppm. It seems that this MATC value is very sensitive when compared to the MATC values of the same pesticides on other fishes such as rohu using other parameters (Babu *et al.* 2009). These NOEC, LOEC, MATC values and Application factor (0.1263) so obtained for this species can also be used to assess tentative water quality status for the pollutants in the natural environment, as the fish chosen for the study is the most representative test animal depicting the field situation of Kuttanad water bodies. Due to their non migratory nature they are prone to all sorts of pollutants added to the aquatic environment of Kuttanad.

The application factor derived from the laboratory studies can be directly applied to the filed situations, as they are the representative samples of Kuttanad. Their usefulness lies predominantly in the insight, which they provide on the toxic action of the contaminant, which can be helpful in establishing the relevance of the effect for fitness and survival. This knowledge of fundamental toxicological and pathological processes is not only important for the regulators of chemicals that are potentially aquatic pollutants, but also for researchers involved in field studies.

Histopathology as a tool for sublethal toxicity studies

The primary objective of environmental hazard

analysis and risk assessment is to arrive at concentration levels, which can protect environmental life even after long-term exposures. Although, a number of comparative studies on the toxicity of chemicals to single species and to communities have shown insignificant differences in the toxicity threshold values, the present knowledge is not sufficient with regard to safe extrapolation from acute and chronic no-effect levels for single species to no-effect levels for entire ecosystem

In almost all the previous works in toxicity studies, the end points used are mortality, survival and growth. In contrast, the present study, the histopathological parameters are evaluated to obtain the NOEC, LOEC and the MATC of the toxicants used.

Histopathology provides, evidences of adaptation to degeneration, and this certainly represents the major advantage of the use of histopathological alterations as biomarkers of environmental pollution by organic chemicals. Paperna and Van As (1983) reported that histopathological changes in fish tissues are the most sensitive parameters for the evaluation of chronic toxicity test effects when compared to biochemical and hematological parameters and also for the derivation of maximum admissible concentrations.

Hence it is suggested that histopathological studies on the gills of fishes could be used as a relatively sensitive method for estimating the harmful effects of different concentration of a specific pollutant and the long term damage to fish exposed to different levels for a limited period as reported by Arumugam *et al*. (2019). It also helps in the water quality monitoring and provides an 'early warning' of the conditions of fishes.

Conclusion

The study shows that the pathological changes are gradually increasing from the lowest concentration to the highest concentration. The Maximum Allowable Toxicant Concentration (MATC) based on the index value is 0.4243 ppm and the Application Factor (AF) is 0.1263. The application factor derived from this sublethal study can be directly applied to the field situations. Histopathology can be used as a tool in assessing the intensity of pesticides even at the sublethal levels. Hence preventive measures can be taken to protect the fishery resources.

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