Toxic effects of Trebon®, a synthetic pyrethroid based insecticide formulation, on Oreochromis mossambicus (Family: Cichlidae)

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Abstract

Trebon® (active ingredient: Etofenprox) is a recently developed potential pyrethroid insecticide formulation and reported to bring about adverse effects to non-targeted organisms in agricultural fields and associated habitats. The toxic effects of Trebon® on fingerlings of tilapia (Oreochromis mossambicus) were studied in the present investigation following standard laboratory experimentation procedures.

Results revealed that the 24-hr and 96-hr LC50s of Trebon® were 0.0657 mg L⁻¹ and 0.0087 mg L⁻¹ respectively which are nearly 23 times and 172 times below its recommended field dose which is 1.5 mg L⁻¹. It therefore appears that Trebon® brings about mortality to tilapia fingerlings at concentrations that are milder than the field dose within a short period of exposure. Gill hyperplasia, deformation and telangiectasis of secondary gill lamella, mucosal cell proliferation, lamella disorganization and fusion were observed in the insecticide exposed test fishes. The liver tissues were also damaged with intracellular vacuolation, aggregation of melanomacrophages, blood congestion and focal necrosis. The severity of these damages increased with the level of dosage and the exposure period. The fingerlings suffered respiratory distress while the body coloration gradually changed into a pale hue upon exposure to Trebon®.

Key words: Etofenprox, field dose, tilapia, toxic effects, Trebon®

Introduction

Etofenprox is a synthetic pyrethroid insecticide that is proved to be very effective in controlling agricultural insect pests. It is highly toxic to insects but is less toxic to mammals as there are no chronic effects reported on their reproduction, growth, or survival even at the highest concentrations tested (EFSA, 2008). However, Etofenprox is found to be toxic to many other non-targeted non-mammals such as fishes and other useful animals in agricultural fields and associated habitats. For example, Yameogo et al. (2001) showed the toxicity effect of Etofenprox (Vectron®) on Tilapia niloticus and Tilapia zillii and found that the 24-hr LC50 were 8.4 mg L⁻¹ and 5.0 mg L⁻¹ respectively. WHO (2007) reported that the LC50 for
rainbow trout (*Oncorhynchus mykiss*) and blue green sunfish (*Lepomis macrochirus*) exposed to Etofenprox were 2.7 µg L⁻¹ and 13.0 µg L⁻¹ respectively. Orrick and Pease (2009) showed that under chronic exposure conditions Etofenprox brings about mortality to Zebra fish (*Brachydanio rerio*), reproductive effects to water fleas (*Daphnia magna*) and decreased growth to midges (*Chironomus riparius*). Sreehari et al. (2009) showed the residual effects of Etofenprox on household animals. Etofenprox remains in the environment for a considerable period of time with a half-life of 1 to 4 weeks in aerobic soil, 4 to 8 weeks in aerobic water bodies and 6 months in anaerobic water bodies including inland reservoirs (EFSA 2008; Orrick and Pease 2009). Etofenprox is widely available in the market under many trade names and Trebon® is one of them. Trebon® was first introduced to Sri Lanka in 2010, and at present it is popular and widely used particularly in the dry zone of the country where paddy cultivation is intensively carried out (pers. com. Agriculture Department, Sri Lanka). In the dry zone there is a large number of reservoirs to provide an uninterrupted water supply to the paddy cultivation. The food fish, tilapia (*Oreochromis mossambicus*, Peters 1852) thrives in these reservoirs and supports a profitable fishery too. Therefore any negative effects, if any, caused by Trebon® on them would be a direct threat to the tilapia fishery, and eventually nutritional and economic status of the rural poor in the dry zone. Therefore the present laboratory investigation was carried out with a view to determine the toxic effect of Trebon® on tilapia with particular emphasis to determine its 24-hr and 96-hr LC₅₀, to assess the histopathological changes in the gills, liver and intestine and to assess the changes of their behaviour and body colouration upon exposure to Trebon® following standard laboratory experimentation procedures.

**Materials and Methods**

Tilapia fingerlings (average length 1.00 cm and weight 0.9 g) were purchased from a commercial fish breeding centre, and were acclimated to laboratory conditions under natural photoperiod for 14 days in aged tap water. During this acclimation period continuous aeration was provided and the fingerlings were fed twice daily with commercially available fish feed at 5% of their body weight. The unconsumed food pellets and faecal matter accumulated at the bottom were siphoned out daily to ensure a proper hygienic condition in the acclimation tank.

The insecticide Trebon® was purchased from a local agrochemical shop. The commercial formulation of Etofenprox in this product is 100 g L⁻¹. According to the manufacturer the recommended field dose of this product is 1.5 mg L⁻¹ (10 L field dose per ha).

Initially, a "range finding test" was conducted to determine the approximate range of Trebon® concentrations that would bring about tilapia mortality. For this test, a series of 10 Trebon® concentrations ranging from 1.5 mg L⁻¹ to 0.003 mg L⁻¹ were prepared in 10 separate 5 L containers by diluting the recommended field dose (i.e. 1.5 mg L⁻¹) with aged tap water as required in a half-wise manner. A control set up containing only the aged tap water was also prepared (Table 1). Immediately after the test solutions were prepared a batch of 5 acclimated tilapia fingerlings were
introduced into each container and exposed for a period of 4 days (96-hr). During this exposure period containers were aerated continuously but the fingerlings were not fed. At the end of each day, the number of dead fingerlings, if present, at each container was counted and the carcasses were discarded together with siphoning out the faecal accumulation on the bottom.

According to the range finding test results, 100% mortality occurred at 1.5 mg L\(^{-1}\), 0.75 mg L\(^{-1}\), 0.375 mg L\(^{-1}\), 0.1875 mg L\(^{-1}\), 0.0938 mg L\(^{-1}\) preparations, while it was 20% in 0.0469 mg L\(^{-1}\), 0.0235 mg L\(^{-1}\), 0.0118 mg L\(^{-1}\), 0.0059 mg L\(^{-1}\) and 0% in the control (Table 1) within the first 24-hr. Results of this range finding test concluded that the Trebon® concentrations for the definitive acute toxicity test should be between 0.003 mg L\(^{-1}\) and 0.0469 mg L\(^{-1}\).

Table 1. Percentage mortality of tilapia at the 24-hr and at 96-hr exposure periods at the 10 different concentrations of Trebon® tested in the range finding test. Note that 1.5 mg L\(^{-1}\) is the recommended field dose.

| Trebon® concentration mg L\(^{-1}\) | Percentage mortality of tilapia |
|-----------------------------------|---------------------------------|
|                                   | 24-hr exposure period. | 96-hr exposure period. |
| 1.5                               | 100                        | 100                     |
| 0.75                              | 100                        | 100                     |
| 0.375                             | 100                        | 100                     |
| 0.1875                            | 100                        | 100                     |
| 0.0938                            | 100                        | 100                     |
| 0.0469                            | 20                         | 100                     |
| 0.0235                            | 20                         | 40                      |
| 0.0118                            | 20                         | 20                      |
| 0.0059                            | 20                         | 20                      |
| 0.003                             | 20                         | 20                      |
| 0.0 Control                       | 0                          | 0                       |

Based on the results of range finding test a definitive acute toxicity test was carried out to determine the exact range of Trebon® concentration that brings about tilapia mortality. In this test, 10 Trebon® solutions where the concentrations ranged from 0.0015 mg L\(^{-1}\) to 0.0469 mg L\(^{-1}\) (i.e. 0.0015 mg L\(^{-1}\), 0.003 mg L\(^{-1}\), 0.0045 mg L\(^{-1}\), 0.0059 mg L\(^{-1}\), 0.0089 mg L\(^{-1}\), 0.0118 mg L\(^{-1}\), 0.0177 mg L\(^{-1}\), 0.0235 mg L\(^{-1}\), 0.0352 mg L\(^{-1}\) and 0.0469 mg L\(^{-1}\)) were prepared in quadrupled replicates in rectangular glass aquaria by diluting the Trebon® stock solution as required with aged tap water to a volume of 30 L each. A control set up with only aged tap water and an additional solution of 0.0015 mg L\(^{-1}\) were also prepared. Immediately after these test solutions were prepared, a batch of 10 acclimated tilapia fingerlings were introduced into each aquarium and exposed for a period of 96-h. As in the range finding test, fingerlings were not fed but the tanks were aerated continuously during the entire 96-hr exposure period. The number of dead fishes, if present, in each aquarium was recorded separately after the 24-hr and 96-hr exposure periods.
Of the tilapia that were moribund after 24-hr and 96-hr in each test solution in the definitive acute toxicity test, one was picked randomly and dissected for its gills, liver, and intestine. These body parts were preserved in freshly prepared buffered formalin and embedded in paraffin wax after standard dehydration procedure. Histological sections were obtained to a thickness of 5µm using a microtone and were stained with Hematoxylin and Eosine following standard staining procedure. Abnormalities of these tissue sections were examined under high power light microscope and compared with healthy tissue sections of the control fish to assess the degree of histopathological changes caused by Trebon®.

It was expected for the fishes to randomly disperse in the test aquaria at each solution in the definitive acute toxicity test, but was noted that they preferred to gather around the aerator stones gulping air bubbles. The total number of tilapia that gathered around the aerator stone in each test solution and elsewhere in the tank were recorded separately immediately at the end of the first 24-hr exposure period. Their body colour changes were also recorded after the 96-hr exposure period. Further, the temperature, dissolved oxygen concentration and pH of the test solution in each aquaria were measured in triplicate readings with a hand held glass thermometer, Dissolved oxygen meter (model; Oxi 315i) and pH meter (model; pH 315i) respectively daily during the entire 96-hr experimentation period.

Fish mortality data were analyzed using Probit analysis (Finney 1971). In this method, the lognormal concentration of Trebon® preparations and the corresponding mortality data of the fingerlings were analysed to obtain the 24-hr LC50 value and the 95% confidence limits. The same procedure was repeated for the remaining three sets of replicates so that an overall LC50 value and 95% confidence limits could be obtained. This analytical procedure was repeated to determine the overall 96-hr LC50 value and 95% confidence limit too. The fingerlings dispersed throughout the test tanks and those remained around the aerator stone in each test concentration for the 24-hr exposure period was analysed with one-way ANOVA after testing for normality. Tukey’s pairwise test was carried out in a pair wise manner when the ANOVA yielded a significant result (p < 0.05). Variation of the pH, temperature and dissolved oxygen between the test concentrations and the exposure periods were analyzed with two-way ANOVA. These experimental data were analyzed in Minitab Statistical Software Package for Windows (ver. 14) at α = 0.05.

Results

Result of the range finding test revealed that the tilapia mortality occurs at Trebon® concentrations between 0.0469 mg L⁻¹ and 0.003 mg L⁻¹. The mean % mortality of tilapia fingerlings in the acute toxicity test at the end of the 24-hr and 96-hr exposure periods are shown respectively in Table 2 and in Figure 1. It is evident that the mortality rate of tilapia fingerlings increased both with the Trebon® concentration and the exposure period.

The 24-hr LC50 values for the four replicates were 0.074 mg L⁻¹, 0.0617 mg L⁻¹, 0.0683 mg L⁻¹ and 0.0586 mg L⁻¹ respectively (Figure 2) and (Table 3). The 95% confidence limits of these four LC50 values are narrow and overlapping so that
LC$_{50}$ values, SE values and 95% confidence limits were pooled separately to calculate the overall mean values. Accordingly, the mean LC$_{50}$ value for the 24-hr exposure period was found to be 0.0657 mg L$^{-1}$ while its SE and the 95% confidence limits were 0.0312 – 2.0327 mg L$^{-1}$ (Table 3).

Table 2. The mean % mortality of tilapia at increasing concentrations of Trebon® in the definitive acute toxicity test at 24-hr and 96-hr exposure periods.

| Trebon® concentration mg L$^{-1}$ | Mean percentage mortality of tilapia (n = 40) |
|----------------------------------|---------------------------------------------|
|                                  | 24-hr exposure period. | 96-hr exposure period. |
| 0.0 Control                      | 0 ± 0                          | 0 ± 0                          |
| 0.0015                           | 0 ± 0                          | 0 ± 0                          |
| 0.003                            | 10 ± 0                         | 20 ± 4.1                       |
| 0.0045                           | 10 ± 0                         | 30 ± 4.1                       |
| 0.0059                           | 10 ± 0                         | 40 ± 4.1                       |
| 0.0089                           | 10 ± 0                         | 47.5 ± 2.5                    |
| 0.0118                           | 12.5 ± 2.5                     | 47.5 ± 2.5                    |
| 0.0177                           | 20 ± 0                         | 90 ± 5                         |
| 0.0235                           | 22.5 ± 2.9                     | 95 ± 5                         |
| 0.0352                           | 35 ± 2.9                       | 100 ± 0                        |
| 0.0469                           | 57.5 ± 2.5                     | 100 ± 0                        |

Table 3. The 24-hr LC$_{50}$ values of Trebon® for tilapia fingerlings, standard errors and the 95% confidence limits for the four replicates.

| Replicate No | LC$_{50}$ (mg L$^{-1}$) | SE  | 95% confidence limit (mg L$^{-1}$) |
|--------------|-------------------------|-----|-----------------------------------|
| 1            | 0.074                   | 0.0474 | 0.0331 – 3.7506                   |
| 2            | 0.0617                  | 0.0334 | 0.0304 – 1.0186                   |
| 3            | 0.0683                  | 0.0419 | 0.0314 – 2.6728                   |
| 4            | 0.0586                  | 0.0298 | 0.0299 – 0.6889                   |
| Mean         | 0.0657                  | 0.0381 | 0.0312 – 2.0327                   |
Figure 1. Variation of mortality of tilapia in the acute toxicity test. Mean % mortality of tilapia ± SE at increasing Trebon® concentration at the 24-hr (black bars) and at 96-hr (white bars) exposure periods are shown in the graph (n = 40).

The 96-hr LC₅₀ for the four replicates were 0.0086 mg L⁻¹, 0.008 mg L⁻¹, 0.0104 mg L⁻¹ and 0.0076 mg L⁻¹ respectively (Figure 3) and (Table 4). The 95% confidence limits for these four replicates are narrow and overlapping so that LC₅₀ values, SE values and 95% confidence limits were pooled separately to calculate the overall mean values. Accordingly, the mean LC₅₀ value for the 96-hr exposure period was 0.0087 mg L⁻¹ while its SE and the 95% confidence limits were 0.0054 – 0.0384 mg L⁻¹ (Table 4).

Table 4. The 96-hr LC₅₀ values of Trebon® for tilapia, standard errors and the 95% confidence limits for the four replicates.

| Replicate No | LC₅₀ (mg L⁻¹) | SE     | 95% CI limit (mg L⁻¹) |
|--------------|--------------|--------|-----------------------|
| 1            | 0.0086       | 0.0021 | 0.0043 – 0.0169       |
| 2            | 0.008        | 0.0014 | 0.0054 – 0.0132       |
| 3            | 0.0104       | 0.0028 | 0.0070 – 0.1109       |
| 4            | 0.0076       | 0.0014 | 0.0049 – 0.0126       |
| Mean         | 0.0087       | 0.0019 | 0.0054 – 0.0384       |
Figure 2. The relationship between the lognormal concentration of Trebon® and the % mortality of tilapia for the four replicates in definitive acute toxicity test at the 24-hr exposure period. The LC₅₀ values for the 4 replicates were 0.074 mg L⁻¹, 0.0617 mg L⁻¹, 0.0683 mg L⁻¹ and 0.0586 mg L⁻¹ respectively.

Immediately after the fingerlings were introduced into the test tanks, it was observed that they began erratic swimming movements. The fingerlings in the control tanks gradually calmed down within a few minutes and later they were found to be freely and randomly swimming throughout the tanks. However, those who were in the test tanks showed a significant preference to gather around the aerator stone gulping for air bubbles than in the controls (P < 0.05; Tukey’s Pairwise test after One-way ANOVA). During the 96-hr exposure period to Trebon®, the body colouration too began to fade. It was observed that their initial body colour was black but it began to fade gradually to a light hue towards the end of the 96-hr exposure period.
Figure 3. The relationship between the lognormal concentration of Trebon® and the % mortality of tilapia for the four replicates in acute toxicity test at the 96-hr exposure period. The LC₅₀ values for the 4 replicates were 0.0086 mg L⁻¹, 0.01 mg L⁻¹, 0.008 mg L⁻¹ and 0.0076 mg L⁻¹ respectively.

The tissue structure of the gills, liver and intestine showed moderate to severe histopathological damages upon exposure to Trebon®. Damages to the liver tissues included severe intracellular vacuolation, melanomacrophages aggregation, blood congestion and focal necrosis (Plate 1). Damages to the gill structure and gill tissues included deformation of secondary lamellae, gill hyperplasia, enlarged gill tips, telangiectasis in gill lamellae, mucosal cell proliferation, disorganization and fusion of secondary gill lamella (Plate 2). Histopathological changes in the intestinal tissue included fusion of the intestinal epithelium and disorganization of connective tissues (Plate 3). It was very interesting to note that the severity of the above histopathological changes increased with the Trebon® concentration and exposure period.
Plate 1. Histopathologic changes of tilapia liver tissues (×400) upon exposure to Trebon®. (A) Normal liver tissue of the control fish with roundish polygonal cell body containing clear spherical shape nucleus, (B) Severe intracellular vacuolation at 24-hr at 0.0469 mg L⁻¹, (C) Melanomacrophages aggregation at 96-hr at 0.0089 mg L⁻¹, (D) Moderate level blood congestion at 24-hr at 0.0059 mg L⁻¹, (E) Severe blood congestion at 24-hr at 0.0469 mg L⁻¹ and (F) Focal necrosis at 24-hr at 0.0469 mg L⁻¹
Plate 2. Histopathological changes of tilapia gills (×400) due to Trebon®. (A) Normal structure of gill filament showing the primary lamella (PL) and secondary lamella (SL) of the control fish, (B) Deformed secondary lamellae at 0.0469 mg L⁻¹, (C) Mild hyperplasia at 24-hr at 0.0059 mg L⁻¹, 0.0045 mg L⁻¹, 0.0030 mg L⁻¹ and 0.0015 mg L⁻¹ and (D) Severe hyperplasia and enlarged gill tip at 24-hr exposure at 0.0469 mg L⁻¹, 0.0352 mg L⁻¹, 0.0235 mg L⁻¹ and 0.0177 mg L⁻¹, (F) Mucosal cell proliferation at 96-hr at 0.0059 mg L⁻¹ and 0.0045 mg L⁻¹ and Lamella disorganization at 24-hr exposure at 0.0469 mg L⁻¹.
Plate 3. Histopathological changes (×400) of the intestine of tilapia upon exposure to Trebon®. (A) Normal structure of intestinal wall and (B) Abnormal appearance in intestinal wall upon exposure to Trebon®.

In the definitive acute toxicity test the physico-chemical parameters (i.e., DO, pH and temperature) varied only within a narrow range among the treatments during the 96-hr exposure period. However, none of these variations were significant (P > 0.05; Two-way ANOVA). Therefore it is obvious that the DO, pH and temperature variations in test aquaria of the present experiment have no significant influence on tilapia mortality, alternations in the gills, liver, and intestinal tissues, alternation in the body colouration and behavioural changes of tilapia fingerlings.

**Discussion**

Extensive use of insecticides to control insect pests began after the Second World War and more and more powerful insecticides have been developed since then. Among these, Etofenprox is a recently developed powerful synthetic pyrethroid and, is widely used all over the world against a broad range of insect pests (EFSA 2008; Orrick and Pease 2009). This insecticide was introduced to Sri Lanka around the year 2010 by the trade name Trebon®. In the present investigation, the toxic effects of Trebon® on the non-targeted tilapia fingerlings were studied following standard laboratory procedures.

Since Trebon® is a pyrethroid, its residues are not as highly persistent as those of organochlorines and organophosphates. In spite of this, Trebon® remains in the environment for a considerable period of time displaying a little mobility and rapidly absorbing into sediment and eventually bio-accumulating in aquatic food chains (Orrick and Pease 2009). This is further aggravated by their improper spray where spraying is done many times above the recommended frequencies and also at higher doses (Peterson and Hulting 2004). Sometimes they are sprayed directly into water. For example, Tsui and Chu (2003) reports a similar situation where pesticides are directly sprayed into sea weed culture ponds. Therefore, there is a high chance of Trebon® to accumulate at extremely high levels in agricultural fields and associated aquatic habitats such as streams, ponds and reservoirs.
The recommended field dose of Trebon® is a very high value indeed where a field dose as high as 1.5 mg L\(^{-1}\) should be sprayed to control insect pests in a paddy field. The present study showed that the 24-hr and 96-hr LC\(_{50}\) of Trebon® against tilapia fingerlings were very low values (i.e. 0.0657 mg L\(^{-1}\) and 0.0087 mg L\(^{-1}\) respectively) and lie well below this recommended field dose. It indicates that Trebon® will bring about tilapia mortality at concentrations 23 times and 172 times milder than the field dose during the 24-hr and 96-hr exposure periods. In addition more sensitive fishes in these aquatic habitats, if not tilapia, too would become easy victims of Trebon® at these very low concentrations. More or less similar results have also been recorded by Yameogo et al. (2001), Boateng et al. (2006), Coimbra et al. (2007), Al-Kahtani (2011) and Davoodi and Abdi (2012).

Ali and Rani (2008) showed that the increase in exposure period decreases the LC\(_{50}\) value. The present study endorses this view where the 96-hr LC\(_{50}\) value (i.e. 0.0087 mg L\(^{-1}\)) is 8 times smaller than the 24-hr LC\(_{50}\) value (i.e. 0.0657 mg L\(^{-1}\)) so that longer the fish is exposed to Trebon®, higher would be its toxicity effect. Therefore fish morality increased in all the test preparations towards the latter part (i.e. 96-hr) of the experiment.

In general, increasing temperature is usually followed by increasing toxicity in toxicological studies (Phommakone 2004). In the present investigation however, physico-chemical parameters such as DO, pH, and temperature of water remained more or less unchanged between the control and the test preparations during the entire experimentation period. This suggests that the physico-chemical parameters of water have no synergy effect on the toxicity of Trebon®. Therefore, it appears that tilapia mortality, changes in their histopathology, behaviour and body colouration were all brought about by the sole toxicity effect of Trebon® but not by changes of any physico-chemical parameters measured.

Changes of the fish behaviour, body colouration, and tissue structure of gills, liver and intestine of the fingerlings were also studied in the present investigation. It was observed that the fingerlings showed erratic swimming movements immediately after they were introduced into test tanks. However, they gradually settled around the aerator stone and later seen in gulping for air bubbles. Generally fishes show the above behaviour in an attempt to obtain more oxygen under stress. However, all the test tanks were vigorously aerated and their dissolved oxygen level remained at a high level around 7.5 mg L\(^{-1}\) throughout the entire experimentation period. Therefore, changes in the fish behaviour, as was seen in the present study, was not due to the oxygen stress but may be due to the histopathological changes in the gills induced by Trebon®. These histopathological changes were observed in the present study. But this particular behaviour was not observed in those in the control tanks where they swam freely and dispersed throughout the tank.

Velisek et al. (2011) suggested that acute effects of pyrethroid pesticides in fish include damage to gills and changes in behaviour because pyrethroids are highly lipophilic and are likely to be strongly absorbed by the gills even when it is present at very low levels. As Trebon® is a pyrethroid insecticide it is therefore expected that the tilapia gill tissues are affected with severe histopathological changes. It was interesting to note that mild, moderate and severe gill hyperplasia
conditions were observed in all the fishes of the present experiment where the severity of the symptoms are Trebon® dose dependent. In hyperplasia, the secondary gill lamellae fuse together making it hard for the fishes to exchange respiratory gasses so that they directly gulp for air, and this results in a change of their behaviour. In addition to gill hyperplasia, deformation and telangiectasis in secondary lamella, mucosal cell proliferation, lamella disorganization and fusion were also observed in the tilapias. Telangiectasis indicates acute toxicity distress (Velisek et al. 2011).

Further to the gills, histological changes in liver tissues were also observed. The liver is a very important organ performing a number of vital functions including detoxification of organic xenobiotics, insecticides and other toxic by-products (Metelev et al. 1971). Liver, owing to its very high metabolic rate, is also one of the most affected organs by insecticide contaminants (Rodrigues and Fanta 1998). It was observed in the present study that Trebon® has induced damages to the liver tissues too, where the damages included cellular degenerations such as intracellular vaculation, aggregation of melanomacrophages, blood congestion, and focal necrosis. The intracellular vacuolation was observed 24-hr after exposure. But melanomacrophages aggregation could only be observed 96-hr after exposure. The above cellular degeneration may have been resulted by the oxygen deficiency as the fishes were unable to obtain oxygen due to initial gill damages so that stasis of blood would occur later (Mohmed 2008). Further, the liver damage was Trebon® dose dependent where the fishes exposed to low concentrations of Trebon® showed less severe damage while those in the higher concentrations showed more severe damages.

In the present study, histological changes in the intestinal wall were also observed upon exposure to Trebon®. The intestinal epithelia were damaged, but the severity of the damage is far milder than those seen in gills and liver. It is noteworthy that these fishes were not fed during exposure period so that there were chances to damage their intestinal tissues by ingestion of Trebon® contaminated food. But the observed damages could be attributed to fingerlings feeding on their own faecal accumulations or feeding on dead individuals in the test tank. However, this area needs further experimentation.

The body colouration of the fingerlings was also changed upon exposure to Trebon® where the initial blackish body colour gradually faded to a light hue towards the latter part of the experiment. Hedberg and Wallin (2010) showed that pesticides inhibit intracellular transport of the melanopores of the fish, *Xenopus laevis* resulting a change in body colouration. Similarly, the change of the body colouration of the tilapia in the present experiment may be due to the toxicity effect of Trebon®.

Tilapia is abundant particularly in the dry zone of Sri Lanka where the paddy cultivation is been carried out intensively. They are perhaps the most important food fish species that provide cheap protein source to the rural poor and contribute significantly to reservoir fishery in the country. Therefore the toxic effects caused by Trebon® to tilapia is a clear indication of the threat to tilapia fishery, and subsequently to nutritional and economic status of the rural poor in the country.
Conclusion

The present study showed that the 24-hr LC$_{50}$ (i.e. 0.0657 mg L$^{-1}$) and 96-hr LC$_{50}$ (i.e. 0.0087 mg L$^{-1}$) of Trebon® against tilapia fingerlings were very low values and lie well below its recommended field dose (i.e. 1.5 mg L$^{-1}$), indicating severe toxic effects even at extremely low concentrations. The tilapia fingerlings preferred to gather around the aerator stone than resting elsewhere in the Trebon® containing test tanks due to the difficulty of obtaining oxygen as the gills were founds to be severely damaged upon exposure to Trebon® or due to stress caused by Trebon®. In addition to the gills, liver tissues were also severely damaged by Trebon®. The severity of these damages was dependent on the Trebon® dose and the exposure period.

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