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Histopathological changes in the gills of common carp *Cyprinus carpio* treated with sublethal concentrations of phorate

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Abstract
Cyprinus carpio (*C. carpio*) fish were exposed to chronic sublethal toxicity (one-tenth of the LC_{50}/96 hours - 0.071 ppm/l) of phorate (CSTP) for 1, 7, 15 and 30 days and the effect of CSTP on gill histopathology of the fish was investigated. On exposure for a period of 1 day to CSTP, mild degenerative changes were observed in the gill of the fish. After 7 days of exposure, a further damage in the structure of the gill was observed and it was progressed up to day 30. The primary and secondary lamellae of the gill showed epithelial hyperplasia and hypertrophy. Atrophy and swelling at the base with desquamation of the hyperplasic epithelia in the secondary gill lamellae was observed. Significant hemorrhagic condition was also noticed in the gill. The findings of the present investigation suggest that the frequency of pathological changes increases with increasing the exposure time to CSTP.

Keywords: *Cyprinus carpio*, Chronic sublethal, Phorate, Lamellae, Hypertrophy, Atrophy

1. Introduction
Toxicology is a branch of biology, chemistry and medicine concerned with the study of the adverse effects of chemicals such as pesticides on living organisms. It is the study of symptoms, mechanisms, treatments and detection of poisoning. Extremely high concentrations of pesticides are more toxic to the biological systems and the severity of damage to animals like fish increases with an increase in the concentration of pesticides [1, 2]. Evaluation of toxicity of a pesticide therefore is necessary to know, because it would help us to know its potentiality and toxic effect on animals like fish. The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic fauna in the near future. The necessity of determining the toxicity of substances like pesticides to commercial aquatic forms has been useful and accepted for water quality management.

Unconscious and reckless handling of pesticides results in several disastrous incidences of pollution and accidental poisoning [3-5]. Hence man has recognized the need for better control of the present use and future development of pesticides. Major purpose of the toxicological investigations is to provide a basis for estimating the maximum dose of a chemical that may be tolerated by animals throughout their life time without manifesting any adverse effect [6]. The pesticides are varying greatly in their action, toxicity and persistence. The toxicity testing on the non-target organisms like fish would definitely help to understand the hazardous nature of pesticides and to improve health condition of mankind on a long run.

Toxicity assessment is the characterization of the toxicological properties and effects of a substance like pesticides, specifically the dose response relationship associated with a particular route of exposure. Several studies have been conducted in assessing the toxicity of pesticides to the aquatic biota especially fishes [7-12]. The wide use of fishes in toxicological investigations is due to their adaptability to the laboratory conditions as well as their availability and varying degree of sensitivity to the toxic substances [13].

Phorate is an organophosphorus insecticide (OPI) and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, nematodes and rootworms [14, 15]. It is used in pine forests and on root and field crops, including paddy and groundnut. It is an important OPI to which the fresh water fishes are frequently exposed due to the indiscriminate use of it by the farmers. Hence the present investigation is aimed to assess the impact of CSTP, which is widely used in the local area to combat pests, on gill histopathology in the fish *C. carpio*, a representative of the aquatic environment.

2. Materials and Methods
2.1 Test Species
The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fish, having great commercial value. Besides its wide availability and commercial importance, this carp fish is known for its adaptability to laboratory conditions and appear to be suitable test animal to toxic studies [16].

2.2 Test Chemical
Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an OPI which is widely used throughout the world and also in India including Andhra Pradesh as a broad spectrum insecticide on numerous crops. Commercial names of phorate are thimet, rampart, granutox, agrimet etc and its molecular formula is $C_7 H_{17} O_2 PS_3$.

2.3 Procurement and maintenance of fish
Fingerlings of *C. carpio* fish were brought from the department of fisheries, Anantapur, Andhra Pradesh and released into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. Then the fish were separated into the batch of having the size of 10 ± 2 gm and

were maintained in static water without any flow [17]. Water was renewed every day to provide freshwater, rich in oxygen. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of animals, size, sex etc., [18], precautions were taken throughout this investigation to control all these factors as far as possible.

2.4 Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the probit method of Finney [19]. One-tenth of the LC_{50}/96 hours (0.071 ppm/l) concentration of phorate was taken as the sublethal concentration for chronic toxicity study.

2.5 Experimental Design

100 fishes were divided into 5 groups comprising of 20 fishes each. The group I was considered as normal control, group II, III, IV and V were experimental groups. The fishes of group II were exposed to CSTP (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm/l) for 1 day, group III for 7 days, group IV for 15 days and group V for 30 days. Then the fish were sacrificed and gill tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

2.6 Histopathology

The histological sections of the gills of the control and chronic toxicity exposed fish were taken by adopting the procedure as described by Humason [20]. The tissues were isolated from control and the phorate treated fish and rinsed with physiological saline solution (0.9% NaCl) to remove blood, mucus and debris adhering to the tissues. They were fixed in Bouin's fluid for 24 hours and the fixative was removed by washing through running tap water overnight. The tissues were processed for dehydration using ethyl alcohol as the dehydrating agent and were passed through a graded series of alcohols, cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with hematoxylin [21] and counter stained with eosin (dissolved in 95% alcohol). Then the sections were mounted in canada balsam after dehydration and cleaning and photomicrographs were taken using the magnus photomicrography equipment.

3. Results and Discussion

3.1 Results

The structure of the gill of normal control *C. carpio* fish is composed of primary and secondary gill lamellae with well-marked inter lamellar spaces. Primary gill lamellae consisted of cartilaginous skeletal structure filament, multilayered epithelium and vascular system. Numerous secondary lamellae were lined up along both sides of primary lamella. Secondary gill lamella was constituted of epithelial cells supported by pillar cells (Fig 1).

3.1.1 Histopathological study in gills

On exposure for a period of 1 day to CSTP, mild degenerative changes were observed in the gill of the fish *C. carpio*. There was mild degree of hypertrophy in the epithelial cells of the gill lamellae but the primary and secondary gill lamellae were distinct with inter lamellar spaces. (Fig 2a). After the exposure period of 7 days to CSTP there was a further damage in the structure of the gill. The primary and secondary lamellae of the gill showed epithelial hyperplasia and hypertrophy. Atrophy and swelling at the base with desquamation of the hyperplasic epithelia in the secondary gill lamellae was observed. Significant hemorrhagic condition was also noticed at the base of the primary gill lamellae (Fig 2b). On exposure for a period of 15 days, heavy swellings in the primary and secondary gill lamellae were observed. Significant aneurism in the gill lamellae and damage in the structure of filament, primary and secondary lamellae with epithelial necrosis and desquamation were observed (Fig 2c). After 30 days of exposure further degeneration in gill structure was observed with significant aneurism. Severe degeneration in the filament, primary and secondary lamellae, cytoplasmic vacuolization along with the epithelial necrosis and desquamation were observed. The lamellar structure was lost with cytoplasmic vacuolization and erosion of gill epithelial cells (Fig 2d).

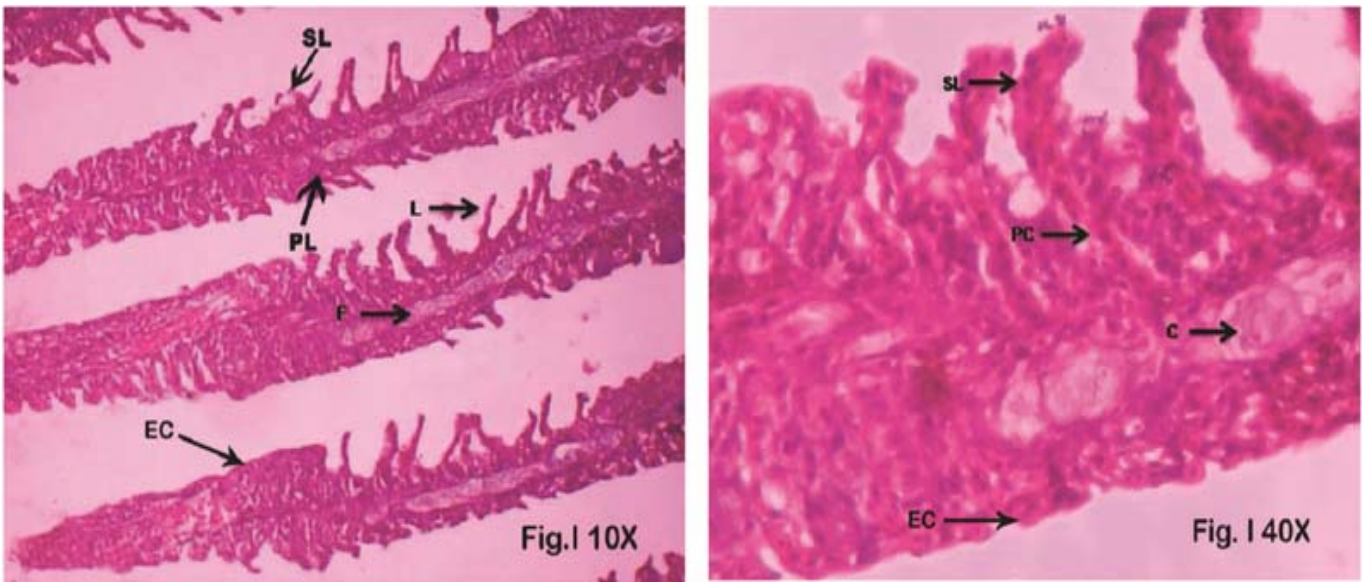

Fig 1: The normal architecture of the gill tissue of control fish showing primary lamellae (PL), secondary lamellae (SL), epithelial cells (EC), lamella (L), filament (F), chondrocytes (C) and pillar cells (PC) with well-marked inter lamellar spaces under lower (10X) and higher magnification (40X) respectively.

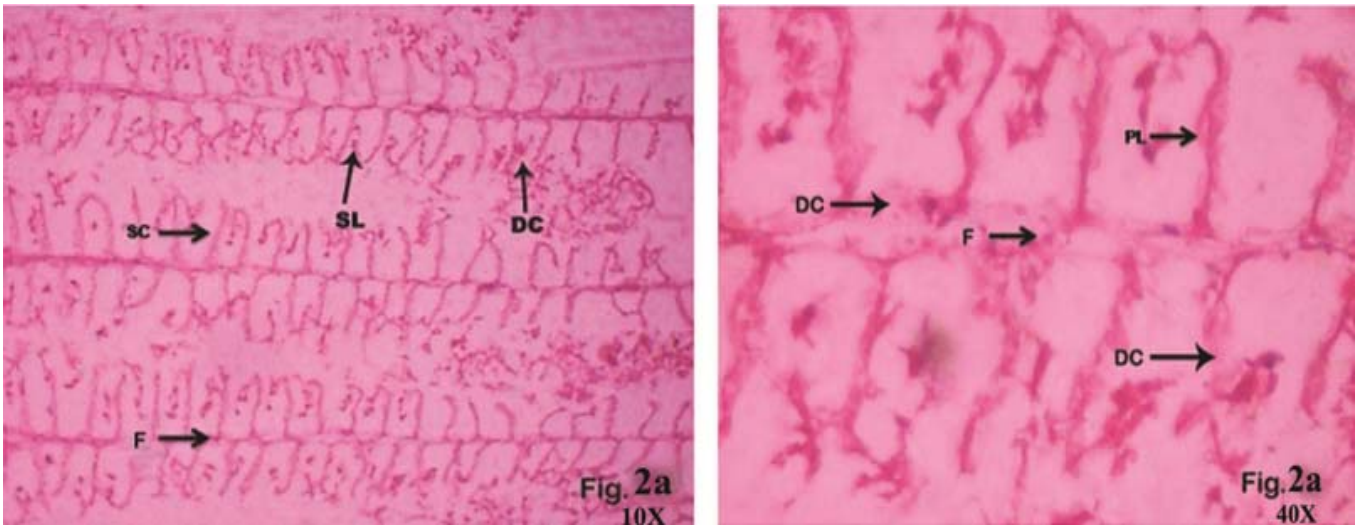

Fig 2a: The gill of the fish exposed to CSTP for 1 day showing structural changes (SC) in primary lamellae (PL), secondary lamellae (SL) and filament (F) with mild degenerative changes (DC) in normal cytoarchitecture with lower (10X) and higher magnification (40X).

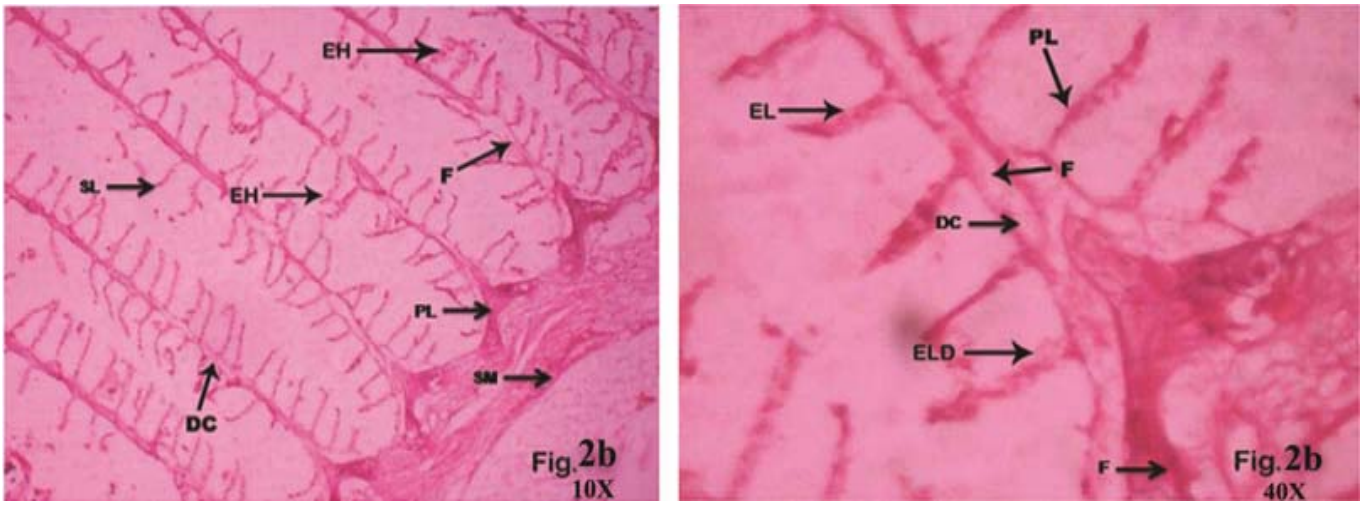

Fig 2b: The gill of the fish exposed to CSTP for 7 days showing further degenerative changes (DC) in primary gill lamellae (PL), secondary gill lamellae (SL), filament (F) and submucosa (SM) along with epithelial lifting (EL), epithelial hyperplasia (EH) and epithelial lifting and desquamation (ELD) with lower (10X) and higher magnification (40X).

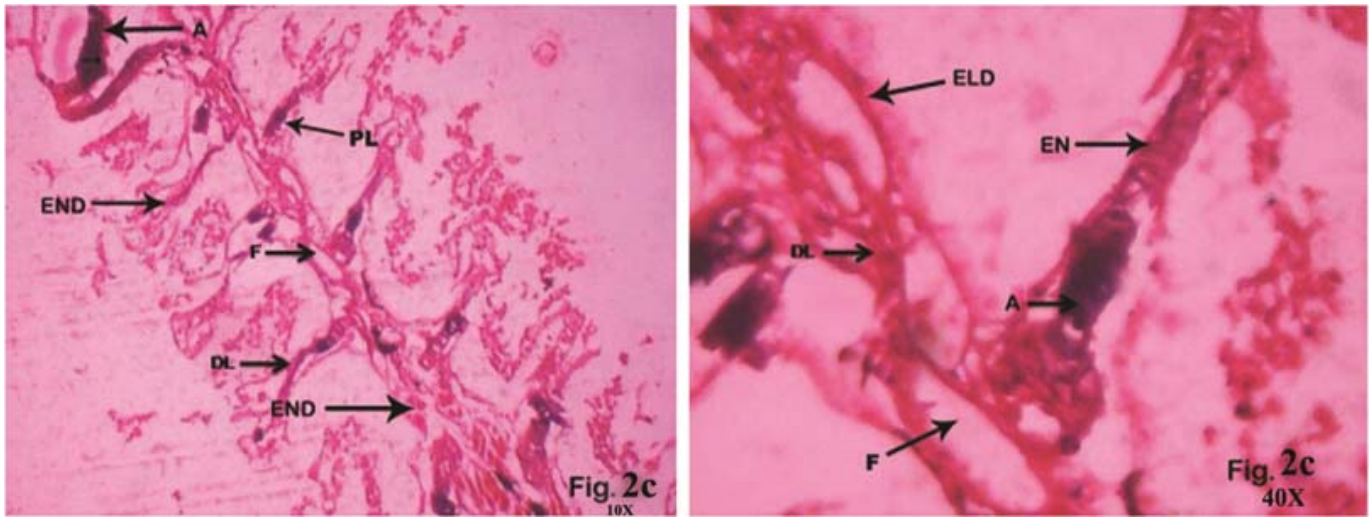

Fig 2c: The gill of the fish exposed to CSTP for 15 days showing degeneration of lamella (DL), filament (F) and primary gill lamellae (PL) with aneurism (A), epithelial necrosis (EN), epithelial necrosis and desquamation (END), epithelial lifting and desquamation (ELD) with lower (10X) and higher magnification (40X).

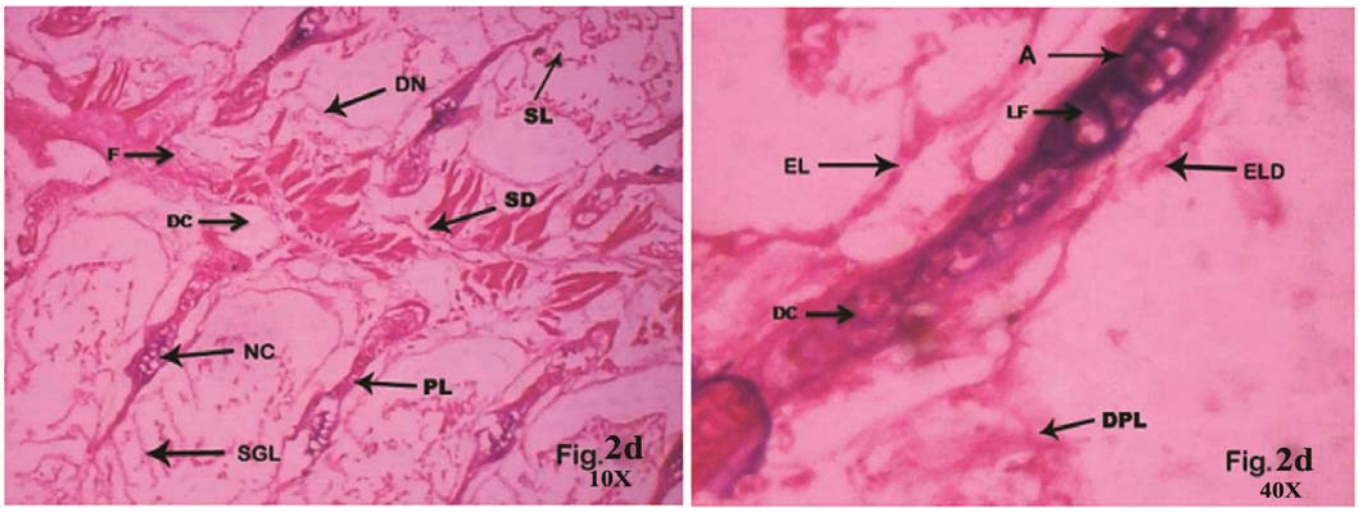

Fig 2d: The gill of the fish exposed to CSTP for 30 days showing degenerative changes (DC) in primary gill lamellae (PL), secondary gill lamellae (SGL) and lamellar filament (LF) with necrotic changes (NC), aneurism (A), epithelial lifting (EL) desquamation (DN), epithelial lifting and desquamation (ELD), structural degeneration (SD) and degeneration in primary lamellae (DPL) with lower (10X) and higher magnification (40X).

3.2 Discussion

The histopathological investigations can provide information about the health and functionality of organs in the animals like fish. Tissue injuries and damages caused by the pesticides in the organs of the fish can result in the reduced survival, growth, fitness and low reproductive success or increase of susceptibility to pathological agents. In the present study phorate has induced pronounced pathological changes in the gills of the fish *C. carpio* exposed to CSTP (Fig 2a to 2d). Similar types of pathological changes were observed by many researchers on exposure to different pesticides. De Silva and Samayawardhena [22] observed irregular appearance of gill lamellae, increased vacuolation in epithelial cells, lamellar fusion and complete destruction of gill lamellae in *Poecilia reticulate* exposed to chlorpyrifos. Cristina *et al.*, [23] observed lesions like epithelial ruptures, secondary gill lamellae fusion and hyperplasia of branchial epithelium in *Carassius auratus gibelio* after acute exposure to malathion. Similar type of pathological changes were also reported in *Gambusia affinis* due to long-term chronic toxicity of malathion [24] and epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema, shortening of secondary lamellae and lamellar fusion in *Cirrhinus mrigala* exposed to dichlorvos [25].

Sandipan Pal *et al.*, [26] reported hyperplasia and hypertrophy of gill epithelium, blood congestion, dilation of marginal channel, epithelial lifting, lamellar fusion, disorganization and aneurysm, rupture of the lamellar epithelium, pillar cells and necrosis in the gill of common carp, *C. carpio*, intoxicated with sub-lethal concentrations of chlorpyrifos pesticide for a period of 14 days. Aswin *et al.*, [12] observed degeneration of epithelial lining and secondary lamella, fusion of secondary lamellae with irregular lamellar spaces, epithelial proliferation and necrosis in the gills of fresh water fish, *Anabas testudineus* exposed to sublethal concentration of quinalphos. The present study revealed the degree of damage caused by this pesticide to the gill tissues of the fish. Various histopathological responses during the chronic toxicity of pesticides could bring a relationship between the level of accumulation of the pesticide and to the various physiological and biochemical activities of the animal. The extent of damage caused by phorate to the gills of the fish was progressive over the period of exposure to CSTP suggest that the histopathological responses depend on concentrations of pesticides and the length of the period of fish exposure to pesticides [12, 26, 27].

The damage occurred to the secondary gill lamellae with light precipitation of mucous and exfoliated nuclei in the fish at day 1 of exposure to CSTP indicate that the pesticide affect the organ systems during the initial period of exposure. These changes may be a part of defense mechanism of fish. It can be speculated that pathological alterations like hyperplasia of epithelial cells, epithelial lifting and lamellar fusion may increase the space of contact of toxicants with the vascular system of the gill, resulting in impairment of respiration as well as fish health. As gills are crucial organs for their respiratory, osmoregulatory and excretory functions in fish, the cellular damage in the gills of the fish *C. carpio* induced by phorate toxicity might also impaired the osmoregulatory function of the fish. Aneurism was observed at day 15 on exposure to CSTP. It occurs due to collapse of pillar cells in the secondary lamellae and rupture of blood vessels, releasing large quantities of blood that push the lamellar epithelium outward. Rupture of the lamellar epithelium, pillar cells and necrosis are the direct deleterious effects induced by phorate exposure.

The histological changes that were taken place in the gills of the fish in the present study, at the initial period of exposure to CSTP, might be a part of defense mechanism of the fish. On prolonged exposure, due to further accumulation of phorate in the gills of the fish, it caused destruction in the gill structure. The slight structural reorganization of the gills of the fish observed at day 30 of exposure gives support to some extent that the ability of the fish to resist the sublethal stress and in repair of the damage caused to the gills by enhancing the protein synthetic potentials and other associated activities of the cell. Probably the fish could excrete or chelated the accumulated phorate over the time of exposure, there by the toxic effect of it might have been gradually decreased. The degree of destruction in the gills of the fish appeared to be linearly proportional to the period of exposure [11, 26].

4. Conclusions

On exposure to CSTP, though initially it caused a mild damage to the gills of the fish at day 1, further exposure for 7, 15 and 30 days it caused a severe damage to these organs. On prolonged exposure for 30 days to CSTP, the fish could develop resistance and replenish the loss by activating the energy cycles. Thus the changes induced by CSTP in the structure and morphology of the gills of the fish *C. carpio* are not only dependent on the concentration of the pesticide but also on the length of the exposure period. Frequency and intensity of tissue lesions depend on the concentration of pesticides and the length of the period of fish exposure to pesticides.

5. References

1. Ayoola SO, Ajani EK. Histopathological Effects of cypermethrin on juvenile African catfish (*Clarias gariepinus*). World Journal of Biological Research, 2008; 001(2):1-14.
2. Ayoola SO. Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. African Journal of Agricultural Research, 2008; 3(12):825-834.
3. Jeyaratnam J. Health problems of pesticide usage in the third world. BMJ. 1985; 42:505.
4. Igbedioh SO. Effects of agricultural pesticides on humans, animals and higher plants in developing countries. Arch Environ Health 1991; 46:218.
5. Forget G. Balancing the need for pesticides with the risk to human health. In: Forget G, Goodman, T, de Villiers A. editors. Impact of Pesticide Use on Health in Developing Countries. IDRC, Ottawa, 1993, 2.
6. Gralla EJ. Scientific considerations in monitoring and evaluation toxicological Research. M.C. Graw Hill International Book Company Washington D.C. New York, London, 1981, 141.
7. Verma SR, Bansal SK, Gupta AK, Pal N, Tyagi AK, Bhatnagar MC *et al*. Bioassay trials with twenty three pesticides to a fresh water teleost, *Saccobranchus fossilis*. Water Research 1982; 16(5):525-529.
8. Ravikrishnan R, Murugan SS, Pillai KS, Murthy PBK. Effect of a sublethal concentration of combination of two pyrethroids on acetylcholinesterase in brain of a freshwater fish, *Tilapia mossambicus*. Journal of Aquatic Sciences. 1997; 12(122):39-41.
9. Vasait JD, Patil VT. The toxic evaluation of organophosphorus insecticide monocrotophos on the edible fish species *Nemacheilus botia*. Ecology Environment and Conservation 2005; 8(1):95-98.
10. Susan Anita T, Sobha K, Tilak KS. A study on acute toxicity, oxygen consumption and behavioral changes in three major carps, *Labeo rohita* (ham), *Catla catla* (ham) and *Cirrhinus mrigala* (ham) exposed to fenvalerate. Bioresearch Bulletin 2010; 1:33-40.
11. Ram Nayan Singh. Histopathological alterations in the kidney of *Cyprinus carpio* after exposure to dimethoate (EC 30%). Ind. J. Sci. Res. 2012; 3(1):127-131.
12. Aswin B, Binu S, kumari, Ravishanker S, Mohan kumar M, Ambikadevi AP, Drishya MK. The effect of quinalphos on histopatholagical changes in the Gills of fresh water fish, *Anabas testudineus*. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). 2016; 10(4):12-16, Ver. II.
13. Verma SR, Rani S, Bansal SK, Delala RC. Effect of pesticide thiotox, dichlorovous and chlorofurun on the fish, *Mystus vittatus*. Water, Air and Soil Pollution 1980; 13(2):229-234.
14. Wagner SL. The acute health hazards of pesticides. In Chemistry, Biochemistry, and Toxicology of Pesticides. Witt, J. M. Ed. Oregon State University Cooperative Extension Service, Corvallis, OR 1989; (1):5-2.
15. Gallo MA, Lawryk NJ. Organic phosphorus pesticides. In Handbook of Pesticide Toxicology. Hayes, WJ, Laws Jr, ER Jr. Eds. Academic Press, New York, NY, 1991, 5-3.
16. Sreenivasan A, Swaminathan, GK. Toxicity of six organophosphorous insecticides to fish *Cyprinus carpio*. Curr. Sci., 1967; 36(15):397-398.
17. Doudoroff P, Anderson BG, Bordwic GE, Galtsolf BS, Hart NB, Patrick R, Strong ER, Surber EW, Vanfforn WH. Bio-assay method for evaluation of acute toxicity of industrial wastes to fish sewage. Indi. Wastes 1951; 23:1380-1397.
18. Sivaramakrishna B, Suresh A, Radhakrishnaiah K. Assessment of mercury toxicity by the changes in oxygen consumption and ion levels in the fresh water snail *Pila clobosa* and the mussel *Lamellidens marginalis*. Bull. Environ. Contamn. Toxicol 1991; 46:913-920.
19. Finney DJ. Probit Analysis, 3rd edition, Cambridge University press, Cambridge, 1971, 333.
20. Humason GL. Animal tissue technique, III Edn. WH. Freeman and Company, San Francisco, 1972.
21. Harris HF. On the rapid conversion of haematoxylin into haemation in staining reaction. J. Appl. Microse. Lab. Meth. 1900; 3:777.
22. De Silva PMCS, Samayawardhena LA. Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early life stages in guppy. Ecotox. Environ. Safe 2002; 53:248-254.
23. Cristina SA, Cristina MM, Anca D. Malathion induced histological modifications in gills and kidney of *Carassius auratus gibelio*. Lucrari stiintifice Zootehnie si Biotehnologii 2008; 41(1):448-453.
24. Cengiz EI, Unlu E. Histopathology of gills in mosquitofish, *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. J. Environ. Sci. Health, Part B. 2003; B38(5):581-589.
25. Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E. Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. Braz. Arch. Biol. Technol 2009; 52(5):1291-1296.
26. Sandipan Pal, Emiko Kokushi, Jiro Koyama, Seiichi Uno, Apurba R. Ghosh. Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. Journal of Environmental Science and Health, Part B. 2012; 47:180-195.
27. Velmurugan B, Selvanayagam M, Cangiz E, Unlu E. The effects of monocrotophos to different tissues of freshwater fish *Cirrhnus mrigala*. Bull. Environ. Contam toxicol 2007; 78(6):450-454.