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Effect Of Bifenthrin, A Synthetic Pyrethroid On The Oxygen Consumption In The Fish *Labeo Rohita* (Hamilton)

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Abstract

The fish *Labeo rohita* a major carp cultivable species is exposed to both technical grade and 10% EC of Bifenthrin, a synthetic pyrethroid (Type I, non-cyanogroup one) experiment in the laboratory (*in vivo*) both in lethal and sublethal concentration and studied the variation in the oxygen consumption, while in the process of inspiration of the physiological process of respiration. The fish due to toxic stress initially had taken more amount of the gas that is dissolved in water and later also had a decrement in its consumption. In the lethal concentration, the fish dies and is one of the reason of its toxic effect and in sublethal concentration which is the situation in the natural conditions of the aquatic environment act as slow poison. Such levels have to be monitored while the fish is cultured and also for its survival in the chronic concentration and the sublethal concentration levels are really lethals in the long run.

Keywords: Bifenthrin, Pyrethroids, 10% EC, *Labeo rohita*, Lethal and Sublethal.

INTRODUCTION

Pesticides, one of the pollutants as toxicants cause when transported into the aquatic environment, deleterious effects. No doubt they are the most potentially beneficial ones targeted on pests but in the defilement act of the situation the non-point of source they effect the non-target ambient aquatic organisms.

Among such usage of the agricultural pesticides, the pyrethroids represent a major class of highly effective insecticides which are considered to be relatively low in its toxic action to birds and mammals (sauropsids) but more toxic to aquatic organisms especially the fish, the connecting link of the food chain to the terrestrial environment (Prusty *et al.* 2015).

Sana Ullah *et al.* (2019), regarding pyrethroids in an extensive way of the studies of the biomarker nature, gave an account of the different representatives of its concentration of soil, cash crops and land and terrestrial organisms across the globe including the products which is important statistically. From this origin point, these chemicals including the one which is present studied includes, reach the water the 'ultimate sink' for all.

One of the biomarker study after carried into the aquatic system for the fishes is none other than respiratory effect study which Kaviraj and Gupta (2014) specifically mentioned apart from Sana Ullah *et al.* (2015). It gives the information how much the targeted test fish, inspire in terms of quantity for mg/body weight, when compared with toxic stress of the chemical in that serve as an indices of the pollutant load even though the concentration need not to be lethal causing the death of the organism and in some, the concentration is termed as the sub-lethal also. But such sub-lethals are not lethal, but in the long run (chronic situation) are really lethal especially for those which are the toxicants. All pollutants are not toxicants but all toxicants are in fact pollutants and all pesticides are toxicants.

Hence, in the present study one of the major carp fish *Labeo rohita* is selected as the test organisms and the biomarker study of oxygen consumption while in toxicant stress is attempted. For heterotrophic organism the metabolism depends on how much oxygen of intake it consumes because growth is related to the process of metabolism especially the cultivable species which is very importantand the present studied belongs to the same.

MATERIALS AND METHODS

Experiments on the oxygen consumption of the fish *Labeo rohita* were carried out in a respiratory apparatus developed by Job (1955). The fish were brought from a local fish farm at Nandivelugu, Guntur district (Andhra Pradesh). They were acclimatized to the laboratory conditions in well aerated water for 10 days. If any in the batch, the mortality is more than 5%, the entire batch is discarded. The water used for fish acclimatization and experimentation was the same and is given in table 1.During the experimental period, the fish were regularly fed, but the feeding was stopped for two days prior to the experimentation.The fish measuring 3 to 5cm in length and 4 to 5gms in weight were used in the experiment. All the precautions mentioned by OECD (2019) and APHA (1998, 2005 & 2012) are followed, for maintaining the fish and conducting the experiments. The fish were exposed to 96h LC_{50} lethal 0.22µg/L and 0.11 µg/Land sublethal ($1/10^{th}$ of 96h LC_{50} i.e., 0.022, 0.011 µg/L), concentrations of synthetic pyrethroid pesticide Bifenthrin technical grade and 10% EC respectively. The samples for estimation were taken from the respiratory chamber, at two hours intervals for a period of 24 hours.

Student t-test was employed to calculate the significance of the differences between control and experimental means. P-values of 0.05 or less were considered statistically significant (Fisher, 1950).

The toxicant selected Bifenthrin technical grade was from M/s. Kalyani Industries, Agrochemical Suppliers, 1202/1204, 12^{th} Floor B-wing, Kailash Business park Ghotopur power) and its 10% formulation is purchased locally from the market.

Description of the respiratory chamber

The chamber used for the measurement of whole animal oxygen consumption is a wide mouthed bottle which is called a respiratory chamber (RC). Its mouth was fitted with a four holed rubber stopper (S) and through one of the holes a thermometer (T) was placed to know the temperature of the medium in the respiratory chamber. From the remaining three holes three glass tubes were passed whose outer ends were fitted with rubber tubes. These three tubes serve as delivery tubes and are designated as T_1, T_2 and T_3 respectively. They were fitted with pinch cocks P_1, P_2 and P_3. $T_1$was connected with the reservoir ('R') and through this water could be drawn (inlet) into the respiratory chamber. T_2 was atmospheric tube; useful for testing the air tightness of the respiratory chamber. Through the T_3 tube (outlet) water samples from the respiratory chamber were collected for estimation of dissolved oxygen. The respiratory chamber was coated black to avoid photo chemical reactions and to keep the animal activity at normal during the experiment.

Setting up of the Apparatus

Only one fish was introduced into each respiratory chamber and was filled with water drawn through T_1 from the reservoir. After checking the air tightness pinch cock P_2 was closed and pinch cock P_3 was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a state of normalcy from the state of experiment, if any, difficulty due to the handling and also to allow the animal to adjust to darkness in the chamber (acclimatization).

Collection of the initial and final samples

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through T_3. After collection of initial sample, the respiratory chamber was closed by closing P_3 first and then P_1 after two hours, the next sample was collected from the respiratory chamber. Likewise, other samples also were collected at the end of each two hours for a period of 24hrs and the amount of oxygen present was estimated by the modified method of Winklers (Golterman and Clymo, 1969).

Along with the experimental fish chamber, one respiratory chamber without fish (control) was maintained. The control serves to estimate initial amount of oxygen. The experiments were conducted in sublethal and lethal concentrations of technical grade and 10% EC of Bifenthrin.

$$\text{O}_2 \text{ consumed by fish/ gram body weight/hour} = \frac{\alpha - \beta \text{X N of hypo x 8 x 1000}}{\text{Volume of the sample taken X Correction factor X Wt. of the fish X time interval for each sample}}$$

α - hypo rundown before exposure
β - hypo rundown after exposure

The difference in the rate of oxygen consumption between the control and the test fish denotes the effect of the toxicant on the oxygen consumption. The data obtained for the lethal and sublethal concentration experiments were compared with that of control along with standard deviation and standard error. Student 't' test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant Fisher (1950).

RESULTS

The comparative data on the whole oxygen consumption of control and experimented fish calculated for gram body weight in lethal and sublethal concentration of the technical grade and 10% EC for *Labeo rohita* respectively are graphically represented as Fig. 1 and 2 as both line and bar graphs, Fig. 3 and 4..

Throughout the experimental period the fish showed severe respiratory distress and rapid opercular movements leading to higher intake of oxygen. As the toxicant intake increased, it resulted the mucus secretion, higher ventilation volume and decrease in oxygen uptake efficiency. The rate of the oxygen consumption in both control and exposed fish were gradually decreased. The starved condition and the reduced metabolic rate are the causes for the decrease of the oxygen consumption in control fish whereas the decrease in exposed can be correlated with the extent of damage in gill epithelium and toxic stress The constant decrease in oxygen consumption as per the Bifenthrin technical grade exposed fish and the oxidative stress varied timely in presence of the toxicant and also for 10% EC. The fish under suffocation of stress media demanded more oxygen consumption, so intake of dissolved oxygen for cellular oxidation naturally more.

DISCUSSION

1. With Synthetic pyrethroid of non-cyanogroup designated as group/type I
Eg: Bifenthrin, Permethrin and Cyhalothrin etc.

In the *Ctenopharyngodon idella*, Sana Ullah *et al.* (2022) using Bifenthrin as a toxicant reported various enzymes functionally important for physiological process of respiration had an impact in respiration and as a result the organism suffered in the metabolism.

While reporting on the Bifenthrin toxicity to *Clarias batracus* Saha and Saha (2021) and Saha *et al.* (2020) in the fish *Oreochromis mossambicus*, Saha *et al.* (2021) in the fish *Heteroneusteus fossils* (Bloch) all reported that due to the toxic stress, in their behavioural alterations of the respective studied fish had changes in the opercular movements and finally respiration had an impact.

Reporting a study of pyrethroids by Mayada R. Fraag*et al.* (2021) too had a mention of the different chemicals of any category in different fish that had impact on respiration and finally the metabolism. Bano *et al.* (2021) in their report on the fish *Labeo rohita* the present tested fish had an effect by pesticides, Bifenthrin (the present tested toxicant) along with an organophosphate chlorpyrifos, damaged the erythrocytes which had an impact on the fish respiration.

Kalavathi Kumari (2020) in her review article of pesticide toxicity to fish mentioned about the gill damage that resulted an intake of oxygen which had drastically effected.

Satyanarayana *et al.* (2020), using the toxicant permethrin to which the present studied toxicant group belongs on the fish *Ctenopharyngodon idella* (the grass carp), exposing to both technical grade as well as 25% EC. Any change in the oxygen uptake due to toxic stress in the heterotrophic metabolism that was definitely effected. The pesticide in the flowing waters of the test medium through gills, which is the contact point as well as the entry point, the fish tried to avert the condition of the act of that situation consumed more oxygen initially and reverted back to normal state after some time. The sustainance of the living organism is possible provided the fish takes its normal quantity of oxygen of the dissolved state for its metabolic needs. This is moreso important, not only for survival but also for the growth which is important for all cultivable species and the fish tested (the test fish) belong to the same category.

Tanzen *et al.* (2019) while studying Bifenthrin toxicity to the fish *Onchorhynchus mykiss* (rainbow trout) reported that the gill had an impact on na^{+}/K^{+}ATpase activity that influenced the respiration especially the intake of oxygen.

Sana Ullah *et al.* (2019) in his review article on pyrethroid toxicity to fish by using different biomarkers they mentioned about the oxidative stress, histopathological devastation in/on the gills, biochemical studies also as some of them for Bifenthrin and cyhalothrin and permethrin of type I and also for Fenvalerate, Deltamethrin and Cypermethrin of type II pyrethroids. The cumulative effects of all the biomarkers study caused in the different fish as reported by the different authors Viera and Reis Martinez (2018) in the fish *Prochidus lineatus* (by alfa cyhalothrin), Clasen *et al.* (2018) in the fish *Cyprinus carpio*. Tu*et al.* (2016) in the fish *Danio rerio* by using Bifenthrin Crago and Schlenle (2016) in the fish *Buchorhynchus mykiss* by using the present tested toxicant are worth mentioning.

Not only that Saumya Biswass *et al.* (2018), Prusty *et al.* (2015) for synthetic pyrethroid and also for other class of pesticides including the pyrethroids by Ullah and Zorriezahara (2015) and Murthy *et al.* (2013) also mention in the respective review articles regarding the respiratory effect by these chemicals.

Agnieszka *et al.* (2018) reported on the pyrethroids, general safety of course on human exposure, the permethrin had an impact on the neuronal system and endocrine functioning that was disturbed andresulted in the respiratory problems. The same can be visualized even with the present studied fish.

Balakrishna Naik *et al.* (2018) too reported using the same toxicant as above on the fish *Cyprinus carpio* using both technical grade as well as 25% EC reiterated the same opinion as earlier. They expressed that the toxicant/s exposed damaged the gill epithelium (squanous Epithelium) that had reduced the diffusion process of the penetration of the respiratory gas while during inspiration apart from latent changes in the blood and gill architecture.

Huma Naz *et al.* (2017), in their study of major carps due to exposure of the mixture of the pesticides in which Bifenthrin is also the one (the present toxicant), the antioxidant enzyme effect made an impact on the oxygen intake and ultimately the metabolism and growth in the cultured carps including the *Labeo rohita*.

In another direction/line of the study by Renuchaudari and Saxena (2016), the exposure of Bioallethrin, another synthetic pyrethroid effect the nucleus of the erythrocytes that had an effect which resulted a profound bearing on the oxygen intake.

Priyanka and Ansari (2014) in the fish *Danio rario* (zebra fish) exposed to three different class of compounds, Endosulfan, an organochlorine, chlorpyrifos an organophosphate and permethrin, the synthetic pyrethroid (also the same group which the present tested toxicant belongs). Initially they focused their main attention to the behavioural/ morphological changes as alterations which paved the way to conclude the fish ultimately effected and due to the respiratory distressand also rapid opercular movements, paved the way for more consumption of the respiratory gas. The same situations of the result prevail even in the present study.

Sapna Devi and Abhik Gupta (2014) in the fish *Anabas testudineus* using to two different toxicants, Deltamethrin (type II synthetic pyrethroid) permethrin (type I synthetic pyrethroid),reported on the effect of oxygen consumption. Both the chemicals as toxicants exerted cumulative stress and interfered in the physiology of the respiratory process. They made an aspect of the growth study also which they proved that it was finally retarded. If such situation is visualized due to the pesticide contamination it will be too dangerous for cultivable species like the one of the present tested.

Maria Christiana *et al.* (2010) in three fish, Prussian carp, blech and perch exposing them to the present tested toxicant Bifenthrin and Fenvalerate in the fish *Heteroneustes fossils* reported a change in the rhythm of the respiration rate that resulted a change in the oxygen consumption.

Velisek *et al.* (2009) in the fish *Oncorhynchus mykiss* rainbow trout using Bifenthrin as the toxicant, while reporting that the chemical not only was strongly toxic to fish but also had alterations of the haematological aspects that resulted a change in the oxygen carrying capacity too.

2.With synthetic pyrethroids of type II

Sana Ullah *et al.* (2019) in their review article reported that the Deltamethrin increased the oxygen intake by reactive oxygen species production and lipid peroxidation in the vital organs and all other antioxidant enzymes had an impact in the fish *Hypothalmicthys moltrix*. They also mentioned that quoting the references of Parlak (2018), inferred that in the fish *Danio rerio* deltamethrin induced the oxidative stress leading to the inhibition of the enzyme Acetyl cholinesterase (AChE) activity. Apart from the above, also as per the report of Abdel-Daim *et al.* (2015) increased the oxidative stress in the fish *Oreochromis niloticus* due to increase in malondialdehyde (LPO) in the liver, kidney and gills and decrease in the other enzymes such as catalase, superoxide dismutase glutathione peroxidase and glutathione. In the fish, *Sparus aurata* deltamethrin has an effect on metabolism that is disturbed and immune system due to oxidative stress as reported by Guardiola *et al.* (2014) that was also mentioned. Finally, they also added the work of Ensibi *et al.* (2013) in the fish *Cyprinus carpio* that the deltamethrin increased the level of malonodialdehyde level in the pancreas with concomitant increase in glutathione S. transferase, catalase and glutathione reductase. By visualizing all the above the present study of the result also can be inferred for changes in oxygen consumption due to the impact of the toxicant.

Saumya Biswas *et al.* (2019) in the review article while referring the reports of Jipsa *et al.*(2014), Logoswamy and Rewia (2009) and Marigoudar*et al.* (2009), the synthetic pyrethroids of group II had alterations in the oxygen consumption of the fish, *Tilapia mossambica*, *Tilapia mossambicus* and *Labeo rohita* respectively. The impact will be more in sub-lethal concentrations and was delayed and extended effect. Quirong *et al.* (2019) referring to deltamethrin toxicity, which was due to oxidative stress referring to all vertebrates of the neurotoxic action.

Srinivasa Rao *et al.* (2018) reported in the fish *Ctenopharyngodon idella*, deltamethrin had a severe impact on the oxygen consumption due to the toxic stress and concluded that it was really the sub-lethal concentrations are lethals.

Neelima *et al.,* (2016) using cypermethrin 25% EC as toxicant which also belong to type II synthetic pyrethroid to the toxicant of evaluation to the fish *Cyprinus carpio* exposed to both lethal and sub-lethal concentrations. The result reported that there was a demand for more oxygen consumption which is more in lethal concentration than to sub-lethal concentrations. The changes are due to respiratory distress as a consequence of the impaired oxidative metabolism. The LC_{50} value calculation method is not continuous flowthrough system that is employed and is only static renewal and the experimentation method was not with technical grade cypermethrin. However, the toxicant belongs to the different class with cyanogroup not similar to the present studied toxicant but experimentation includes only the EC.

Lenin Suvetha *et al.* (2015) while reporting on the deltamethrin toxicity to the fish *Labeo rohita*, the toxicity effect was due to hormones and enzymes. particularly the cholinesterase that disturbed the oxygen metabolism. Guardiola *et al.* (2014) opined also in the fish *Sparius aurela* L., deltamethrin as a toxicant effected the fish due to the oxidative stress.

Hashibur Rahman *et al.* (2014) in the review article of deltamethrin, the synthetic pyrethroid mentioned that it is highly toxic to fish and due to the resultant of gill arches flaring and as well as difference in osmomolarity that brought changes in the oxygen uptake.

Mukundan and Kulkarni (2014) reported that the acute toxicity of cypermethrin, a synthetic pyrethroid to Estuarine clam *Katelysiaopima* (Gmelin) and its effect on the oxygen consumption. The study reported on the rate of oxygen consumption which fluctuated with an increase in the exposure period of the toxicant. The decrease they observed is attributed to variation in the volume of water that is ventilated through the gills caused by the intermittent closure and opening of the shell valves. They reported that the main reason/factor responsible for decreased oxygen uptake was accumulation of mucus on gills due to Cypermethrin exposure which caused reduction in the effective transfer of oxygen to internal tissues and that adversely affected the absorption of oxygen from the ambient medium. The bivalves usually try to avoid toxicants and in doing so they can minimize the metabolic activity due to stress of the toxicant which resulted a decrease in the oxygen consumption. This was also confirmed by histopathological structure of the gills where in the organ showed inefficiency. Thus, mucus secretion, stress, impaired oxidative metabolism and architectural changes in the gills resulting pathological condition there by reduced efficiency of diffusion of gases all culminate the effect. The present study also with the same concepts even through the quantities of the variations of oxygen are different as well as organisms tested. The main entry, that is the first entry and also the first damaged organ which all are none other than the gills and as a consequence, oxygen consumption is not being normal and is altered and even in the present study which can be visualised.

Jipsa *et al.*, (2014) reported on the impact of a cypermethrin insecticide on oxygen consumption and certain biochemical constituents of the fish *Tilapia mossambica*. The study objective also includes to observe the impact of sub-lethal concentration of the toxicant on the rate of oxygen consumption of the fish. The values obtained in the tested media of water, in the control and exposed to 24, 48, 72 and 96 h LC_{50} values also showed marked changes while demanding more oxygen due to stress. They reported consumption that all the parameters of biochemical nature except glucose which all are decreased. The increased values of glucose are due to failure of metabolism where the substrate is not subjected to anabolism due to the failure of the oxygen intake.

Manjula and Veeraiah (2014) too reported the same concept in the effect of cypermethrin on oxygen consumption of freshwater fish *Cirrhinus mrigala* (Hamilton) exposed to sub-lethal concentration 1/10 of 96 hour LC_{50} value. They also are of the opinion that one of the earliest symptoms of acute pesticide poisoning as the respiratory stress. The fish tested is the bottom feeder, acclimatized to live in low oxygen concentration, hence showing least indices in pesticide toxicity. On the contrary, the present study fish *Cyprinus carpio* the common carp, an omnivorous require more oxygen and can be a good indices of toxicity. In both cases of studies common point was due to severe respiratory distress and rapid movements of the covering of gills that led to the higher amount of toxicant uptake, increased mucus secretion higher ventilation volume decreased the oxygen uptake efficiency labored breathing and engulfing air through mouth when exposed to the toxicant.

Paritha Bhanu and Deepak (2014) reported that the toxicity of cypermethrin influenced by pH and temperature on fresh water fish *Orechromis mossambicus*. They opined pH and temperature influence the toxicity and dissolved oxygen is dependent on temperature which not only effect the toxicity and decreased oxygen consumption that impaired the respiratory activities. The present study of bifenthrin was conducted at low temperature and accordingly LC_{50} values are calculated that to in continuous flowthrough system where methodology is different that too in sub-lethal concentration. Hence, the results that are obtained due to the elevation of pH which lead to acidosis in fishes which would decrease the oxygen carrying capacity of blood. The present study at the specific pH of water a decreasing trend is reported and the hydrographical conditions are such that no situation of acidosis like the work of above which resulted an enhanced oxygen uptake in stress due to the toxicant exposure.

Anita Susan *et al.,* (2012 & 2010) reported a study on the acute toxicity, oxygen consumption and behavioural changes in the three major carps, *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) *Cirrhinus mrigala* (Hamilton) exposed to Fenvalerate another type II synthetic pyrethroid with cyanogroup. The study revealed that, 20% EC was found to be more toxic than to technical grade and at sublethal exposure had profound effect not only on the behaviour but also in the oxygen consumption. During experimentation due to severe respiratory distress and rapid opercular movements that resulted a change in respiration in the fish experimented. Finally, the report of the work concluded that due to the effect of the toxicant on respiratory centers of the brain or on the tissues involved in breathing had change in oxygen consumption. The total oxygen consumption is one of indicators of the general healthy and active fish. The damage inflicted on the animal in the gill epithelium could either increase or decrease the oxygen uptake. The same study also revealed that in *Labeo rohita* and *Catla catla* a significant increase in oxygen consumption as compared to the controls throughout the experimentation period. The exposed fish *Cirrhinus mrigala*, the toxicant resulted a decrease on the contrary, which is a bottom feeder akin to live in low levels of O_2.

Huynh *et al.* (2012) reported the deltamethrin toxicity, wherein they emphasized that in black tiger Shrimp (*Penacus monodon*) due to environment factors of temperature and salinity that were what interact resulting oxidative stress which lead the changes finally, in respiration particularly in the oxygen consumption.

Velisek *et al.* (2011, 2007 & 2006) while working on deltamethrin toxicity to fish, too opined that alterations in the oxygen uptake were possible due to the toxic stress in the fish, *Onchorhynchus mykiss*, *Cyprinus carpio* etc.

Marigoudar *et al.,* (2009) reported cypermethrin induced respiratory and behavioural responses of the freshwater teleost *Labeo rohita* (Hamilton). They opined that main causative factors of alterations in oxygen consumption are due to the respiratory distress and also the subsequent consequences of impairment in oxidative metabolism. The methodology is only static renewal basing on which sub-lethal and lethal concentrations that are calculated, and exposure is for four days in lethal concentrations and in sub-lethal concentrations 1, 5, 10 and 15 days. But overall result due to stress of the toxicant can be considered where there is a demand for more oxygen as in the present study.

Deshponde *et al.,* (2007) reported that the pyrethroids induced respiratory changes in the fish *Labeo rohita*. The fish is exposed to two pyrethroids as toxicants which belong to type II, fenvalerate and cypermethrin in both lethal and sub-lethal concentration wherein the experimented method for calculation of LC_{50} is the static test and the fingerlings of the fish are only tested. They opined first, increased rate of oxygen may be because of increased ventilation, volume in order to compensate the drop-in oxygen content in water and also due to reduction in permeability of the gills. Their study revealed that oxygen consumption fluctuated between 0.0175 and 0.216 mg/l/g/h weight of the body during to 0 to 96h for cypermethrin and for fenvalerate from 0.240 to 0.320 mg/l/g/h body weight. The present study of bifenthrin is only within 24 hours, but not up to 96 hours of the report. Methodology of the toxicant in determination of the quality of LC_{50} and exposure is different not only that the fish is different with the present study. The present toxicant is also toxic to the fish than the other two that is fenvalerate and cypermethrin and at the same time, increased ventilation, mucous secretion and stress are the common factors that were reported for alterations in oxygen uptake. In the report they mentioned on the impact on behavioural responses in the freshwater Teleost *Labeo rohita* (Hamilton), and opined that the toxicosis of the cypermethrin on behaviour resulted due to hyper or hypo operculum activity, resulting alterations in O_2 intake indirectly. The same behavioural responses are observed even in the present study as a result change in oxygen intake.

Tilak and Satyavardhan (2002) reported that the effect of fenvalerate on oxygen consumption and haematological parameters in the fish *Channa punctatus* (Bloch). A comparison is made between the sub-lethal concentration of technical grade and 20% active ingredient (EC) to the fish for every two hours. The experimentation is similar to that of the present study and both the toxicants are of different group. The consumption of oxygen is decreased when the time of exposure to the toxicant is increased. There is a significant decrease of oxygen in water due to more oxygen consumption by the fish due to stress to overcome the situation.

Bradbury and Coats (1989) and Haya and Waiwood (1983) too reported in the similar lines for the synthetic pyrethroids. Bradbury *et al.*, (1986) stated that greater decrease in the rate of oxygen consumption in the fish *Cirrhinus mrigala* may be due to internal action of the pesticide as the toxicant that alters the metabolic cycle at subcellular level. Changes in the architecture of the gill under stress would alter diffusing capacity with consequent hypoxic/anoxic conditions and thus respiration may become problematic task for the fish that was what the above reports concluded, which can also be similar even in the present study.

3.With organophosphates (OP) and others

Organophosphates and synthetic pyrethroid share a common aspect apart from short persistence inhibit the enzyme Acetyl cholinesterase. The different types of the OP compounds had an impact on respiration and the following are important studies.

Balquees (2018), reported in the freshwater fish due to toxic stress of chlorofate, at four different concentrations of the toxicant. The fish that were tested includes *Gambusia affinis, Cyprinus carpio* and *Ctenopharyngodon idella* by taking LC_{50} values of 24 h only. Histopathological damage of the gill, the mucus deposition over the gills and over all failure of the harmony of biochemical imbalance combinely/cumulatively responsible for the change in the respiration particularly the oxygen in take, the physiological phenomenon which had a devasting consequences. As per the studies of the present fish the same is true even in the present case.

Lokhande (2017) while studying in the fish, *Rasbora daniconius* exposed to the toxicant, dimethoate, organo-phosphate, reported on the oxygen consumption a pattern similar to the result of the present study. The behaviour pattern itself and a change in operculum movement that was responsible for the result they got.

Sathwick *et al.* (2017) in the fish *Mugil cephalus* when exposed to 10% of the lethal concentrations of 96h LC_{50} value as sub-lethal, for 10, 20and 30 days and reported the decrement of the oxygen uptake. The reasons for such effect which they explained were: (1) due to microcytic anaemia of the gills, (2) as a protective measure to cope the situation of lowering the intake of the toxic substance and (3) also, due to the respiratory inhibitory action of the enzymes that all are being responsible for such actions. The initial period the increase and later a decline as in the present study can be taken into justification of the present result. The present study that resulted even in the 10% EC formulation also which by the action of the additive toxicity resulted much variation not similar to the technical grade.

Ravindra and Patel (2016) while studying an organochlorine, endosulphan in the fish *Channa punctata* exposed to sub-lethal concentrations. The pH of the water, temperature at which experiments were conducted and the sub-lethal concentration value as consideration being different (static in the case of their study and continuous flow through system as in the present study) and measurement of quantity of oxygen 6 at end of 24, 48, 72, 96 h not as in the present study of the 2 h interval period all these aspects cannot be a good comparison of the their study, yet the reasons they mentioned that the architectural damage of the gill which they focused as the reason for the less uptake as consumption which may also in agreement of the present study but during initial period, the perturbation of the toxic molecules caused them suffocation and showed hyperactive aspect to intake more gas and later then was a decrement.

Similarly, Kharat *et al.* (2016) in the fish *Rasbora daniconius* while studying on the effect of glyphosate herbiside, by the static bioassay, only considered $1/10^{th}$ of the 96 h LC_{50} value not similar of the present study (but in the present study the tests not by the static method determined value that is considered but $1/10^{th}$ of Continuous flow through test of 96 h method) reported a decreased respiratory rate of the toxicant effect. Due to the toxic stress during the initial period the fish consumed more oxygen to cope the toxic stress and that required more extra energy to tackle the toxic situation.

Padmanabhan *et al.* (2015) reported on the oxygen consumption due to chlorpyrifos toxic action where the chemical which tested they opined in the similar lines for the same result as in the present study in the fish *Oxochromis niloticus.*

Kulkarni and Bhilave (2015), reported for the fish *Labeo rohita* due to exposure to diazinon, an organo-phosphate.The method of LC_{50} value taken into consideration was static only, different from the present continuous flow through test method, $1/10^{th}$ 96 h LC_{50} value apart from the differing in the physical and chemical parameters that were different of the medium of the experimentation. The accumulation of the mucus interfered in the gases (Oxygen Carbon-di-oxide) exchange by the phenomenon of the diffusion. The operculum movement that covers the gills, decreased and as a consequence less amount of the gas in its consumption.

Ram Narayan Singh (2014) reported in the common carp *(Cyprinus carpio)* due to dimethoate (EC 30%) toxic action an electrolyte imbalance that existed was mainly responsible for the oxygen intake in lesser quantity as the time progress which is different at the initial period.

Maharajan *et al.* (2013) reported that the profenofos an organophosphate toxic action in the fish, *Catla catla* which had a profound bearing on the consumption of the oxygen only. By taking into consideration of only 24 h LC_{50} value of the static tests the oxygen consumption effect was aimed and the result of decrement in the consumption was due to the architectural damage of the gill, increased mucus secretion and also higher ventilation volume' which all resulted a cumulative aspect of the oxygen in take efficiency and all the reasons are even true even in the present study. Even Rao *et al.* (2013) in the fish *Oreochromis niloticus* exposed to chlorpyrifos and the tested toxicant reported in the similar lines of the present study.

Jothinarendran (2012) also reported in the fish *Channa punctata* by using dimethoate another organophosphate as toxicant. The fish were kept in the different concentrations ranging from 0.15 ppm to 0.6 ppm, up to 96 h duration. The rate of the consumption except in the initial period of the exposure is the concentration dependant only, initially elevated and the stability reached at 72 hrs. The 'defection' of the normal gill surface area reduced to extent to such that which can result a decrease in the oxygen diffusion possible. The oxidative, acceleration towards the enhanced metabolism in the initial period that resulted an increase of the uptake of oxygen in the toxic stress.The results of this can also reiterate the results of the resent study. Even in the fresh water bivalve mollusk, *Lamellidens corsianus* exposed to an organochlorine, thiodan, which was reported by Kumar *et al.* (2012) and they mentioned that the stress factor as a cause and as a consequence of it the metabolism enhanced to cope the energy demand that finally resulted the variations in the oxygen consumption.

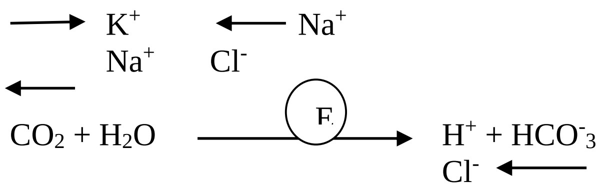
Chebbi and David (2010) in the fish *Cyprinus carpio* using Quinolphos as the toxicant, Shareena *et al.* (2009) in the fish, *Tilapia mossambica* using Dimethoate as the toxicant reported the same result of the present study.

Illavazhahan *et al.* (2010) reported that the synergistic concept of the impairment of oxygen intake in the fish *Labeo rohita.* The mucus along with bacterial accumulation too interfere in the diffusion of gas oxygen while during inspiration.

Tilak and Swarna Kumari (2009) in the fish *Ctenopharyngodon idella* with Nuvan (an organophosphate) as the toxicant, Vineeth Kumar and David (2008) in the fish *Labeo rohita* using Malathion, an organophosphate as the toxicant, Tilak and Koteswara Rao (2003) using chlorpyrifos in the three major carps, all, as the cause that reported in their respective studies emphasized only the stress factor resulted changes in the oxygen consumption and used this as a study of the biomarker for assessing the toxicant action. They all, also mentioned mainly the accumulation of the mucus on the gills and the architectural damage of the branchial filaments, the primary and secondary lamellae to be the valid reasons for such alterations in the oxygen consumption due to the stress and is same might be even for the present study also.

Evans (2005 & 1987) explained the mechanism of the gas exchange while during respiration. The toxic stress leads to the damage of the gill epithelium and as a consequence the epithelial transport of the ions is also affected. There are cells called chloride cells found in the lamellar epithelium. Their role in the ion transport, the afferent artery brings deoxygenated blood at its place where efferent one also at that same point, normal process takes place if anything happens the diffusion got impaired. There is a branchial epithelium that is sand-witched between serosal and mucosal blood. There are cat-ions K^+, Na with Cl^- ions. When Na' goes out Cl^- ions are more inside and externally Na^+ will be more. Positivity in the serosal blood, as ion in the afferent artery. Later, CO_2 when diffused inside with water forms H^+ and HCO_3^- after the dissociation of the

weak carbonic acid. When HCO_3^- goes out one Cl ion gets inside. The osmo-regulatory, acid base and blood dynamic actions if it not
affected by the environment pollutants. This can be explained as follows:



ATPase enzymes, Carbonic anhydrase (E) and Na^+, K^+ ions as in-flux and outflux will be disturbed. The mechanism of actions is clearly explained in the article Reported by Evans (2005 & 1987) and the above diagrammatic representation explains and if it is altered the O_2 diffusion is curtailed. Such similar mechanism is in operation even in the present work.

CONCLUSION

Oxygen is a parameter of life for its sustenance in living organisms and for the heterotrophs (animals-Fish) was a must for their metabolism. All such anabolism and catabolism reactive activities depend on the purification of the blood via the elimination of the metabolites, including gaseous carbon-dioxide. Gill is the entry point of the toxicant and is again at the same point, the exchange by diffusion of respiration takes place. The reasons of the alteration of the consumption of the oxygen, at one end can be explained by the architectural damage of the respiring organ apart from the excretory organ, too, and at the other and the biochemical impediments of the blood, due to the failure of which only, not, to have the normal quantity of the oxygen to be carried to all cells/tissues/organs and that can be a good reason to be explained. All is well for the fish but it not, the health condition, disease prone, toxic stress even in sub-lethal concentrations have made them to suffer. It is a study of the biomarker in ecotoxicology and is also the indices of the toxic action.

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Table 1

Physical and Chemical properties of water used for the present study

S. No.	Water Characteristic / Parameter	Quantity
1	Turbidity	8 silica units
2	Electrical conductivity at 28°C	816Micro ohms/cm
3	pH at 28°C	8.1
4a*	Alkalinity: Phenolphthalein	Nil
B*	Alkalinity: Methyl orange	472
5*	Total Hardness (as $CaCO_3$)	232
6*	Carbonate Hardness (as $CaCO_3$)	232
7*	Non Carbonate Hardness (as $CaCO_3$)	Nil
8*	Calcium Hardness (as $CaCO_3$)	52
9*	Magnesium Hardness	40
10*	Nitrite Nitrogen (as N)	Nil
11*	Sulphate (as SO_4^{2-})	Trace
12*	Chloride (as Cl^-)	40
13*	Fluoride (as F^-)	1.8
14*	Iron (as Fe)	Nil
15	Dissolved Oxygen	8–10ppm
16	Temperature	28±2°C

* The sample water used for experiments was clear, colorless and odorless.

* The following results were in mg/l

Figure 1

The amount of oxygen consumed in mg/gr body weight /hr of the fish *Labeo rohita* exposed to Sublethal and lethal concentrations of Bifenthrin technical grade

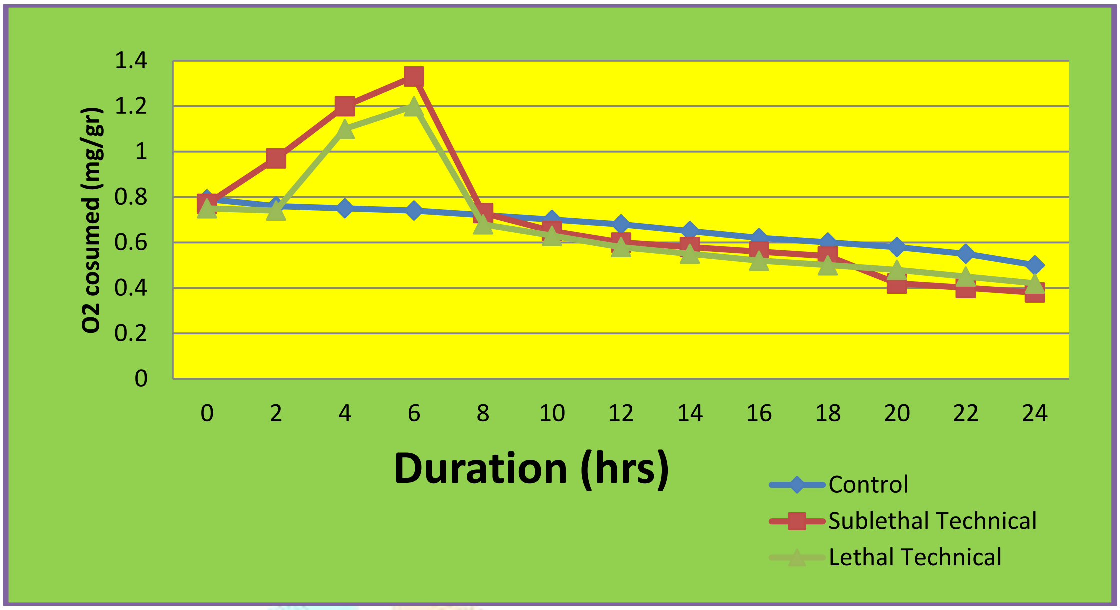

Figure 2

The amount of oxygen consumed in mg/gr body weight /hr of the fish *Labeo rohita* exposed to Sublethal and lethal concentrations of Bifenthrin technical grade

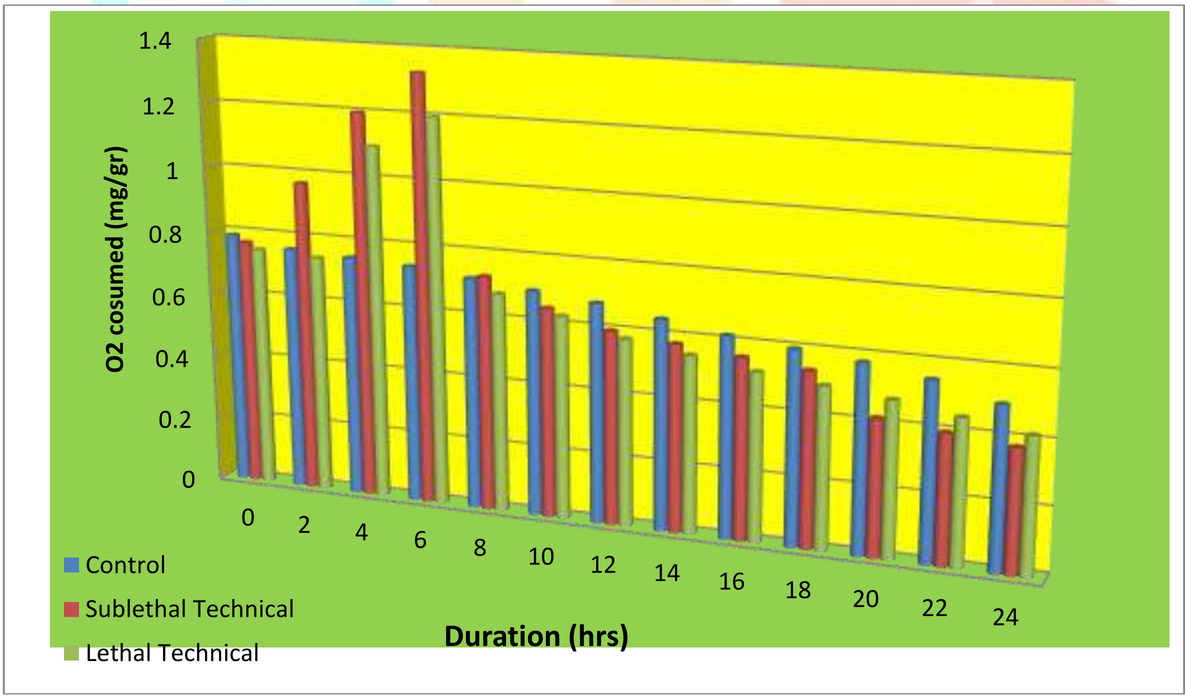

Figure 3

The amount of oxygen consumed in mg/g body weight/hr of the fresh water fish *Labeo rohita* exposed to sublethal and lethal concentrations of Bifenthrin 10% EC

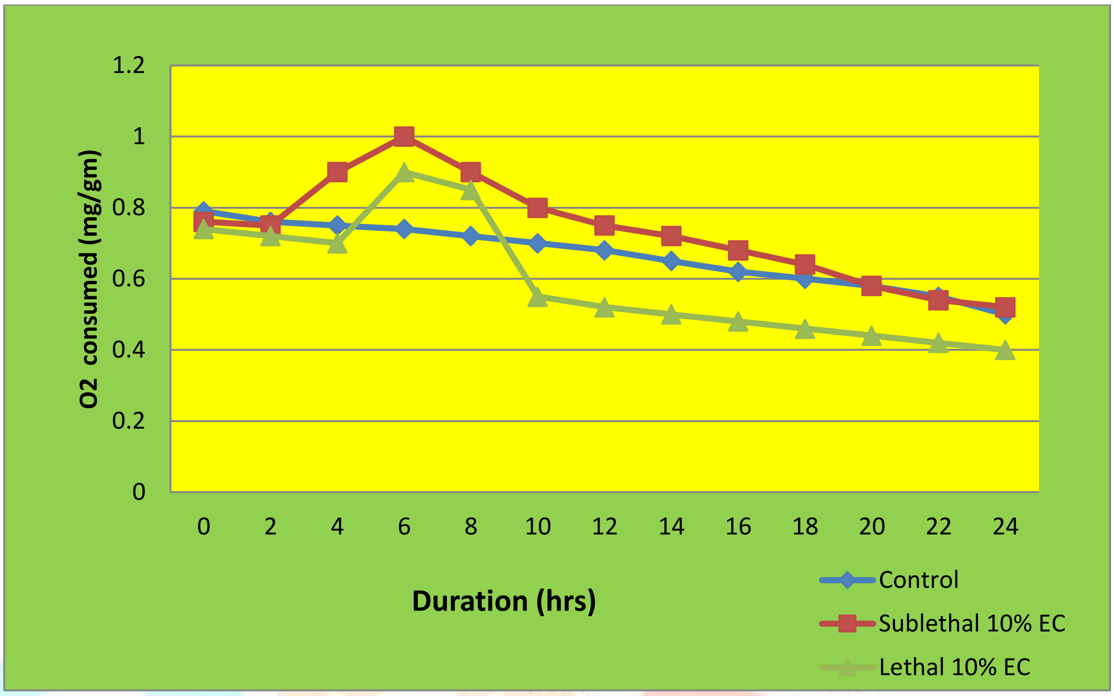

Figure 4

The amount of oxygen consumed in mg/g body weight/hr of the fresh water fish *Labeo rohita* exposed to sublethal and lethal concentrations of Bifenthrin 10% EC