TOXICITY AND EFFECT OF TECHNICAL GRADE AND 11% EC OF DELTAMETHRIN TO THE FISH *CTENOPHARYNGODON IDELLA* (GRASS CARP)

G. Srinivasa Rao, SatyanarayanaS,R. Bala Krishna Naikand Gopala Rao, N.*
Department of Zoology and Aquaculture, Acharya Nagarjuna University

G. Srinivasa Rao, M.Sc.
Department of Zoology and Aquaculture
Acharya Nagarjuna University
Nagarjuna Nagar 522510, AP India
sreegollu@gmail.com, gsr.anu.ac@gmail.com

S. Satyanarayana M.Sc.
Principal
Govt Junior College
Chagallu,
West Godavari
satyanarayana28sunnam@gmail.com

R. Bala Krishna Naik, M.Sc.
Senior Research Fellow, UGC
Department of Zoology and Aquaculture
Acharya Nagarjuna University
Nagarjuna Nagar 522510, AP India
razikrishna@gmail.com

*Dr. N. Gopala Rao, Msc., Ph.D.,
Asst. Professor
Department of Zoology and Aquaculture
Acharya Nagarjuna University
Nagarjuna Nagar-522 510, A.P. India
nagallagopalarao@yahoo.com

Abstract

Toxicity of a synthetic pyrethroid, technical grade and 11% E.C. of Deltamethrin is determined by employing both static and continuous flow through systems to the fish *Ctenopharyngodon idella.* The LC_{50}values of 24 hours for both technical grade and 11% EC are 0.510,0.519 µg/L^{-1} respectively in static system, 0.390,0.379µg/L^{-1} respectively in continuous flow through system. The fish are tested for oxygen consumption during respiration in which the toxicants exerted more demand for the consumptionof oxygen due to stress. During exposure duration of 24 hrs, the effect of 11% EC is more when compared in technical grade Deltamethrin.

Key words:*Ctenopharyngodon idella*, Deltamethrin, 11% EC, Static and Continues Flow through System, Respiratory Effect.

INTRODUCTION

The 'dogma' of Ecotoxicology is, pesticides, as insecticides either indirectly or directly are transported into the water or directly when sprayedinto the fish farms during aquaculture practices in disease management. The resultant situation is defilement of water and effect the ecosystem, (Figen Esin Kanghan *et al.* 2013). Being shown their presence, they not only change the ambient chemical characteristics, but also effect the non-target organism, fish. The piscine model in assessing the status of the pesticidal pollution is to determine the concentration at which they are killed or some times the levels are not that much termed as lethal and sublethal concentrations imparting the acute and chronic toxicity respectively. The well recognized such models are there for different chemicals including synthetic pyrethroids which arein use due to their production reached significant levels after the second world war which rose sharply from approximately 500000 t/a in the 1950 to over 3 million t/a at the beginning of 21st century, [Kaushik Mondal *et al.* 2015]. They are safe to birds and mammals but toxic to fish (Ahrar Khan et al 2012).Hence, the toxicity of the pesticides to fish received much attention andare reviewed by Ullah and Jallil 2015; Hasibur Rehman *et al.* 2014 and Shankar Murthy *et al.* 2013, wherein the toxicity and other effects to fish are recordedby different researchers.

Hasibur Rehman *et al* (2014)mentioned that the synthetic pyrethroidsare of two types one without cyanogroup as type 1 and with cyano group termed as type II. The examples are permethrin,etc,for typeI and cypermethrin, fenvalerate and deltamethrin etc., are for type II. Toxicity of type II are determined by individual researchers, Velisek (2011, 2007 and 2006), Anitha Susan(2012) and Satyavardhan (2013), Neelima *et al.* 2016a. Much work is on Cypermethrin, followed by Fenvalerate and least for Deltamethrin as reviewed by Bhattacharjeeand Das (2014). The work on the toxicity to the fish *Ctenopharyngodon idella* appears to be not recorded

much in the laboratory as *in vivo*studies. Hence, an attempt ismade to determine the toxicity and its effect during respiration ofthe inspiration,as consumption of oxygen which will serve as indices of pesticidalpollution in the science of ecotoxicology.

MATERIALS AND METHODS

The freshwater fish *Ctenopharyngodon idella*(both sexes, length 3-5 cms., weight 4-5 g) haven been used as the test organisms for the present investigation.Healthy and active fish were obtained from local fish form, at Nandivelugu, Guntur district, A.P, India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at room temperature 28± 1^{o}C and 12-12 h dark and light cycle. Water was renewed every day. During the period of acclimatization, the fish were fed (ad libitum) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions recommended by APHA on toxicity tests to aquatic organisms (APHA, 1998, 2005 and 2012) were followed. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

Physical & Chemical properties of water used for the present experiments are (in mg/L^{-1}): Turbidity - 8 silica units, Electrical conductivity at 28^{0}C-816 Micro ohms/cm, P^{H} at 28^{0}C-8.2, **Alkalinity**: Phenolpthalene- Nil, Methyl orange as $CaCO_3$-472, Total Hardness-320, Calcium Hardness-80, Magnesium Hardness-40, Nitrite nitrogen (as N)- Nil, Sulphate (As SO_4) – Trace, Chloride (as Cl^-) – 40, Fluoride (as F^-) - 1.8, Iron (as Fe) –Nil, Dissolved Oxygen - 8-10 ppm, Temperature - 28± 2^{o}C.

Acute Toxicity Test: Experiments were conducted to determine the acute toxicity of Deltarmethrin in various concentrations of technical and commercial grade formulation of 11% EC with five replicates for each concentration along with the control group. Control groups were added with the equal quantity of acetone used for preparation of highest deltamethrin working solutions. The concentrations of the test chemical used in short term definitive tests were in between lowest concentration at which there was no mortality and the highest concentration at which 100 per cent mortality resulted. The data on the mortality rate of fish was recorded for 24h. The number of dead fish at each concentration are noted, but takena precaution to remove dead fish immediately. The toxic tests were conducted to choose the mortality range from 10% to 90% for 24h. Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. The respective probit values were taken from Fisher and Yates (1938). For the determination of the 95% confidence limits, LC_{50} values and a normal variate of 1.96 were taken into consideration. .

Further the data is also analysed by probit analysis and computer generated output is taken which has given 24h LC50, lower and upper limits, regression equation, slope, and R^zvalues (Finney 1952) and is found that it is a GOODFIT.

Oxygen: Experiment on the oxygen consumption of the fish *Ctenopharyngodon idella* was 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 10 days. The water used for fish acclimatization and experimentation was the same. The fish measuring in the same length and in weight were used in the experiment as 24h toxicity experiments. All the precautions laid down by APHA (1998, 2005, 2012) are followed, for maintaining the fish. The samples for estimation were taken from the respiratory chamber, for every two hours of intervals up to at 24 hours. The amount of dissolved oxygen consumption was calculated per gram body weight per hour.

$$O_2 \text{ consumed by fish / gram body weight/hour} = \frac{\alpha - \beta \text{ x N of hypo x 8 x 1000}}{\text{Vol. of the sample x Correction factor x Wt. of the fish x Time interval for sample.}}$$

α = hypo rundown before exposure
β = hypo rundown after exposure

Students 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.

RESULTS AND DISCUSSION

As per the report of Prusthy *et al*., (2015) a review article on synthetic pyrethroids to the fresh water fish: Perils and mitigations, being used widely used and as agricultural runoff or drift from aerial or ground based spraying in fish farms which cause defilement are known to be less tolerant as those pose a threat to fishes. The non-cyano group type I and with cyano group as type II have toxic impact on them.

The fish *Ctenopharyngodon idella* with fenvalerate, Tilak and Yacobu (2002) reported the 96 hours LC_{50} value as 2.60 mg/l (0.26 µl) which cannot be compared with the present study as the toxicant is different but the fish and methodology are one and the same. Anitha*et al*., (2012) also reported with the same methodology to the major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* to determine LC_{50} values of fenvalerate are the other research contributions for fenvalerate Mushigiri (2003); Tilak and Yocobu (2004)

also. For cypermethrin to the different fish, wherein the methodology andfish are different are by Andems *et al.* (2016), Morolli *et al*., (2006); Sree Veni and Veeraiah (2014), Sailendra Kumar, Singh *et al.* (2010)for different fishes.

For Deltamethrin,reported toxicity studies are by Lord Tertese Angular (2017); Anilava and Gupta (2014); Sharma and Ansari (2013); Velisek (2011,2007&2006); Morolli *et al.* (2006),Josephine *et al*. (2006); Catla and Ural (2004), Venkata Ramudu (2008) and also Yildrim *et al* (2006).

In the present studyas per table 1, the methodology is totally different of the tested and resulted values of the LC_{50} of 24 hrs. The toxic sensitivity range of the quantity of toxicants technical grade and 11% EC deltamethrinis varied in the dissolved solvent and is 0.1 ml in and 0.2 ml respectively. . The formulation is only nearly 10% of 96% concentration of the technical grade. The static values are higher whereas lower in continuous flow through system. The lotic and lentic waters in natural ecosystem can be considered as of a static and continuous flow through system of freshwater which show all the difference in concentrations and the values in both tests media decreased as the duration of the exposure increases. It may be a factor of resistance or immune response to achieve this being cold blooded/ poikilothermic. The pyrethroids are not that much toxic to birds and mammals as they are warm blooded, the circulating fluid shows all the difference. According to Prusty *et al*., (2015) the toxicant is not likely to evaporate into the air or dissolve easily into water and is moderately to high toxic under laboratory conditions.

The toxicity values of the fish generally lies below 10 µg/L, due to lower rates of metabolism and removal in fish than those other groups of land vertebrates (Bradbury 1989a, b, 1985). The oxidative stress is the main cause of toxicity to fishes according to Sayeed *et al*., 2003, Abdollahi *et al*,, 2004 and Diana et al., (2009). De Assis (2009) and Pimpo *et al*., (2008) reported that the fish are very sensitive to Deltamethrin and even as per the WHO (1996) report is moderate to highly toxic to fishand is in the range of 0.91 -3.50 µg/l, close to the present study.

Prusty *et al*., (2015) mentioned that Deltamethrin being lipophillic which is the characteristic feature to have a high sensitivity to fish.. According to them the LC_{50} values to different species are given in the table as Table 2.. By viewing the table, it is clear that Deltamethrin showed species specific toxicity which depends on various factors. Even though the *Ctenopharyngodon idella* is first in the list, given as 24, 48, 96 h, values, in the present experiment of its size range being less and a different methodology of the hydrographical conditions which are varied in the different laboratories,so also, results.The toxicity data of the present simply can be used as a record to contemplate permissible limits for environmental monitoring and toxicity evaluation.

Not only that, Velisek *et al*., (2011) a chapter on effects of pyrethroid and triazine pesticides on fish. physiology, mentioned about the report of Dobsikova*et al*(2006), where in the toxicity of Deltamethrin to the fish rainbow trout and common carp, deltamethrinis more toxic than the other fellow toxicants of type II, Cypermethrin and Fenvalerate. Yildrim *et al*., (2006) reportedthat the 24h LC_{50}value of the nile tilapia (*Oreochromis niloticus*, L.) in static system as5.14µg/L and for 48 hrsas4.85µg/L and such trend is evidenteven in the present study, while the duration increases the toxicity value decreased.

Viran *et al*., (2003) reported on the toxicity of Deltamethrin and proved that it is a potential toxicant to the fish *Pochilia reticulate*, LC_{50} value for 48 h is 5.13 µg/L and mentioned mucous secretion on the gills is the causative factor impairing the normal functioning of them, ultimately effect..
Caglon Karasu Benll *et al*., (2009) reported Deltamethrin toxicity to the fish *Oreochromis niloticus*(L. 1758) Larvae and fry for 48 hrs, 1.17 µg/L and 1.70 µg/L respectively. In the culture practices, fry is important for sustainance of population stock. that has to be maintained. The present studyon fish is also, a candidate for culture, hence all necessaryprecautions have to be taken in monitoring pollution. The formulations are to be viewed seriously and at the same time a strict quality control is required.

Suvetha *et al*., (2015) reported on another culturable species, *Labeo rohita* where in for 24&96 hrs LC_{50} values are 0.438 and 0.38 mg L^{-1} respectively, in static bioassay. They opined that the toxicant exerted toxic effect due to the high rate of gill absorption, lipophilicity and deficiency in the fish enzyme system. Not only that the insecticides act mainly on the voltage dependant Na channels of the nerve cell membrane and induce the toxicity.

The fish dies – why? May be the answer lies partially or completely due to respiratory impairment wherein the nektonic, cold blooded/poikilothermic heterotrophic fish requires oxygen and in take during the process of ventilation, through gills the pharyngeal modifications partly from ectoderm and endoderm germ layers origin. The chloride cells in fresh water environment have to achieve homeo- stasis as it is subjected to, endosmosis process that have to be overcome, where the accumulated salts have to be secreted at one end and diffusion of gases through lamellar squamous epithelium supported by columnar cells (Pillar cells), may be viewed in this way also.[Velisek, 2006]

The fish when subjected to stress, the normal functioning of gills is not in a position to achieve and is deprived off. The present study on the fish respiration,, of intake oxygen is required to meet the demand of energy for active swimming and feeding. The data presented in the graphical representation as figure 2 which depicts the quantity of uptake is more and later due to oxidative stress the consumption is less hence more demand for oxygen to cope the onus situation. The formulations effect more in this stich,the fish are suffered.

The same points are reiterated by earlier reports of Bradbury *et al*., (1989a, b &1985), Anitha *et al*., (2012), Velisek *et al*., (2007), Satyavardhan (2013), Tilak and Yacobu(2002) and Neelima *et al*., (2016b) directly during respiration and indirectly by altering the haematological parameters and have bearing on oxygen carrying capacity. The fish have nucleated RBC, wherein the count is decreased as a consequence less amount of oxygen consumptionand demand for more oxygen eventhough the concentrations are sublethal, which are really lethal in chronic toxicity owing to this (Velisek 2012). If we consume such contaminated fish can cause health hazard to human beings as reported by Neelima*et al.* (2016b) as a cautious note.

The commercial formulations purchased locally are more toxic than the technical grade because of the ingredients that are mixed in 11% EC exert cumulative or additive toxicity. The formulation LC_{50} value is nearly same to the technical grade value but it contains only 11% technical grade substance and this is in agreement with the study of Bradbury *et al*., 1985 in which, it was observed for fenvalerate, another type of class II type synthetic pyrethroid.

The oxygen consumption due to toxic stress as per the figures1 &2, the fish showed toxicity effect by taking more oxygen and as the duration of the exposure increased the demand for more oxygen consumption. Due to severe stress the fish consumed less food and it is going to be more detrimental in culture media where growth is an important parameter. The same point of opinion is reiterated by Anitha *et al* (2012); Velisik *et al.*(2007& 2006) and Tilak and Yacobu (2002).

The toxicant on fish study, is first of its kind IN THE PRESENT METHODOLOGY among synthetic pyrethroids, of type II in both static and continuous flow through tests as a comparisonto give any candidate species for culture, needs to be monitored in an environmental policy drafting and planning to provide various strategies to be adopted.

CONCLUSION

Along with Cypermethrin and Fenvalerate, Deltamethrin the type II synthetic pyrethroid with cyano group are toxic to fish. Due to stress there is a demand for more oxygen, as evidenced by the present study. The formulations are with some ingredients which exert toxic action, cumulative or additive.Toxic action ofthe toxicant has an effect causing death or no death immediately in acute and chronic toxicity, it is dependent on the fish, size and also the nature of the toxicant including formulations. In nature, the existence of Deltamethrin as a single contaminant never arise, cumulative effects of many such toxicants. It requires further investigation to understand the toxic action and respiratory distress.in the laboratory experiments for combined toxicity.But the present study on the deltamethrin to the fish is helpful to document the data and can be used for toxicity tests as tool by the toxicant.

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Table 1. 24h LC_{50} values of Deltamethrin Technical Grade and 11% EC in static and continuous flow through system in the freshwater fish *Ctenopharyngodon idella*

Duration of Exposure	LC_{50} (µg/L)				Regression equation			
	Static		CFT		Static		CFT	
	Technical	11% EC	Technical	11% EC	Technical	11% EC	Technical	11% EC
24h	0.510 (0.503-0.516)*	0.519 (0.507-0.532)*	0.390 (0.383-0.396)*	0.379 (0.367-0.392)*	Y=72.4x +26.20 (72.347)** (26.68)** (R^2=0.994)**	Y=36.88x +15.5 (36.818)** (15.479)** (R^2=0.994)**	Y=55.3x +27.67 1(55.300)** 2(27.629)** 3(R^2=0.994)**	Y=28.3x +16.91 (28.197)** (16.8721)** (R^2=0.974)**

*Values in the parentheses are lower and upper limits
**Values in the parentheses are 1.Slope 2.Intercept and 3.R^2 respectively

Figure 1. The amount of oxygen consumed in mg/gr body weight /hr of the fish *Ctenopharyngodon idella* exposed to Sub lethal and lethal concentrations of Deltamethrin technical grade

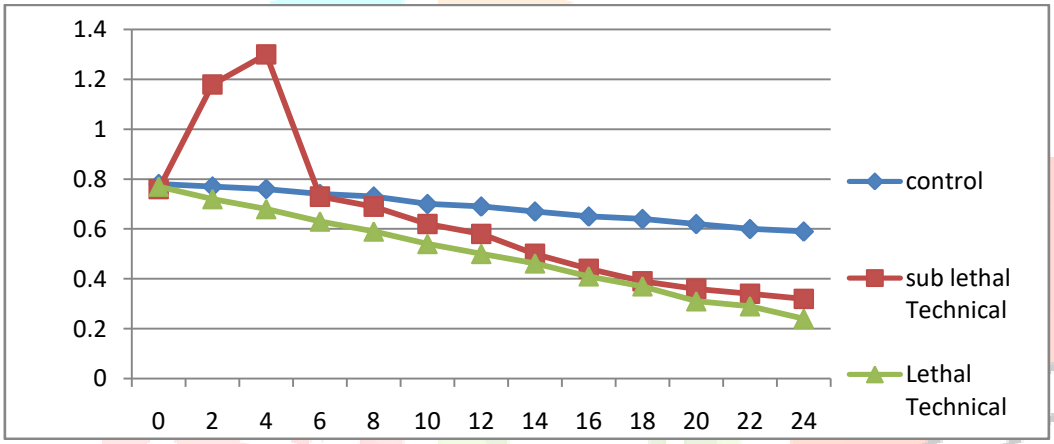

Figure 2. The amount of oxygen consumed in mg/gr body weight /hr of the fish *Ctenopharyngodon idella* exposed to Sub lethal and lethal concentrations of Deltamethrin 11%EC

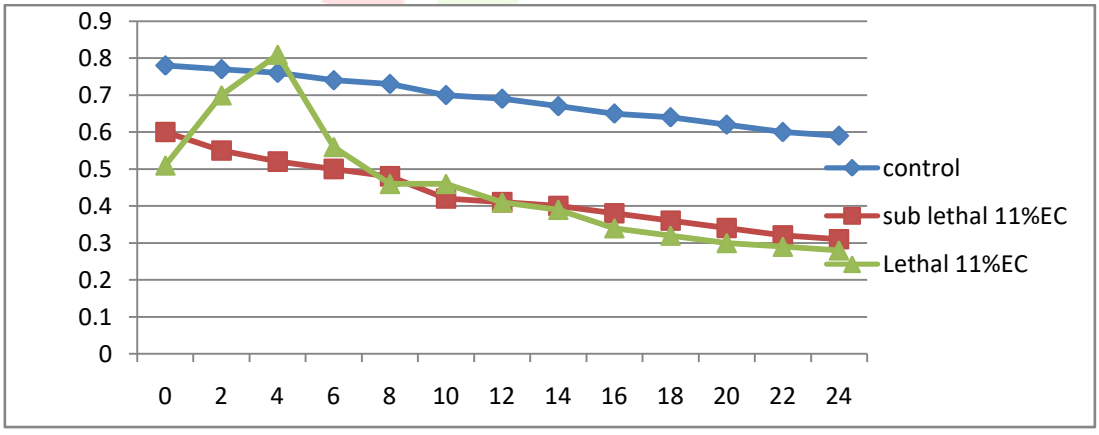

Table 2. Toxicity studies on the effects of deltamethrin on various species
Source: Preesty *et al*. (2015). Int.Aquatic Res. 7: 163-191.

Scientific name	LC_{50} value	References
Ctenopharyngodon idella	155.0 µg/L (24 h) 96.0 µg/L (48 h) 91.0 µg/L (96 h)	Rao *et al*., (1983)
Cyprinodon macularious	0.60 µg/L (24 h) 0.60 µg/L (48 h)	Mulla *et al*., (1978)
Cyprinus carpio	3.5 µg/L (24 h) 3.5. µg/L (48 h) 3.5 µg/L (96 h)	Lakota *et al.*, (1989)
Cyprinus carpio	4.00 µg/L (48 h) 2.30 µg/L (96 h)	Sun (1987)
Cyprinus carpio	91.0 µg/L (24 h) 89.0 µg/L (48 h) 78.0 µg/L (96 h)	Rao *et al.*, (1983)
Cyprinus carpio	0.058 µg/L (96 h)	Svobodoya *et al.*, (2003)
Cyprinus carpio	9.41 µg/L (24 h) 4.47 µg/L (48 h) 2,.37 µg/L (72 h) 1.65 µg/L (96 h)	Catla and Ural (2004)
Cyprinus carpio	0.213 µg/L (48 h) 0.074 µg/L (48 h)	Koprucu and Aydin (2004)
Esox lucious	44.0 µg/L (24 h) 30.0 µg/L (48 h) 23.0 µg/L (96 h)	Rso *et al.*, (1983)
Gambusia affinis	1.00 µg/L (24 h) 1.00 µg/L (48 h)	Malla *et al.*, (1978)
Oncorhynchus mykiss	0.50 µg/L (24 h) 0.70 µg/L (48 h)	Malla *et al.*, (1978)
Oncorhynchus mykiss	2.50 µg/L (24 h) 2.30µg/L (48 h) 2.30 µg/L (96 h)	Lakota *et al.*, (1989)
Oncorhynchus mykiss	8 µg/L (12 h) 10 µg/L (24 h0 12 µg/L (48 h) 25 µg/L (72 h) 50 µg/L (96 h)	Ural and Sanglam (2005)
Tilapia mosambica	0.80 µg/L (24 h) 0.80 µg/L (48 h)	Mulla *et al.*, (1978)
Tilapia nilotica	16.0 µg/L (24 h) 15.0 µg/L (48 h) 14.5 µg/L (96 h)	Golow and Godzi (1994)
Oreochromis niloticus	1.17 µg/L (48 h) 1.70 µg/L (48 h) 1.45 µg/L 4.85 µg/L	Karasu benli *et al.*, (2009) Kan *et al.*, (2012) Yildirin *et al.*, (2005)
Poecilia reticulata	24.0 µg/L (24 h) 21.0 µg/L (48 h) 20.0 µg/L (72 h) 19.0 µg/L (96 h) 18.0 µg/L (120 h)	Stalin *et al.* (2008)