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Toxic Impact of Phosphamidon on Lipid Content in the Freshwater Fish, *Labeo rohita*

T. Joselinfreeda[1,2*] and B.Venkadesh[1 2 3]**
[1]Department of Zoology, Muslim Arts College. Department of Zoology, Thiruvithancode - 629 174, Tamil Nadu.
[2]Manonmaniam Sundaranar University, Abishekapatti,Tirunelveli -627 012, Tamil Nadu.
3.Government Arts College for Men (Autonomous), Nandanem, Chennai – 600 035., Tamil Nadu.
** Corresponding author: E-mail: profvenki68@gmail.com (Dr. B. Venkadesh)
*Research Scholar, Reg No:9249

ABSTRACT
The insecticide Phosphamidon was used for the present study. The fishes were exposed to different concentrations of Phosphamidon to evaluate the acute toxicity and calculate the LC_{50} value. The computed LC_{50} values for 12, 24, 48, 72 and 96 hours were found to be 7.714, 6.560, 6.344, 5.681 and 5.149 mg/L respectively. The fishes were exposed to sublethal concentrations for 45 days. At the end of each exposure period, fishes were sacrificed and tissues such as liver, muscle and intestine were removed and analyzed for lipid content. It showed decreased value of lipid content in all the tissues when compared to control.
Keywords:*Sublethal concentration; Phosphamidon,Labeo rohita,Lipid.*

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INTRODUCTION

The pesticides usage is desirable for the control of pests in agro and household environment; but it causing environmental pollution in various level[1-3].During agriculture practices of pesticides (surface runoff and aerial spraying) are the major source for translocating pesticides into aquatic ecosystems. The contamination of water by pesticides may affects on non-target organisms like fish and other aquatic organism such as alga, *Lemna* and *Daphnia* [4-6]. Circulation of these pesticides in a water body may lead to accumulation in fish and other aquatic plants [7-8]. Upon penetrating into an organism, pesticides rapidly spread in it and become selectively accumulated in separate parts or organs of a body [9-10]. As considerable amount of pesticides entersinto the fish body, through the food chain, where they are distributed and metabolised depending upon the detoxifying ability of the fish, and elicit some responses in fish which depends on the nature and concentration of pesticides [11-12].

Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by the water pollution [13]. Biochemical studies of fish tissues are of considerable interest for their specificity in relation to food of fish and for evaluating their physiological need at different periods of life. The energy demand during pesticide stress has been observed by Nanda et al[14]. Hence the present study showed the effect of insecticide Phosphamidon on the lipid content of the fresh water fish *Labeo rohita* .

MATERIAL AND METHODS

The pesticide Phosphamidon, has been selected for the present study, as it is being indiscriminately used in Kanyakumari district and also there is a lack of information on the toxicity of Phosphamidon in *Labeorohita.*

Well acclimatized *Labeorohita*, approximately (8±2 g) were selected from the stock and exposed to different concentrations of Phosphamidon individually for the static bioassay test. The experiments were conducted in 100 litre tanks with 10 fishes each, starved for 24 hours prior to the experiment. The experimental medium was renewed daily till the end of the experiment. The mortality of fishes in different concentrations was noted at 12, 24, 48, 72 and 96 hours, and the dead fish were removed during observation. Fishes showing no respiratory movement and response to tactile stimuli were considered as dead. Then 12, 24, 48, 72and 96 hrs LC_{50} values of Phosphamidon were computed [15]. The experiment was repeated three times and the mean values recorded separately for each test fish. Simultaneously ten fishes were reared in pesticide-free medium and are treated as control for each set of experiment.

Estimation of lipid

The sublethal concentration was selected from the 96 hours LC_{50} values.The sub-lethal concentrations, *viz* 1.029($1/5^{th}$) mg/L and 0.514 mg/L ($1/10^{th}$) were chosen to expose the fish for biochemical study. The fishes were kept at each concentration for a period of 45 days. All the fishes were regularly fed with nodules prepared from ground oil cake and rice bran during the experimental period. After 15, 30, and 45 days exposure, the fishes were taken out and the following tissues, *viz.*, liver, muscle and intestine, were dissected out under aseptic condition. Using the wet sample, total lipid was estimated by Folch et al [16]. Two-way analysis of variance (ANOVA) was applied to find out the significance of variation caused by concentration of the pesticide and exposure periods.

Procedure

At 50 mg experimental tissue sample was taken and homogenized well with 4 ml of chloroform methanol mixture. After mixing well, 0.2 ml of 0.9% sodium chloride was added thenit kept without disturbance overnight. The lower layer of lipid was dissolved in concentrated sulphuric acid by keeping in boiling water bath for 10 minutes. From the prepared total lipid sample, 0.2 ml was taken in a test tube and add 5 ml of sulphophospho vanillin reagent, shaken well and kept undisturbed for 30 minutes. The intensity of red colour was measured at 520 nm in a spectrophotometer.

RESULT

Acute toxicity

Present study mortality was recorded after 12, 24, 48, 72 and 96 h and LC_{50} values and its confidence levels were calculated. The computed LC_{50} values for 12, 24, 48, 72 and 96 hours were found to be 7.714, 6.560, 6.344, 5.681 and 5.149 mg/L respectively Table 1.

Toxicity curve constructed by plotting LC_{50} values against time Fig 1. The peak LC_{50} values for Phosphamidon was at 7.714 mg/L (12 hours LC_{50}) and the LC_{50} values decreased with increased exposure hours. The minimum LC_{50} values 5.149 mg/L at 96 hours.

Lipid

The sublethal concentration of Phosphamidon treated with *L.rohita* were subjected to biochemical estimations. The fish organs (liver, muscle and intestine) were studied inlipid level at different time interval, the results showed that duration of exposure period increased the lipid content of the organ was declined. The changes in the level of lipid contents in muscle, liver and intestine of the fish, *Labeo rohita,* were given in the Table 2 -4 and Figure 2a-4a.

The percentage decreased lipid content of muscle was on day 15 (21.09 & 35.16%), on day 30 (33.86 & 41.43%) and on day 45 (44.30& 55.06%)at the concentration 0.541 and 1.029mg/L respectively (Table 2). The concentration dependant activity was recorded (Table 2a)

The percentage decreased lipid content of liver was on day 15 (20.13 & 37.84%), on day 30 (31.61 & 8.39%) and on day 45 (50.60 & 57.23%)at the concentration 0.541 and 1.029 mg/L respectively (Table 3). The concentration dependant activity was recorded (Table 3a)

The percentage decreased lipid content of liver was on day 15 (20.13 &37.84%), on day 30 (31.61 &48.39%) and on day 45 (50.60 &57.23%)at the concentration 0.541 and 1.029mg/L respectively (Table 3). The concentration dependant activity was recorded (Table 3a)
The percentage decreased lipid content of intestine was on day 15 (11.84 &19.74%), on day 30 (24.09 &32.53%) and on day 45 (36.26&42.86%)at the concentration 0.541 and 1.029mg/L respectively (Table 4). The concentration dependant activity was recorded (Table 4a)
To find out the effect of pesticide concentrations and the duration of exposure times on the Lipid change in the tissues of *Labeo rohita,* two -way analysis of variance (ANOVA) was carried out(Table 2a – 4a).

Table 1 Calculation of log – dose profit Regression line for mortality experiments (96 hours) in which same sized *Lebeorohita* were exposed to different concentration of the pesticide in the Basvine technique.

(1) Dose %	(2) No. %	(5) Mor. dose	(6) Log Pro.	(7) Emp. Pro.	(8) Exp. Pro.	(9) Work Coef.	(10) Wt. w	(11) Weight	(12) wx	(13) wy	(14) y
4.00	10	20.00	1.60	4.16	3.86	4.20	0.41	4.05	6.49	17.00	3.77
4.50	10	30.00	1.65	4.48	4.44	4.48	0.56	5.58	9.22	25.00	4.34
5.00	10	40.00	1.70	4.75	4.95	4.75	0.64	6.37	10.82	30.28	4.86
5.50	10	60.00	1.74	5.25	5.42	5.25	0.60	6.01	10.46	31.56	5.32
6.00	10	70.00	1.78	5.52	5.84	5.50	0.50	5.03	8.94	27.64	5.75
6.50	10	80.00	1.81	5.84	6.23	5.76	0.37	3.70	6.71	21.31	6.14
7.00	10	100	1.84	7.33	6.59	7.01	0.24	2.38	4.39	16.68	6.50

STATISTICS

SW= 33.120 SWX= 57.034 X Bar= 1.722 SWY=169.483 Y Bar= 5.117
SWX*X= 98.388 SWY*Y= 884.694 SWXY= 293.494
b Value = 11.240
Regression Equation y = 11.240x -14.24
If y=5.0 then x = 1.712 This corresponds to dose of 5.149
Varaiance 0.0003 Chi-square 1.89 (with 5 Deg. of freedom p)
Lower Limit 1.6753 Log Dose 1.7116 Upper Limit 1.7479

Fig.1. Toxicity curve of pesticide against *Lebeorohita*

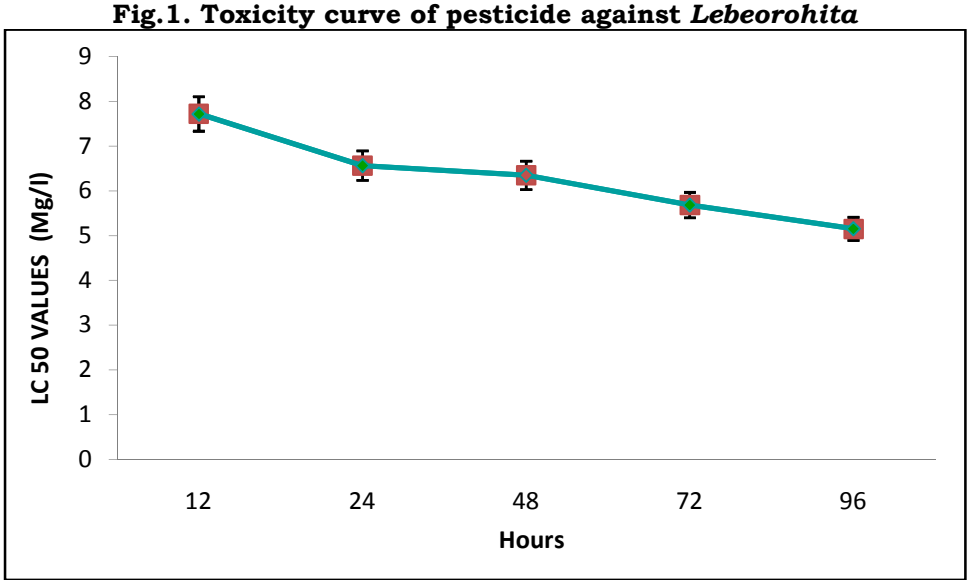

Table 2. Changes in the level of Lipid content (mg / 100 mg wet tissue) in the Muscle tissue of *Labeo rohita* exposed to sub lethal concentrations of Phosphamidon

Days	**Control**	**Concentrations Phosphamidon (mg/L)**	
		0.541	**1.029**
15	1.28±0.02	1.01±0.03 (21.09)	0.83±0.03 (35.16)
30	1.40±0.01	0.94±0.02 (33.86)	0.82± 0.02 (41.43)
45	1.58±0.02	0.88±0.02 (44.30)	0.71 ± 0.01 (55.06)

Table 2a. Two- way ANOVA of lipid content (mg / 100 mg wet tissue) in the muscle tissue in *Labeo rohita* exposed to different concentrations of Phosphamidon

Source of Variation	**Sum of squares**	**Degrees of freedom**	**Mean sum squares**	**F-Value**	**P-value**
Total Variance	0.7158	8			
Variance due to exposure duration	0.000467	2	0.000233	0.014941	(P>0.05)
Variance due to concentration	0.652867	2	0.326433	20.90288	** (P<=0.01)
Error variance	0.062467	4	0.015617		

Table 3 Changes in the level of Lipid content (mg / 100 mg wet tissue) in the Liver tissue of *Labeorohita* exposed to sub lethal concentrations of Phosphamidon

Days	**Control**	**Concentrations Phosphamidon (mg/L)**	
		0.541	**1.029**
15	1.48±0.02	1.17±0.03 (20.13)	0.92±0.02 (37.84)
30	1.55±0.01	1.06±0.03 (31.61)	0.80± 0.02 (48.39)
45	1.66±0.02	0.82±0.02 (50.60)	0.71 ± 0.01 (57.23)

Table 3 a. Two- way ANOVA of lipid content (mg / 100 mg wet tissue) in the liver tissue in *Labeo rohita* exposed to different concentrations of Phosphamidon

Source of Variation	**Sum of squares**	**Degrees of freedom**	**Mean sum squares**	**F-Value**	**P-value**
Total Variance	1.0118	8			
Variance due to exposure duration	0.024267	2	0.012133	0.618522	(P>0.05)
Variance due to concentration	0.909067	2	0.454533	23.17077	** (P<=0.01)
Error variance	0.078467	4	0.019617		

Table 4 Changes in the level of Lipid content (mg / 100 mg wet tissue) in the Intestine tissue of *labeo rohita* exposed to sub lethal concentrations of Phosphamidon

Days	**Control**	**Concentrations Phosphamidon (mg/L)**	
		0.541	**1.029**
15	0.76±0.01	0.67±0.02 (11.84)	0.61±0.02 (19.74)
30	0.83±0.02	0.63±0.03 (24.09)	0.56± 0.04 (32.53)
45	0.91±0.02	0.68±0.03 (36.26)	0.52 ± 0.01 (42.86)

Table 4 a. Two- way ANOVA of lipid content (mg / 100 mg wet tissue) in the Intestinal tissues in *Labeo rohita* exposed to different concentrations of Phosphamidon

Source of Variation	Sum of squares	Degrees of freedom	Mean sum squares	F-Value	P-value
Total Variance	0.129022	8			
Variance due to exposure duration	0.001489	2	0.000744	0.195335	(P>0.05)
Variance due to concentration	0.112289	2	0.056144	14.73178	* (P<=0.05)
Error variance	0.015244	4	0.003811		

DISCUSSION

Acute toxicity

In the present study the computed LC_{50} values for 12, 24, 48, 72 and 96 hours were found to be 7.714, 6.560, 6.344, 5.681 and 5.149 mg/L respectively.Similar results were also reported many researcher against fish [17-20]. Mustafa et al [21] reported that poisoning/toxicity is categorised as either acute or chronic and the determination of the median lethal concentration (LC_{50}) is considered to be the preliminary step for studies into the extent of acute or chronic toxicity. Different pesticides, have different LC_{50} values in different organisms [22]. Thus, Pentachlorophenol (PCP) has an LC_{50} value of 0.58 ppm in *H. fossilis* [23], Likewise in common carp, acute toxicity of profenofos (LC_{50}) and triazophos (LC_{100}) was determined as occurring at 62.4 ppb and 1.00 ppm, respectively in two different studies [24]. Assis et al [17] determined the LC_{50} values for carbaryl and carbofuran as 33.8 µmol/L and 0.92 µmol/L, respectively. Pandey et al [25] observed acute toxicity of profenofos to *Channa punctuates*observed as 2.68 µg/L while, in *Catla catla,* the LC_{50} values of Phorate was calculated as 0.53mg [26]. Mahboob et al [21] studied the toxicity of triazophos, profenofos, carbofuran andcarbaryl pesticide showed LC_{50} value of 1.05, 0.21, 0.49 and 4.75 mg/L respectively against *Cirrhinus mrigala*.

It has been pointed out that the toxicity of a pesticide can be modified by various factors including the physico-chemical characteristics of the medium, and the biological behavioursand status of test animal [27]. The status of test fishes includes size, weight, age, sex and life cycle stage. The physico-chemical factors such as temperature, pH,alkalinity and hardness also influence the toxicity [28].

In the present study the lipid level in the tested tissues decreased and the decreasing trend was directly proportional to the concentration of the toxicant and periods of exposure. Similar results were observed by Saradhamani and Kumari, [29] noticed significant decrease in glycogen, protein and lipid in some tissues of the fish, *O. Mossambicus* after long term exposure of chlorpyrifos. Veeraiah et al [28] observed decrease biochemical parameters of freshwater fish *Labeo rohita* exposed to lethal and sublethal concentrations of indoxacarb. Puvaneswari et al [30] reported serum biochemical alterations in fish *Mugil cephalus* in different pollutants. Amali *et al.* [31] suggested that lipid content of fish reduced with increasing concentration of pollution. They showed that reduction in lipid content might be due to utilization of lipid as a source of energy during stressful condition. Gupta et al [32] has also reported that lipid content decreased in various tissues of *Channa punctatus* with increasing concentration of vegetable oil factory effluent.

Olaganathan and Patterson [33] stated that lipolytic activity may increase to meet the increased energy demands during pollution stress. The depletion of lipid content may be due to the inhibition of lipidsynthesis or comparatively more utilization of the stored lipids.

Decrease in total lipids of various organisms after acute and chronic treatment may be due to increased activity of lipases involved in oxidation of lipids or due to reduction in fatty acid synthesizing enzymes [34]. Tilak and Yacobu [35] reported that disturbance of fat metabolism is an indication of impaired pancreatic functions. Decrease in tissue lipid might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes, and cell organelles present in the cytoplasm [32].

CONCLUSION

In the present study, clearly indicates that the pesticide at sublethal concentration level influence the biochemical of the freshwater fish when duration of the exposure period was increased. From this result application of pesticides could be reduced in future, for sustainable environment.

REFERENCES

1. Sexana, K.K., Chauhan, R.R.S. (1996). Oxygen consumption in fish *Labeorohita* caused by distillery effluent. Poll. Res. 15(3): 277-280.
2. Fanta, E., Rios, F.S.,Romao, S.,Vianna, A.C.,Freiberger, S. (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotox. Environ. Safe.54: 119-130.
3. Dixit, Y.B. (2005). Biochemical changes in the liver of a freshwater teleost, *Heteropneustesfossilis*(Bloch) exposed to rogor. J. Zool.25:51-53.
4. Amali, A.A. (1995). Biochemical and enzymological studies on the toxicity of pesticides (quinolphosand padan) in the fresh water fish, *Labeorohita* (Ham). Ph.D thesis, University of Madras, Tamil Nadu, India.
5. Natarajan, C.R.(1997). Sublethal effects of selected pesticide on an edible fish *Cyprinus carpio*var. Communis. Ph.D thesis, M.S. University, Tamil Nadu.
6. Ayoola, S.O. (2008). Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromisniloticus*) juvenile. Afr. J. Agric. Res. 3(12): 825-834.
7. Mishra, J., Srivastava, A.K. (1983). Malathion induced haematological and biochemical changes in the Indian catfish, *Heteropneustesfolssilis*. Enviro. Res. 30: 393-398.
8. Brodeur JC, Sanchez, M. Castro L, Rojas DE, Cristos D, Damonte MJ, Poliserpi MB, D'Andrea MF, Andriulo AE. 2017. Accumulation of current-use pesticides, cholinesterase inhibition and reduced body condition in juvenile one-sided livebearer fish (*Jenynsiamultidentata)* from the agricultural Pampa region of Argentina. Chemosphere 185, 36-46.
9. Sethuraman, A., Kiros, S., Tomass, Z. (2013). Residues of organochlorine pesticides in fishes from the Mumbai west coast of India. Int. J. Pure Appl. Zool. 1(1): 109-116,
10. Unyimadu JP, Osibanjo, O., Babayemi JO 2018. Levels of Organochlorine Pesticides in Brackish Water Fish from Niger River, Nigeria. Journal of Environmental and Public Health, Article ID 2658306, 9 https://doi.org/10.1155/2018/2658306a
11. Kumar, R. (1999). Effect of ammonia stress on protein of Indian murrel, *Channa punctuate*s (Bloch). J. Environ.Pollution 6(4):273-275.
12. Kaur,I., Mathur, R.P., Tandon, S.N., Dureja, P. (1997) Identification of metabolites of malathion in plant, water and soil by GC-M.S. Biomed Chromatogr. 11(6):352-355.
13. Prado, R.,Rioboo, C., Herrero, C., Cid, A. (2009). The herbicide paraquat induces alterations in the elemental and biochemical composition of non-target microalgal species. Chemosphere, 76: 1440-1444.
14. Nanda, P., Panda, B.N.,Behra, M.K. (2000). Nickel induced alterations in protein level of some tissues of*Heteropneustesfossilis*. J. Environ. Biol. 21(2): 117-119.
15. Finney, D.J. (1971) : Probit Analysis. University Press Cambridge. pp.333.
16. Folch, J., Less, M., Stantley, G.H.S.(1957). A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226(1):497-509.
17. Assis, C.R., Castro,P.F., Amaral, I.P., Carvalho,E.V., Carvalho, L.B.Jr., Bezerra, R.S. (2010). Characterization of acetylcholinesterase from the brain of the *Amazonian tambaqui* (*Colossomamacropomum*) and *in vitro* effect of organophosphorus and carbamate pesticides. Environ. Toxicol. Chem. 29 (10): 2243-2248.
18. Ansari, S., Ansari, B.A.(2011). Embryo and fingerling toxicity of dimethoate and effect on fecundity, viability, hatchability and survival of zebrafish, *Danio rerio* (Cyprinidae). World J. and MarineSci. 3(2): 167-173.
19. Vajargah, M.F.,Hossaini, S.A., Hedayati, A.(2013). Acute toxicity of two pesticides, diazinon and deltamethrin on spirlin, (*Alburnoidesbipunctatus*) larvae and fingerling. J. Toxicol. Environ Hlth.Sci.5(6): 106-110.
20. Mustafa, G., Mahboob, S., Al-Ghanim, K.A., Sultana, S., Al- Balawi, H.F.A., Sultana, T., Misned, F.A., Ahmed, Z. (2014). Acute toxicity I: Effect of profenofos and triazophos (organophosphates) and carbofuran and carbaryl (carbamates) to *Labeorohita*. Toxicol. Env. Chem.96:3, 466-473.
21. Mahboob, S., Ghazala, Al-Ghanim, K.A., Sultana, S., Al-Balawi, H.F.A. (2015).A Study on acute toxicity of triazophos, profenofos, carbofuran and carbaryl pesticides on *Cirrhinusmrigala*. Pakistan J. Zool. 47(2): 461-466.
22. Gupta, N.S., Mathur, P., Mukesh, D., Bhaduric, S.(2006).Kinetic investigations of the mechanism of dihydrogen driven catalytic reduction of methylene blue, safranine O, methyl viologen and

ferricyanide using platinum carbonyl cluster anions (Chini-clusters) as catalyst. InorganicaChimica Acta359 (12): 3895-3902.
23. Farah, M.A., Ateeq, B., Ali,M.N., Sabir,R., Ahmad, W. (2004). Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere 55(2): 257-265.
24. Ismail, M., Ali, R., Waheed, U., Khan, Q.M. (2009) Evaluation of the acute toxicity of profenofos and its effects on the behavioral pattern of fingerling common carp (*Cyprinus carpio* L.,1758). Bull. Environ. Contam. Toxicol. 82(5): 569-573.
25. Pandey, B.K., Srivastava, S., Singh, M., Ghosh, J.K. (2011). Inducing toxicity by introducing a leucine-zipper-like motif in frog antimicrobial peptide, magainin 2. Biochem. J. 436(3): 609-620.
26. Naik, D.V., Naik, L.S., Jawahar, S., Naik, M.J. (2016). Assessment of acute toxicity and behavioral changes in freshwater fish *Catla catla* exposed to organophosphate pesticide phorate 10% CG. Europeanjournal of Pharmaceutical and Medical Research. 3(11): 429-434.
27. Holcombe, G.W., Phipps, G.L., Tanner, D.K. (1982). The acute toxicity of kelthane, dursban, disulfoton, pydrin, and permethrin to fathead minnows *Pimephales promelas* and rainbow trout, *Salmo gairdneri*. Environmental Pollution Series A, Ecological and Biological 29 (3):167-178.
28. Veeraiah, K., Rao, P.S., Rani, A.S., Dhilleswarao, H. (2013). Changes in biochemical parameters of freshwater fish *Labeo rohita* exposed to lethal and sublethal concentrations of indoxacarb. International Journal of Bioassays 2(10):1382-1387
29. Saradhamani, N., Kumari, S.B. (2011). Impact of (Chlorpyrifos) on the biochemical components of the fish, *Oreochromis mossambicus* (Peters, 1852). Current Biotica 5(1): 79-84.
30. Puvaneswari, S., Jiyavudeen, M., Venkatesh, P. (2017). Serum biochemical alteration in fish *Mugil cephalus* collected from Uppanar river, Cuddalore, Tamil Nadu, India. Life Science Archives. 3(2): 1029-1034.
31. Amali, A., Kumar, C.A. Jayanthi, E., Selvanayagam, M. (1996). Quinolphos induced biochemical abnormalities in *Cirrhinus mrigala* (Ham). J. Environ. Biol. 17(2): 121-124.
32. Gupta, A.K, Dutt, D., Ananth, M., Dalela, R.C. (1994) Combined toxicity of chlordance, malathion and furadon to test fish, *Notopterus notopterus* (Mor). J. Environ. Biol. 15(1): 1-6.
33. Olaganathan, R., Patterson, J. (2013). Effect of Anthraquinone dyes on the carbohydrate , protein and lipid content in the muscle of *Channa punctatus* and *Cyprinus carpio*. . International Journal of Pharmaceutical Applications. 4(1): 11-18.
34. Raj, S.J., Joseph, B. (2015). Kumar L.M and Askar, R.K. 1994. Impact of Acetamiprid toxicity on biochemical biomarkers (Protein and Carbohydrate) in some tissues of the fish *Oreochromis mossambicus*. International Journal of Zoological Research 11 (5): 222-227.
35. Tilak, K.S., Yacobu, K. (2000). Toxicity and effect of Fenvalerate on fish *Ctenopharyngodon idellus*. J. Ecotoxicol. Environ. Monit. 12(1): 9-15.