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Effect of Flubendiamide Pesticide on Morphology, Behaviour and Acetylcholinesterase Activity in Fish, *Catia catla* (Hamilton)

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ABSTRACT

The fish *Catla catla* fingerlings were exposed to the test toxicant an insecticide Flubendiamide for 24, 48, 72 and 96 hrs and the LC50 values were calculated and were reported to be 3.566, 3.456, 3.0221 and 2.892 mg5˜¹ respectively for 24, 48, 72 and 96 hrs. After determination of LC50 values the fish were exposed to sub-lethal concentration (1/10 th of 96 hrs LC50 value i.e., 0.289 mg5′¹) for 24, 48, 72, 96 hrs and 8 days in the Indian major carp *Catla catla* was studied. Fingerlings were exposed to sub-lethal (0.289 mg5-¹) and lethal (2.892 mg5′¹) concentrations of flubendiamide for 24 hrs, 48 hrs, 96 hrs and only to the sub-lethal concentration exposed for 8 days. The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh (Kopecka *et al*., 2004). AChE was identified as the enzyme responsible for termination of cholinergic transmission by cleavage of AChE to acetate and choline; AChE is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction, and the target tissues of the parasympathetic system (Silman and Sussman, 2005). The AChE activity is necessary to maintain normal behaviour and muscular function and represents a prime target called esteric site on which some toxicants can exert a detrimental effect by binding to it. Inhibition of the AChE activity results in a build-up process of acetylcholine causing prolonged excitatory postsynaptic potential. This results in repeated, uncontrolled firing of neurons leading to hyper stimulation of the nerve/muscle fibres, which leads paralysis, and eventual death. In the event of decreased ATPase system, phosphorylation may be preceded by activated phosphates to catalyse the liberation of inorganic phosphates from phosphate esters. Acetyl cholinesterase (AChE) activity is a frequently used parameter in environmental monitoring, usually in areas contaminated with pesticides, heavy metals and effluents. It is an enzyme that catalyses three hydrolysis of acetylcholine to choline and acetate in synaptic cleft. The aim of this study was to verify the relationship between the lethal and sub lethal concentration flubendiamide and the activity levels of Acetylcholinesterase (AChE).

Key words: *Flubendiamide, Catla catla, Acetylcholenesterase*

Introduction

After green revolution during sixties especially in India, pesticides emerged as knight armours for crops (Srivastava and Singh, 2014). Pesticides has credited with economic potential to enhance production of food and fibre and ameliorated in vector-borne diseases, the long-term use has caused effects on human health and the environment including aquatic ecosystem that evolved new branch of aquatic toxicology (Igbedioh, 1991; Forget, 1993; Aktar *et al.,* 2009). Pesticides have been found to be highly toxic not only to fish but also to the other organisms which constitute the food chain. Agricultural run-off from near water bodies is the major cause of deposition of pesticides in aquatic ecosystem. The qualitative and quantitative chemical usage is of great concern ecologically. The discriminate use of chemicals is for the control of insect pests by elimination of target species whereas indiscriminate usage posed the problem on non-target organisms including man. Wide and indiscriminate use of pesticides in modern agricultural practices throughout the world has indirectly created problem of pollution of aquatic ecosystems (Ganeshwade, 2012). These chemicals impair water quality which becomes unsuitable for all aquatic organisms due to their toxicity, persistence, bioaccumulation, and biomagnifications in food chain and ecological balance (Subramani Lavanya *et al.,* 2011). Fish accumulate these pollutants directly or indirectly from polluted waters and food chain (Jabeen *et al*., 2016). In India, flubendiamide, a rynoid pesticide is extensively used in rice and pulse crops to control pests. The excess pesticides used in the farms reach the water bodies by the surface run off and are known to cause ill effects on aquatic organisms.

Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids are in turn oxidized to give energy for body function (Saravanan *et al*., 2000). Enzymes are exceedingly efficient and very specific in terms of nature of reaction catalysed and the substrate utilized. The synthesis and final concentration of enzymes is under genetic control and is greatly influenced by very small molecules of substrate.

Materials and Methods

The fingerlings of the test fish *Catla catla* of size 6-8 ± 1D2 cm and weight 6-7 ± 1D2 gm were procured from local fish hatcheries of Nandivelugu, Tenali mandal, Guntur district, Andhra Pradesh. The fish were acclimated at (28±2 ^{o}C) in the laboratory conditions for two weeks. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms were followed (Annon, 1975). Fish were regularly fed with rice bran and one day prior to the experimentation feeding was stopped. After each exposure period, tissues such as liver, gill, muscle, brain and kidney were dissected and removed. In to a ringer solution, muscle, brain, liver, gill and kidney tissues were Dissected immediately after the fish were sacrificed. The blood in excess was cleaned in 0.15 M KCl (ice cold) and then weight was measured. The potter-Elvehjam homogenizer fitted with Teflon pestle was used to homogenize the above said tissues in (10% w/v) in 0.1 M, pH 8 tris HCl buffer. After homogenation the homogenates were centrifused at 5000 rpm for about 10 minutes. The supernatant after the homogenation was re centrifused at 5000 rpm for nearly 10 minutes. Later the supernatants were kept in ice and for the estimation of ACh.E activity this was used as source of enzyme. The total preparations of enzymes were carried out at 0-4 ^{o}C. Lowry *et al.* (1951) method was followed with the use of bovine serum albumin as standard for the estimation of protein content in enzyme preparations.

Estimation of Acetyl Cholinesterase

Ellman *et al.* (1961) method was followed for ACh.E enzyme assays. The principle of method depends on the hydrolysis of acetylcholine, and the extent of the enzyme produced of thiocoline is measured. This is followed by continuous reaction of thiol with 5:5 dithiobis–nitrobenzoate ion for the production of the yellow colour anion of 5-thio-2-nitro benzoic acid.

$H_2O + (CH_3)_3\ NCH_2\ CH_2\ SCO\ CH_3 \rightarrow (CH_3)_3\ NCH_2\ CH_2S + CH_3\ COO +_2H$

$(CH_3)_3\ NCH_2CH_2S + RSSR \rightarrow (CH_3)_3\ NCH_2\ CH_2\ SSR = RSR = = O_2\ N$

With the help of spectro photometer the rate of colour production was read at 412 nm. The reaction with the thiol is rapid and hence, it is not to be rate limiting in the measurement the enzyme and in the concentration used cannot inhibit the hydrolysis of the enzyme. The hydrolysis rate of enzyme is recorded with the help of a recorder (Ellman *et al.,* 1961).

Results and Disscussion

In the present study the static LC_{50} values of flubendiamide to the fish, *Catla catla* were 3.566, 3.456, 3.221 and 2.892 mg/l for for 24, 48, 72 and 96 h respectively. These results of the present study were in agreement with the results of the earlier authors. Nirmala *et al.* (2016) observed that when the freshwater fish *Labeo rohita* were exposed to different concentrations of flubendiamide. The obtained LC_{50} values for 24 h (17 mg/l), 48 h (15 mg/l), 72 h (13 mg/l) and 96 h (11 mg/l) respectively. The toxicity may be influenced by the source and size of the fish, exposure conditions, formulation and water quality.

Behavioural Changes

The aquatic vertebrates are prone to non-target effects because of their limited mobility and also because of the slow pesticide dispersion, which causes an increase in the period of exposure (Ecobichon, 1991). Particularly fish are sensitive to a diverse range of pesticide chemicals, and the quantities of hazardous substances are only going to increase not only as a result of excessive spillage from agricultural operations but also from a variety of other sources. As the fish reacts with a high sensitivity to the changes in the aquatic environment, fish will be selected as a species of bio-indicator in the process of evaluating pollution. Fish exhibits an increasingly key role in the evaluation of water pollution. Fish will be chosen as a species of bio-indicator. At sublethal exposure conditions, the effects of pollutants can be evaluated by analysing the biochemical, physiological, or histological responses of the organism fish. If a fish dies as a result of pollution, the level of pollution is said to be severe.

The gills are the first organ to be affected by numerous waterborne contaminants due to the fact that they are in continuous touch with the surrounding environment and are also the site of toxicant absorption (Veeraiah *et al.,* 2002). Throughout the beginning of the exposure period, there was a rise in the frequency of opercular movements; however, this trend reversed itself as the duration of the exposure period grew. The fish eventually got restless and agitated, and it also began to secrete an abnormally large amount of mucus all over its body. Because of this, a few of the fish became overly enthusiastic, which caused them to move in an irregular manner.

As part of their avoidance behaviour, the fish exhibited jerky movements and frequently came to the surface to take large breaths of air. It was reported by Madhu Sharma (2019) that a fish died shortly after losing its equilibrium, which caused the fish to sink to the bottom of the tank and roll its body before passing away. Hussain *et al.* (2015) discovered excessive mucus secretion, a change in pigmentation, muscle fasciculation, refusal of feeding, and respiratory distress in Catla catla as a response to dimethoate. These findings were found in Catla catla as a result of dimethoate. Other researchers have observed similar results in different fish species in response to various toxicants.

The freshwater fish Catla catla was selected for the present investigation to be exposed to a sublethal concentration (1/10th of static, 96 h LC_{50}) of flubendiamide over a period of 8 days; and several alterations in behaviour, including swimming irregularly, were detected. It was discovered that they were in a state of toxic stress. Around the time of the second day, the motions in the operculum are discovered to be faster, and the phenomenon of surfacing, for air gasping, is observed; yet, the difficulty in taking breaths seems to have been alleviated. In the test fish, Catla catla, over excitement, loss of equilibrium, an increase in the rate of coughing, flaring of gills, and production of mucus from the gills were seen, as well as movements that resembled darting and hitting movements against the walls of the test tanks.

Nagaraju *et al.* (2017) reported in the fish *Labeo rohita* exposed to indoxacarb showed severe respiratory distress, higher ventilation volume, increased secretion of mucus, erratic movement, engulfing of air through the mouth, loss of buoyancy, decreased oxygen uptake efficiency, labored breathing, jerky movements, losing scales, hyperactivity, hyper excitability, and muscular incoordination.

Al-Otaibi *et al.* (2019) reported that in the catfish, *Clarias gariepinus*, exposed to three sublethal levels of diazinon for 30 days showed abnormal behavior such as gulping of air, erratic swimming, restlessness, high opercular movement, burst swimming, hanging in the water column. Maurya *et al.* (2019) elucidated the effect of pesticides from industrial wastewater at five different concentrations on the fish H. fossilis on 1st, 5th, 10th, and 20th days and observed that the fish showed uncoordinated behavioral responses, erratic and jerky swimming, histologic changes and biochemical changes, mucus secretion, increased opercular movement, and increased mucous secretion all over the body.

Behavioral changes observed in the present study of the exposed fish, appear to be the manifestation of flubendiamide toxicity. They exhibited behavioral changes which reflect its direct effect on nervous system of exposed fish. Further studies on toxicity of flubendiamide and its combinations with other pesticides on mortality and behavior of fish in the laboratory and field conditions are required. The morphological and behavioral changes exhibited by the fish can be taken as useful parameter as a bio-indicator in assessing the extent of aquatic pollution.

Changes in Acetylcholinesterase Activity (Ach.e)

In the current study, research has been done on ACh.E activity in the gills, brain, liver, kidney, and muscle of the fish *Catla catla* as well as changes in the fish's behaviour. Flubendiamide's deadly concentration suppressed the ACh.E activity in fish *Catla catla* more so than its sublethal concentration did in the current investigation.

The largest percent drop in ACh.E during sublethal flubendiamide exposure for 24 hours was seen in the brain (15.80), whereas the smallest percent was seen in the gills (8.15). Greatest percent of reduction was noted in the brain (26.43) after 24 hours of exposure to deadly doses of flubendiamide, while lowest percent was seen in the gills (13.81).

Under exposure to sublethal concentrations of flubendiamide for 48 h, maximum percent decrease of ACh.E was in noticed in brain (21.67) and minimum percent was observed in gill (10.43). Greatest percent of reduction was noted in the brain (34.83) after 24 hours of exposure to deadly doses of flubendiamide, while lowest percent was seen in the gills (20.33).

Under exposure to sublethal concentrations of flubendiamide for 96 h,maximum percent of decrease of ACh.E was in noticed in brain (30.81) and minimum percent was observed in gill (16.85). Under exposure to lethal concentrations of flubendiamide for 96 h, maximum percent of decrease of ACh.E was in noticed in brain (43.89) and minimum percent was observed in kidney (30.33).

Under exposure to sublethal concentration of flubendiamide for 8 days, maximum percent of decrease of Ach.E was in noticed in brain (37.71) and minimum percent was observed in kidney (25.00). In the current study, research has been done on ACh.E activity in the gills, brain, liver, kidney, and muscle of the fish Catla catla as well as changes in the fish's behaviour. Flubendiamide's deadly concentration suppressed the ACh.E activity in fish Catla catla more so than its sublethal concentration did in the current investigation.

Tissue	Control	Sub-lethal	Percent Change	Lethal	Percent Change
Gill	3.68±0.186	3.38±0.106	8.15	3.12±0.104	15.21
Brain	6.96±0.127	5.86±0.124	15.80	5.12±0.182	26.43
Liver	3.96±0.134	3.48±0.176	12.12	3.18±0.174	19.69
Kidney	3.62±0.185	3.32±0.214	8.28	3.12±0.163	13.81
Muscle	4.26±0.134	3.76±0.128	11.73	3.58±0.124	15.96

Results are the mean values of four observations
Standard Deviation is indicated as (±)
Vaules are significant at $p<0.05$

Tissue	Control	Sub-lethal	Percent Change	Lethal	Percent Change
Gill	3.64±0.186	3.26±0.106	10.43	2.84±0.104	21.97
Brain	6.92±0.127	5.42±0.124	21.67	4.42±0.182	34.80
Liver	3.92±0.134	3.24±0.176	17.32	2.96±0.174	24.48
Kidney	3.58±0.185	3.12±0.214	11.86	2.82±0.163	20.33
Muscle	4.22±0.134	3.56±0.128	15.63	3.24±0.124	23.22

Results are the mean values of four observations
Standard Deviation is indicated as (±)
Vaules are significant at $p<0.05$

Brain and muscle tissues of the test fish *Catla catla* evidenced a highly significant decrease in the activity levels of acetyl cholinesterase under sublethal and lethal exposure of flubendiamide for 24 and 96 h. The effect of pesticides on the brain and muscular tissue may be the cause of this. Additionally, it was noted that OP and carbamate chemicals frequently target brain ACh.E activity. Acute CNS and PNS symptoms are either indirectly caused by its suppression or directly caused by it (Bakshi *et al.,* 2013).

Mahidi Banaee *et al.* (2019) reported decreased ACh.E activity in snail *Galba truncatula* exposed to cadmium chloride and dimethoate for 14 days. Akter *et al.,* (2020) significant inhibition ($p < 0.05$) in ACh.E activity in brain of freshwater stinging catfish, *Heteropneustes fossilis* exposed to organophosphate pesticide, envoy 50% SC.

Lixiao Wang *et al.,* (2017) exposed zebra fish (Danio rerio) organo-phosphorous pesticides, melathion, atrazine and heavy metal, cadmium for 96 h. They reported that the ACh.E activity in muscle and brain of zebrafish was significantly ($p< 0.05$) inhibited when treated with melathion, atrazine and cadmium. Singh *et al*. (2018) reported dose dependent as well as tissue specific inhibition in the acetylcholinesterase activity in muscle, gills and brain of Channa punctatus exposed to triazophos and deltamethrin. Pala (2019) reported significant decrease in Acetylcholinesterase (ACh.E) activity in freshwater amphipod, Gammarus pulex (crustacean) exposed to sublethal concentrations (10, 20 and 40 µg/l) of the determined glyphosate-based herbicide (GBH) for 24 and 96 h.

Flubendiamide was used in the current investigation to reduce the ACh.E activity in the fish *Catla catla* for 24, 48, 96, and 8 days. This inhibition was more pronounced at fatal doses than at sublethal values. Under sublethal and lethal exposure to flubendiamide for 24 and 96 hours, the test fish Catla catla's brain and muscle tissues demonstrated a highly substantial drop in the activity levels of acetyl cholinesterase. The effect of pesticides on the brain and muscular tissue may be the cause of this.

Throughout the exposure period, as the fish is continuously bathing in the pesticide medium, the accumulation of pesticide residue is a cumulative process and as a consequence the inhibition of ACh.E is also a cumulative process which is time dependent. The more is the exposure period, the more will be the accumulation of acetyl choline and inhibition of ACh.E activity. The perfluorinated functional group occupied the active site of ACh.E, causing the formation of the enzyme-inhibitor complex and the suppression of ACh.E activity seen in the current work. Then, in a process similar to the acetylation of ACh.E, the serine hydroxyl group in the enzyme active site gets carbamylated. Acetyl choline's serine hydroxyl group is prevented from participating in the hydrolysis process by a caramyl moiety (inhibited enzyme) (Fukuto, 1990).

Alterations in specific activity levels of Acetyl choline esterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/g tissue/min) and % change over control in different tissue of the fish *Catla catla* exposed to sub-lethal and lethal concentrations of flubendiamide for 24 h.

Alterations in specific activity levels of acetyl choline esterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/g tissue/min) and % change over control in different tissue of the fish *Catla catla* exposed to sub-lethal and lethal concentrations of flubendiamide for 48 h

Alterations in specific activity levels of Acetyl choline esterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/g tissue/min) and % change over control in different tissue of the fish *Catla catla* exposed to sub-lethal and lethal concentrations of flubendiamide for 96 h.

Alterations in specific activity levels of Acetyl choline esterase (ACh.E) (µ moles of acetyl

Tissue	Control	Sub-lethal	Percent Change	Lethal	Percent Change
Gill	3.62±0.186	2.96±0.106	18.23	2.46±0.104	32.04
Brain	6.88±0.127	4.76±0.124	30.81	3.86±0.182	43.89
Liver	3.89±0.134	2.96±0.176	23.90	2.46±0.174	36.76
Kidney	3.56±0.185	2.96±0.214	16.85	2.48±0.163	30.33
Muscle	4.18±0.134	3.38±0.128	19.13	2.76±0.124	33.97

Results are the mean values of four observations
Standard Deviation is indicated as (±)
Vaules are significant at $p<0.05$

Tissue	Control	Sub-lethal	Percent Change
Gill	3.56±0.186	2.56±0.106	28.09
Brain	6.84±0.127	4.26±0.124	37.71
Liver	3.86±0.134	2.68±0.176	30.56
Kidney	3.52±0.185	2.64±0.214	25.00
Muscle	4.12±0.134	2.92±0.128	29.12

Results are the mean values of four observations
Standard Deviation is indicated as (±)
Vaues are significant at $p<0.05$

thiocholine iodide hydrolysed/g tissue/min) and % change over control in different tissue of the fish *Catla catla* exposed to sub-lethal and lethal concentrations of flubendiamide for 8 days.

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Conflict of Interest- None

References

Aktar, W., Sengupta, D. and Chowdhury, A. 2009. Impact of pesticides use in agriculture their benefits and hazards. *Interdiscip. Toxicol.* 2: 1-12.

Akter, R., Pervin, M.A., Jahan, H., Rakhi, S.F., Mohsinul Reza and A.H.M. and Zakir Hossain, 2020. Toxic effects of an organophosphate pesticide, envoy 50 SC on the histopathological, hematological, and brain acetylcholinesterase activities in stinging catfish (*Heteropneustes fossilis*. *Jo. BAZ*. 81: 47.

Anon, 1975. Committee on methods of toxicity tests with fish macro invertebrates and amkand dephibians. EPA, Oregon, 61.

Al-Otaibia, M., Al-Balawia, H.F.A., Ahmada, Z. and Sulimana, E.M. 2019. Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, *Clarias gariepinus*. *Braz. J. Biol*. 79(2): 326-336.

Balakrishna Naik, R., Gopal Rao, N. and Srinivasa, Rao G. 2019. Toxicity and behavioural changes in the fresh water common carp cyprinus carpio exposed to permethrin a synthetic pyrethroid of class i type. *Asian Journal of Microbiology Biotechnology and Environmental Sciences.* 20(4): 1200-1220.

Bantu, Nagaraju Venkata rathnamma Vakita and Somaiah Karra, 2013. Effect of chlorantraniliprole on biochemical and certain biomarkers in various tissues of freshwater fish *Labeo rohita* (Hamilton). *Environment and Ecology Research.* 1: 205-215.

Ecobichon, D.J. 1991. Toxic effects of pesticides". In: *Casarett and Doull's Toxicology* (eds) M.O. Amdur, *et al.* The basic science of poisons, 4th ed. Mc Graw-Hill, Inc., New York. 565- 622.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. 1961. A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochemical. Pharmacol*. 7: 88-95.

Forget, G. 1993. Balancing the need for pesticides with the risk to human health. In: *Impact of Pesticide Use on Health in Developing Countries* (eds. Forget G, Goodman T and De Villiers A.): 2-16. IDRC, Ottawa.

Fukuto, T.R. 1990. Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives*. 87: 245-254.

Ganeshwade, R.M. 2012. Histopathological changes in the gills of *Puntius Ticto* (Ham) under dimethoate toxicity. *The Bio Scan.* 7 (3): 423-426.

Harit, G. and Srivastava, N. 2018. Behavioural alterations in Channa punctatus after exposure to endosulfan. *IJFAS.* 6(2): 51-55.

Hussain, A., Mkpojiogu, E.O.C. and Kamal, F.M. 2015. Eliciting user satisfying requirements for an e-health awareness system using Kano model". Proceedings of the 14th WSEAS *International Conference on Computer and Computational Science (ACACOS'15)***,** *Kuala Lumpur, Malaysia*.

Igbedioh, S.O. 1991. Effects of agricultural pesticides on humans, animals and higher plants in developing countries. *Arch. Environ. Hlth*. 46: 218-224.

Jabeen,F., Chaudhry, A.S., Manzoor, S. and Shaheen, T. 2016. Carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks. *Environ. Monit. Assess.* 187 (2): 29.

Kopecka, J., Rybakowas, A., Barsiene, J. and Pempkowiak, J. 2004. AChE levels in mussels and sh collected o Lithuania and Poland (Southern Baltic). *Oceanologia.* 46: 405–418.

Lixiao Wang, Abeer Ghazie Azize Al-sawafi and Yunjun Yan, 2017. Biochemical Changes in the tissues of zebrafish (*Danio rerio*) exposed to organophosphorous pesticides and heavy metal cadmium. *International Journal of Environmental Science and Development.* 8 (10): 707-714.

Lowry, O.M., Rosebrough, N.J., Farr, A.C. and Randall, R.F. 1951. Protein estimation with folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Madhu, Sharma, Thakur, J. and Verma, S. 2019. Behavioural responses in effect to chemical stress in fish: A review. *International Journal of Fisheries and Aquatic Studies.* 7.1 01-05.

Mahdi Banaee, Antoni Sureda, Somayeh Taheri and Fariba Hedayatzadeh, 2019. Sublethal effects of dimethoate alone and in combination with cadmium on biochemical parameters in freshwater snail, Galba truncatula. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 220. 62-70.

Maurya, N., Maurya, J.K., Singh, U. K., Dohare, R., Zafaryab, M., Moshahid Alam Rizvi, M. and Patel, R. 2019. *In vitro* cytotoxicity and interaction of noscapine with human serum albumin: Effect on structure and esterase activity of HSA. *Molecular Pharmaceutics*. 16 (3): 952-966.

Nagaraju Bantu, Ravi Kumar, K. and Venkatarathnamma Vakita, 2017. Acute toxicity and respiratory responses in freshwater fish, *Labeo rohita* exposed to an agrochemical indoxacarb. *International Journal of Sciences*. 4 (6): 1-5.

Nirmala Kallagadda, Anil Kumar, K., Lalitha, V. and Venkata Rathnamma, V. 2016. Toxicity Evaluation and Haematological Studies of Flubendiamide on fresh water fish *Labeo rohita*. *EJPMR.* 3(6): 345-348

Pala, A. 2019. The effect of a glyphosate-based herbicide on acetylcholinesterase (ACh.E) activity, oxidative stress, and antioxidant status in freshwater amphipod: *Gammarus pulex* (Crustacean). *Environ Sci Pollut Res*. 26: 36869-36877.

Priyatha and Chitra, 2018. Acute toxicity of triclosan on the native freshwater fish, Anabas testudineus (Bloch, 1792): behavioral alterations and histopathologica lesions. *Int. J. of Life Sciences*. 6 (1): 166-172, ISSN:2320-7817(p).

Saravanan, T.S., Aneez Mohamed, M. and Harikrishnan, R. 2000. Studies on the chronic effects of Endosulfan on blood and liver of *Oreochromis mossambicus*. *J Ecol Res Biocon.* 1: 24-27.

Singh Shikha, Rishikesh K. Tiwari and Ravi S. Pandey, 2018. Evaluation of acute toxicity of triazophos and deltamethrin and their inhibitory effect on ACh.E activity in *Channa punctatus*. *Toxicology Reports.* 5: 85-89.

Srivastava, P. and Singh, A. 2014. Fate of fungicides on fish, Clarias batrachus- a complete study. LAP LAMBERT Academic Publishing, Germany. 134 p

Subramani Lavanya, Mathan Ramesh., Chokkalingam Kavitha and Annamalai Malarvizhi, 2011. Hematological, biochemical and ion regulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere*. 82:977-985.

Veeraiah, K. 2002. *Cypermethrin toxicity and its impact on histochemical and histological changes in the Indian major carp Labeo rohita (Hamilton*)". Ph.D. Thesis submitted to Nagarjuna University, Nagarjuna Nagar, Guntur, A.P. India.