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ACETYL CHOLINESTERASE (ACHE) AND ACID PHOSPHATASE ACTIVITY IN *CATLA CATLA* EXPOSED TO PYRACLOSTROBIN (20%WG)

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ABSTRACT

Exposure to agricultural pesticide throughout the world is a separate health concern in the form of acute and chronic toxicity. People of all ages and occupations can be exposed to chemicals through air, water food. Sub-lethal and lethal exposure to Pyraclostrobin for 24h, AChE depletion was found in all the tissues of test fish *Catla catla*, in Brain(6.11), (6.84), liver(5.13), (6.93), gill (3.69), (5.98), kidney(3.53), (4.99)and (5.82),(8.22). Under sub-lethal exposure to Pyraclostrobin for 4 and 8 days the Ach.E depletion was in all the tissues of test fish *Catla catla*, maximum depletion was in brain (5.12), (4.67), muscle (6.63), (4.25), kidney(3.98), (3.78),gill(2.74),(2.57) liver(2.68), (2.51). Under sub-lethaland lethal exposure to Pyraclostrobin for 24h, ACP depletion was found in all the tissues of test fish *Catla catla*, in liver(4.92), (5.23), muscle(4.53), (4.87), kidney(3.45), (3.61), gill (2.91), (2.98),and in brain(2.59), (2.78). Under sub-lethal exposure to Pyraclostrobin for 4 and 8 days the ACP depletion of test fish *Catla catla*, muscle (3.89), (3.87), kidney (3.72) (3.48), liver (3.24), (2.72) brain (3.21), (3.15), and gill (2.67), (2.68).

Introduction:

Impact of environmental pollution has drastically affected on food chain, leading to extreme food shortage. Pesticide toxicity depends on the compound family and is generally greater for the older compounds; in humans, they are responsible for acute poisonings as well as for long term health effects, including cancer and adverse effects on reproduction. Man depends on natural ecosystems to meet his basic physical needs for clean air and water, food, waste assimilation, medicinal compounds, and for many les tangible needs like outdoor recreation and aesthetic beauty to renew our spirits (Shyamal Kumar Paul *et al*.,2021).

Chemical pesticides can harm agricultural workers who do not wear the proper safety gear and can also be dangerous for nearby communities. Organization such as the US Environmental Protection Agency (EPA) and World Health Organization (WHO) have established threshold exposure levels for many pollutants, but these permissible levels are exceeded in countless locations throughout the world, where exposure can lead to debilitating health impacts (Pruss-Uatus *et al.*,2011). Despite high usage of large qualitative of these pesticides in India, minimal effort has been to assess their fate in the environment. The popularity of OPs has also persisted to the present, and among pesticides overall, the herbicide glyphosate steadily became the most applied compound by 2000, (Atwood and paisley – Jones 2017; Simon-Delso *et al.*, 2015).

Acutely poisonous events most notably fish kills which were relatively mutual few decades ago are now rarely experiential in most industrialized countries; however, even sublethal toxicity could leads to plain impacts on entire populations (Richard *et al.*, 2012). Agriculture in Andhra Pradesh, India forms a main portion of the state economy. Large number of individuals of this state depends on agriculture provide with the required food grains. The production of paddy, chilies, tobacco, cotton, and other different kinds of crops arecultivated in wetland religions.

Major part of cultivated lands is below Krishna River (Lat 15^0 18^1-16^0 50^1, month long. $70^0 10^1$-80^0 55^1east) area, which include Krishna and Guntur Districts of Andhra Pradesh. In the current past the local agriculture officers advise the farmers to decrease indiscriminate pesticide scattering and abate the usage of banned insecticides. From the upland areas of this locality, the pesticides are washed to the low land water bodies through surface runoff, where the aquaculture actions are taken up by the farmers. Since both Krishna and Guntur Districts are maximum pesticides consuming places, the water is polluted by pesticides. It is important to know the impact of water qualities and the effect of pesticides to aquatic organisms, the more than a few pesticides used, the effect of Ethion and Phenthoate on the non-target organisms are not so far investigation locally. Hence efforts have made to the toxicity of pyraclostrobin 20%(wg)to Indian major carp *Catla catla.*

Materials and Methods:

Fish *Catla catla* of size 6±7 cm and 6.5± 2 g weight were brought from a local fish farm Kuchipudi, Guntur District of Andhra Pradesh, India and acclimatized at 28 ± 2^0C in the laboratory for 15days. Such acclimatized fish were exposed to sublethal and lethal concentrations of pyraclostrobin(20%WG) commercial grade for 24h, 4 and 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation o Acetyl chlorine esterase (AChE), AChE enzyme assays were performed spectrophometrically by the method (filament *et al.*,1961) and Acid phosphatase (ACP), The activity of acid phosphates was estimated by the method of Bodansky (1932).

RESULT AND DISCUSSION

The variation in activity levels of Acetyl cholinesterase in different tissues of fish suggests the variations in neural activities of those particular organs. Under exposures to sub-lethal and lethal concentrations of Pyraclostrobin for 24 h, the activity levels of ACh. E was to decrease in all the test tissues compared withcontrols. The lyotropic series in terms of decrement in Ache activity levels is:

24 h Sub-Lethal: Liver> Gill> Kidney> Muscle > Brain

24 h Lethal: Brain > Muscle > Kidney > Gill > Liver

Under exposure to sub-lethal concentrations of Pyraclostrobin for 4 and 8 days, the activity levels of Ach. E was found to decrease in all tissues. The lyotropic series in terms of decrement in Ach. E activity levels are:

4 Days Control: Gill> Liver > Kidney > Muscle> Brain

4 Days Sub-Lethal : Brain > Muscle > Liver > Gill > Kidney

8 Days Control: Liver > Gill> Kidney> Muscle> Brain

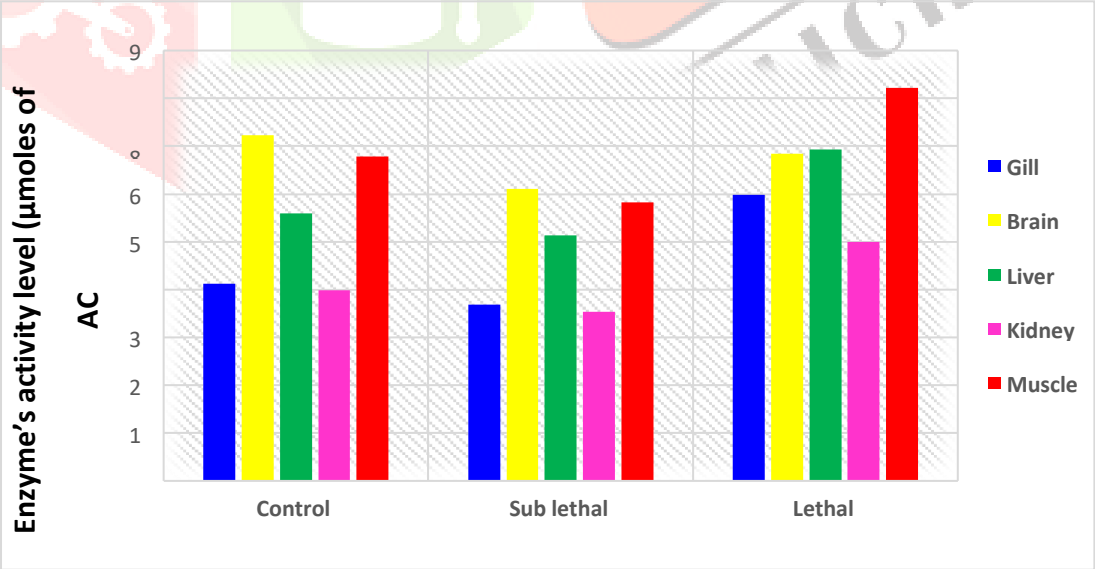
8 Days Sub-Lethal : Brain > Muscle > Gill > Liver > Kidney

Changes in the specific activity levels of acetyl cholinesterase (AChE)(µmoles of acetyl thiocholine iodide hydrolysed/gm tissue/min) of the freshwater fish,*Catla catla* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24h.

Tissues	Control mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Lethal (mg/g)	% Change (mg/g)
Gill	4.12±0.0.21	3.69±0.023	10.43	5.98±0.039	-45.14
Brain	7.23±0.013	6.11±0.012	15.49	6.84±0.032	-10.37
Liver	5.59±0.045	5.13±0.033	8.22	6.93±0.012	-23.97
Kidney	3.98±0.067	3.53±0.045	11.30	4.99±0.018	-25.37
Muscle	6.78±0.032	5.82±0.018	14.15	8.22±0.019	-21.23

Values are the mean of five observations ;(±) indicates the standard deviation: Values are significantly at P< 0.05

Fig. Changes in the specific activity levels of acetyl cholinesterase (ACh.E) (µmoles of acetyl thiocholine iodide hydrolysed / gm/ tissue min) of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24h.



Acetyl Cholinesterase (ACh.E) is an enzyme that catalysis the hydrolysis of acetylcholine to choline and acetate in the synaptic cleft, a key enzyme of the nervous system (Petek Piner and Nevin Uner, 2012; Kumar *et al.*, 2019,.2020). When inhibition occurs in ACh.E activity, the neurotransmitter acetylcholine is not hydrolysed in the nerve synapses and neuromuscular junctions, causing an abnormal amount of Ach.E in the areas, which leads to an over activation of the brain and

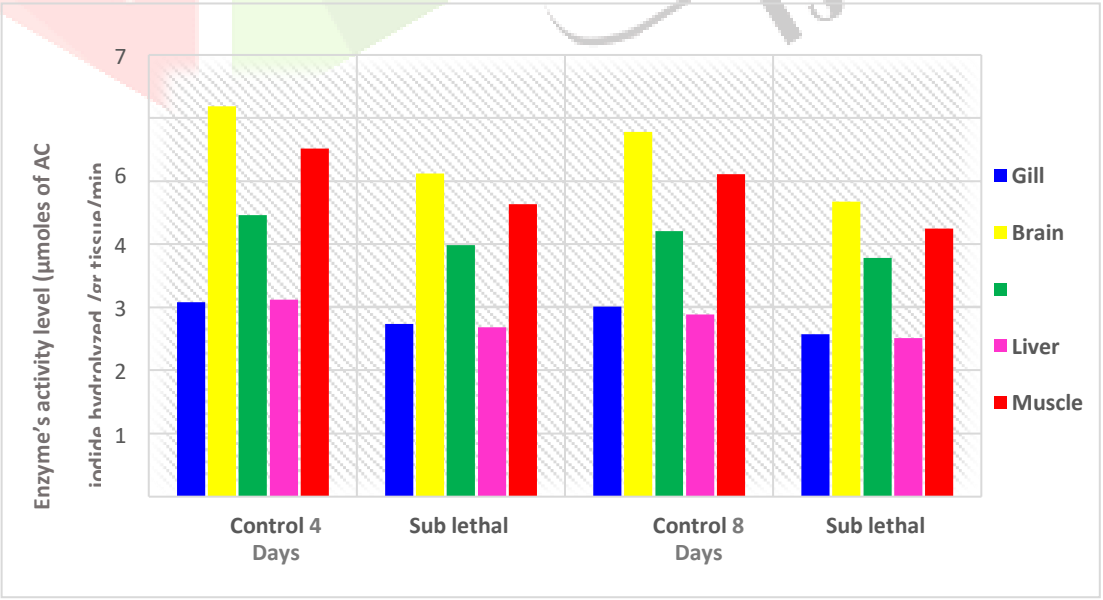
muscular organs (Candida Toni *et al.,* 2011; Warner & Andreescu, 2016), the enzyme acetyl cholinesterase activity is an important neurotoxic parameter used to evaluate the pesticide toxicity in fish (Toni *et al*., 2011). High toxicity of synthetic pesticides has been found to aquatic, zooplankton and mammalian species (Mossa and Swelam, 2015).

Changes in the specific activity levels of acetyl cholinesterase (AChE) (µ moles of acetyl thiocholine iodide hydrolysed/gm tissue/min) of the freshwater fish, *Catla catla* exposed to sub-lethal concentrations of Pyraclostrobin (20%WG) for4 and 8 days.

Tissues	4 Days			8 Days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)
Gill	3.08±0.011	2.74±0.087	11.03	3.01±0.014	2.57±0.012	14.61
Brain	6.18±0.025	5.12±0.023	17.15	5.78±0.023	4.67±0.017	19.20
Kidney	4.46±0.015	3.98±0.017	10.76	4.21±0.066	3.78±0.043	10.21
Liver	3.12±0.034	2.68±0.098	14.10	2.89±0.012	2.51±0.087	13.14
Muscle	5.52±0.056	4.63±0.023	16.12	5.11±0.002	4.25±0.033	16.82

Values are the mean of five observations ;(±) indicates the standard deviation: Values are significantly at P< 0.05

Fig. Changes in the specific activity levels of acetyl cholinesterase (ACh.E)(µ moles of acetylthiocholineiodide hydrolysed / gm tissue / min) of the freshwater fish, *Catla catla* exposed to sub-lethal concentrations of Pyraclostrobin(20%WG) for4 and 8 days.



Activity of AChE inhibition can affect growth, feeding and reproductive behaviour of fish exposed to different types of environmental contaminants **(**Kumar *et al.*,2020). Fish exposure to various pollutants is frequently assessed by determining the alterations in AChE in brain, muscle,

plasma and other tissues probably gamma-amino butaric acid (GABA) activity in brain (Banaee,2010,2012.,2013).

Thus, AChE inhibition is considered specific biomarker of exposure to carbamate insecticides like, *sporocarp*, (Cong *et al*.,2008; Banaee *et al*., 2011; Zhang *et al.,* 2017). In treated fish, alterations in various behavioural patterns were observed with increasing concentrations of both the pesticides as compared to control (Shikha Singh, *et al*,2018). Nagaraju and Rathnamma, (2013) an inhibition of AChE activity in fish *Labeo rohita* under lethal and sub-lethal concentrations of profenofos and carbosulfan, brain and muscle AChE was susceptible of being inhibited by both pesticides, and therefore be responsible for some of the locomotor symptoms observed in the present study through muscular disfunction (Nagaraju and Rathnamma, (2013).

On the other hand, the brain as centre of the nervous systems in fish contains low levels of enzymatic and non-enzymatic antioxidant and higher level of oxidizable unsaturated lipid and catecholamine (Belinskaia *et al.,* 2020). Patek pinner and Nevin Uner,(2012, showed that inhibition of AChE activity in liver and brain tissues of *Oreochromis niloticus* after treated with Spinosad pesticide, AChE is predominant in brain tissue, more inhibition was observed in liver due to destroying the pesticide close to the major site of metabolic production, therefore preventing it from reaching to the brain. Acetylcholine at the nerve endings, thus carbamate increases the cholinergic effects of the neurotransmitter's acetylcholine in the body and depolarization of neural transmission (Dhouib *et al.,*(2016).

Acetyl cholinesterase activity (AChE) in the red blood cells into butyryl cholinesterase activity in plasma have been used to monitor the extent of organophosphates and carbamate exposure (Duchnowicz *et al.,*2018). Reductions of Brain AChE enzyme activity leads to an accumulation of acetylcholine in the brain tissue, interfering with energy metabolism in the nervous system and preventing transmission of nerve impulses, thereby causing behavioural alterations (Hill *et al.,* 2018; Hou *et al.,*2019). An inhibition of AChE activity in fish *Catla catla* under lethal and sub-lethal concentrations of Pyraclostrobin in this study.

Brain and muscle AChE was susceptible of being inhibited by pesticide, and therefore be responsible for some of the loco motor symptoms observed in the present study through muscular disfunction. (Belinskaia *et al.,*2020) brain AChE inhibition caused by exposure to high concentrations of endosulfan for 96h in spotted murrel (*C. punctatus*), respectively. So, insolvent of this enzyme in the neurotoxic effects of Pyraclostrobin was deeply reduced in brain tissue of *Ctenopharngdon idella* exposed to Pyraclostrobin. similar result was obtained in different tissues of other fish species(Marigoudar *et al.,*2009; Dhouib *et al.,* 2016; Kurmar *et al.,* 2020).

Many researchers independently of tissues and species (Methion and deltamethrin in *Cyprinus carpio*); chlorpyrifos in channel catfish (*Ictalurus puncatatus*) and fenthion in European eel (*Anguilla Anguilla*) used are similar in the AChE inhibitory effects (Sancho *et al.*,2009; Duchnowicz *et al.*,2018). In accordance with earlier observations made (Elif, 2007; Modesto and Martinez, 2010; 2019; Kumar *et al.*,2020). Thus, Pyraclostrobin reduced instinctive behavioural responses and effected morphological features by depression of AChE activity.

Proper functioning of swimming skills and sensorial system is essential for detection, attack and capture of prey, as well as for predator evasion (Whitford *et al.*, 2019). A decrease of AChE activity in brain and blood of silver catfish (*Rhamdiaquelen*) exposed to different types of salinities, acetylcholine degraded when AChE decreases, it accumulates within synapses, which prevent them from functioning normally (Ribe *etal.*, 2018).

In nerve impulses inhibition of AChE results as nerves become permeable to sodium, allowing sodium to flow into the nerve (Marigoudar *et al.*, 2009). Inhibition brain AChE activity is more than that of all other tissues; this might be due to the Pyraclostrobin, where the inhibition of activity was also maximum. As the exposed fish is continuously bathing in the pesticide medium throughout the exposure period, the accumulation of pesticide residue is accumulative process; consequently, the inhibition is also accumulative and is time dependent (Chen *et al.*, 2019). Alterations in AChE activity can affect locomotion and equilibrium in fish exposed to different types of pollutants, mainly impact on swimming patterns, impair feeding, escape, and reproductive behaviours (Dhouib *et al.*,2016; Belinskaia *et al.*,2020).

This group of pesticides interferes with the process of synaptic transmission by inhibiting the activity of acetylcholinesterase (Rajkov *et al.*,2018). Its effects on nervous system are well known through the inhibition of the acetylcholinesterase enzyme, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine into choline and acetate. (Ghelichpour *et al.*, 2017.,2020).

Acid Phosphatase (ACP) activity

Under exposure to sub-lethal and lethal concentrations of Pyraclostrobin for 24 h, change in the acid phosphatase activity is in the order of:

24 h Sub-lethal: Brain > Gill > Kidney> Muscle> Liver 24 h lethal : Brain > Muscle > Kidney>gill > Liver

Under exposure to sub-lethal concentrations of Pyraclostrobin for 4 and 8 days, the percent change in acid phosphatase activity is in the order of:

4 Days Control : Gill > Brain >Kidney >Liver > Muscle 4 Days Sublethal : Muscle > Liver > Kidney >Brain > Gill 8 Days Control : Gill > Liver > Brain > Kidney > Muscle8 Days Sublethal : Liver > Gill > Kidney > Muscle > Brain

The Changes in the specific activity levels of Acid phosphatase (ACP) (mg/pi/gram protein/hr) and percent change over control in different tissue of the fish it the increased ACP activity observed. Present study Increase in acid phosphatase activity can be interpreted as a shift of the tissues emphasis on energy breakdown pathway from normal ATPase system to phosphatases system which include phosphorylation. In the event of decreased ATPase system, phosphorylation might be preceded by activated phosphatases to catalysed the liberation of inorganic pesticides on the elevation of ACP activities in all the tissues of freshwater *Catla catla*, exposed to Pyraclostrobin pesticide induced alterations in proliferation of smooth endoplasmic reticulum (Prakash and Verma, 2020). Thus, the Pyraclostrobin intoxication caused elevation in the activity levels of ACP in different tissues of freshwater fish *Catla catla* exposed to lethal and sub-lethal concentrations of pesticide Pyraclostrobin for period 0f 24hrs 4days 8days.

Increased ACP levels were observed in liver of *Labeo rohita* treated with sublethal concentration of organophosphorus insecticide methyl parathion (Rode, T. M. and Hovda, *et al.*,2016). (Selamoglu Talas *et al.*, 2014) observed that enzymes activity decreased in the arsenic group compared to the control in *Cyprinus carpio*. Exposed to sub lethal and lethal concentrations of profenofos for a period of 4 days, adversely affected the AChE and ACP in different organs of freshwater fish, *Catla catla* and these alterations might be attributed to increased autolysis in the tissues due to cytotoxicity (Haribabu Gogula, *et al.*,2018).

The decreased alkaline phosphatase (ACP) activity in muscle, intestine and liver tissue of the fish *Cirrhinus mrigala* exposed to predetermined values of toxicant, indicates that the Chlorantraniliprole act on plasma membrane and alter' the membrane transport (Pooja V. *et al.*,2020; C.A. Jawale.,2016). Observed the fish *Gambusia affinis* exposed to chlorpyrifos disturb the chemical constituents of the fish which leads to cell damages and finally death of fishes (Sharma *et al.*,2016). Many researchers observed the decreased ACP in the lethal and sub lethal concentration. Anitha A.,(2015), Observed decrease ACP content in fish *Labeo rohita* exposed to Pyraclostrobin. (Nirmala.K.,2016) Observed decrease ACP content in freshwater fish *Catla catla* exposed to phosalone.

Decreased ACP content was observed in freshwater fish *Catla catla* exposed Profenofos, Hari babu.G.(2017). Rajeswari., (2020) ACP content was decreased in *Catla catla* exposed to Cyhalothrin. Ch Prassana (2018) decreased ACP content was observed Ethion exposed to *Labeo rohita* lethal and sub lethal concentration. Decreased ACP content in *Labeo rohita* exposed to Profenofos and Carbosulfan. Nagaraju *et al.,*(2014).

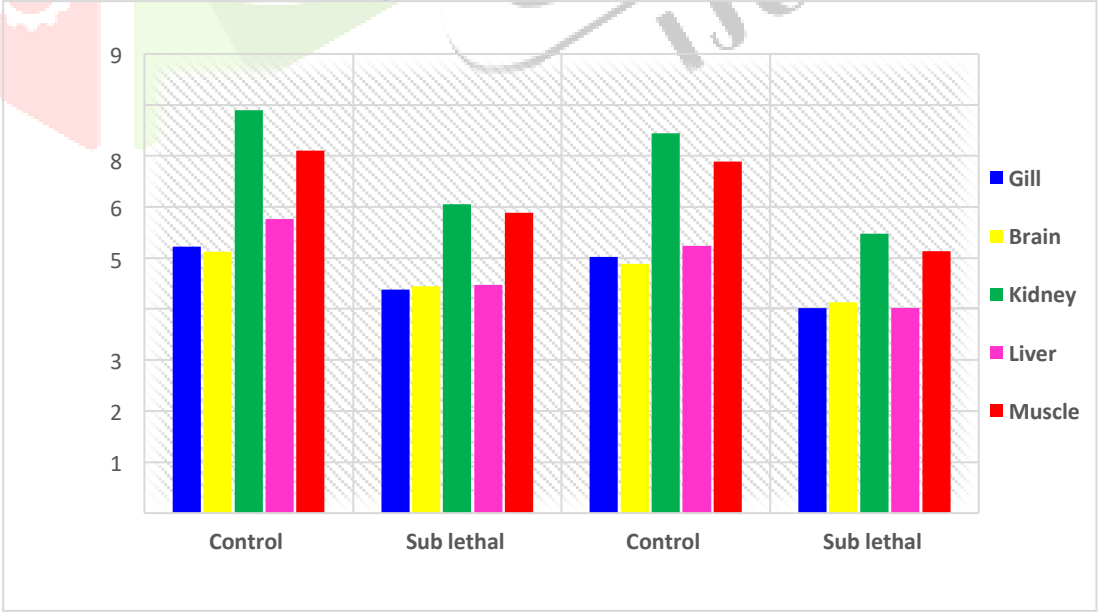
Changes in the specific activity levels of Acid phosphatase(ACP) (mg/pi/gram protein/hr) and percent change over control in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24h.

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Lethal (mg/g)	% Change (mg/g)
Gill	2.56±0.0.22	2.91±0.011	-13.67	2.98±0.012	-16.40
Brain	2.14±0.043	2.59±0.034	-21.02	2.78±0.044	-29.90
Liver	4.23±0.023	4.92±0.087	=16.31	5.23±0.008	-23.64
Kidney	3.11±0.018	3.45±0.013	-10.93	3.61±0.003	-16.07
Muscle	3.87±0.013	4.53±0.098	-17.05	4.87±0.023	-25.83

Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at P< 0.05

Changes in the specific activity levels of Acid phosphatase (ACP) (mg/pi/gram protein/hr) and percent change over control in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24h.

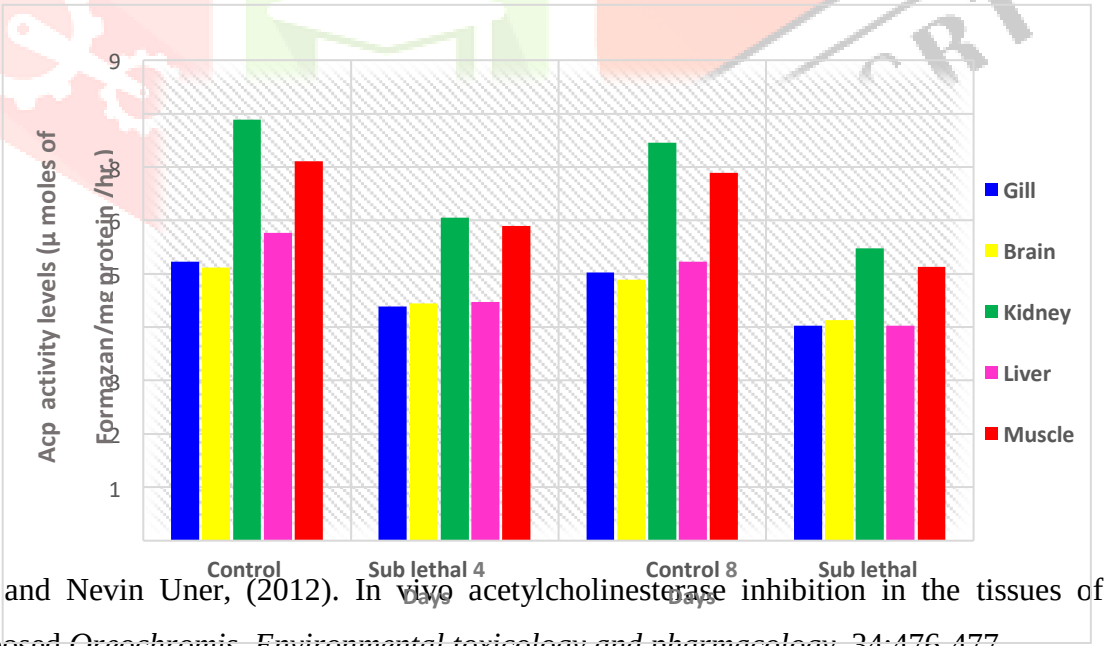


Changes in the specific activity levels of Acid phosphatase(ACP) (mg/pi/gram protein/hr) and percent change over control in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal concentrations of Pyraclostrobin (20%WG) for 4 and 8 days.

Tissues	4 Days			8 Days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)
Gill	2.32±0.088	2.67±0.028	-15.08	2.23±0.043	2.68±0.086	-20.17
Brain	2.61±0.034	3.21±0.056	-22.98	2.43±0.056	3.15±0.023	-29.62
Kidney	3.12±0.008	3.72±0.098	-19.23	2.87±0.012	3.48±0.014	-21.25
Liver	2.87±0.014	3.24±0.043	-12.89	2.36±0.048	2.72±0.025	-15.25
Muscle	3.21±0.028	3.89±0.023	-21.18	3.14±0.087	3.87±0.008	-23.24

Values are the mean of five observations ;(±) indicates the standard deviation: Values are significantly at P< 0.05

Changes in the specific activity levels of Acid phosphatase(ACP) (mg/pi/gram protein/hr) and percent change over control in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal concentrations of Pyraclostrobin (20%WG) for 4 and 8 days.



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