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RESEARCH ARTICLE

EFFECT OF DICHLORVOS TECHNICAL GRADE AND 76% EC (NUVAN) THAT INDUCED THE INHIBITION OF THE ENZYME ACETYL CHOLINESTERASE (ACHE) IN THE FISH *CATLA CATLA* (HAMILTON) EXPOSED TO BOTH SUBLETHAL AND LETHAL CONCENTRATIONS

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Catla catla, Dichlorvos Organophosphate, Technical Grade, 76% EC, Acetyl Cholinesterase Enzyme (AChE), Inhibition

Abstract

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The fish *Catla catla*, one of the major carp and a candidate species for aquaculture practices is selected for a study, to know the impact of Dichlorvos an organophosphate both technical as well as 76% EC in sublethal and lethal concentrations exposure in the laboratory (*in vivo*). After the exposure, in lethal for 4 days at a concentration of 1.778 µg/L of technical grade and 1.445 µg/L of 76% EC and in sublethal for 10 days of technical grade and 76% EC as in 1/10th of LC_{50} value i.e. 0.177 and 0.1445 µg/L respectively and the variations are studied. The inhibition of the enzyme, Acetyl Cholinesterase (AChE) is resulted in the organs/tissues of the fish. In lethal concentrations, brain is effected whereas in sublethal both brain and muscle are effected in the exposures of both the toxicants technical grade as well as 76% EC. The active swimming activity was effected thereby the fish cannot feed metabolism is effected and inturn growth is impaired, which will result a great loss to the venture of the aquaculture.

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Introduction:-

All the cells, the fundamental units of the tissues which form the organs of the organisms, biochemically perform different functions. The living organisms have various physiological procenes like, nutrition, respiration, excretion where the three important organs like, liver, gill and kidneys perform, due to main instructions/action potentials of the brain which controls the organs through muscles only. It is all due to interactions of these organs, the sustenance of the organism depends in spite of not having all the properties of the constituent cells that make the tissues but only due to coordinated actions of them only. Each cell with different cell organelles, plasma membrane, endoplasmic reticulum of smooth and rough, golgi complex, lysosomes, ribosomes, nucleus with chromatic reticulum made of chemically the nucleic acids (DNA & RNA) apart from chemically made of protein (CHON), carbohydrates (CHO) of conjugated ones lipoproteins, glycoproteins, glycolipids, phospholipids and also the nucleo proteins. The functions of the tissues are not present in these chemical constituents of all of them but will be resulted by the interactions of all of specific nature. This, as an organizational hierarchy as of completely at different levels, cells, tissues and organs of the organism which will be in control by the genetic material only and that is how the biochemistry, biochemical aspects in the biological organisms all as life, living biota of the biology, in the studies which comes under story of life and Evolution of them on the earth.

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The concentration of the environment especially more in the hydrosphere because more component of it on the planet earth poses, a threat to the living organisms and as such threat to biological process and finally via the genetic material the evolution. Many pollutants, as toxicants existing either in acute or chronic doses of concentration endanger the biodiversity 'hotspots'.

Such alarming aspects were echoed in the earth summits and one such contributory role was by the agrichemicals the pesticides which are used to control the damage for the produce by the pests, insects, bacteria, weeds etc. Tang *et al.* (2021, Butu *et al.* (2020), Kelly Macnamara (2019), Anket Sarma *et al.* (2019), all made a good contribution in understanding the world usage and the pesticides contamination of the global aspects not only, but also on the ecosystems.

Where such chemicals used to combat pests to increase the yield of produce targeted on them damaging the crops etc., but when present in natural aquatic water bodies, the inhabiting organisms including the fish, the non-target organisms got effected by several ways. One such study, the biochemical aspects when the concentration as either lethal or sublethal bring certain changes/alterations serve as the indices of toxicity (Sana Ullah *et al.* 2019 and Anilava Kaviraja and Gupta 2014). Such reports of the biochemical studies of earlier were already mentioned in their respective review articles for other pesticides [Ullah and Zorrizahara (2015) and Murty *et al.* (2013)] for chlorpyrifos, [Styanova *et al.*, 2020, Sunanda *et al.* 2016, Pallavi Srivastava *et al.* 2016; Deb and Das 2013] and for Dichlorvos the present studied one by Chandrasekhara Rao *et al.* (2017) and Sanjay Deka and Rita Mehanta (2015).

Biochemical toxicity effects were vividly explained and reported by Swarnima kumari (2019) and also by Pallavi Srivastava *et al.* (2014).

Most of studies reported earlier includes only with commercial formulation, but very few with technical grade for different fish and the reports of such nature of *Catla catla* are not studied much. Hence, an attempt is made to study such changes in the acetyl Cholinesterase (AChE) enzyme, the neurotransmitter functioning one.

Most of the studies reported earlier were with commercial formulation, but very few with technical grade and 76% EC (Nuvan) also is attempted to study the enzyme (AChE) alterations in the fish *Catla catla* exposed, both in sublethal and lethal concentrations.

Materials and Methods:-

The grass carp, *Catla catla,* size range of about 2 to 2.5 cm and 2 to 3 grams of weight, irrespective of their sex, were procured for the present investigation. The healthy and active fish were obtained from the local fish farms. The fish were then acclimatized before experimentation to the laboratory conditions in large plastic water tanks as per the guidelines of APHA (1998, 2005 & 2012) for three weeks at a room temperature of 28 ± 1º C. Periodically the water was renewed every day with 12-12 h cycle. During the period of acclimatization, the fish were fed (*ad libitum*) with groundnut oil cake and rice bran. But the feeding was stopped one day prior to the actual toxicity test. All the precautions laid by the committee on toxicity tests to aquatic organisms (APHA, 2012; 2005; 1998) were followed and such acclimatized fish are only were used for experimentation. In that process if the mortality by 5%, occurs in any batch of fish the entire batch was discarded. The fish were exposed for 4 days in lethal and 10 days in sub lethal concentrations of both the technical grade as well as 76% EC by considering the 96 hrs LC_{50} values only that were resulted in the toxicity experiments. The exposed concentrations are lethal 1,77 µg/L, sub lethal ($1/10^{th}$ of 96h LC_{50}) 0.177µg/L for technical grade and for also 76% EC lethal 1.4 mg/L^{-1} and 76% EC sublethal ($1/10^{th}$ of LC_{50} value) 0.14 mg/L^{-1} as per recommendations of APHA (1998, 2005 and 2012).

From such exposed fish only the survived ones after 4 days (96 hrs) in lethal concentrations and after 10 days in sublethal concentrations of both technical grade and 76% EC (Nuvan) are studied for the enzyme AChE, in gill, liver, brain, kidney and muscle tissues of the fish.

Estimation of Acetyl Cholinesterase Activity (AChE)

AChE enzyme assay was performed spectrophotometrically by the method that was described by Ellman *et al*, (1961). The principle of this method is the measurement of the rate of the production of thiocholine as acetylcholine which is hydrolysed. This is accomplished by the continuous reaction of the thiol with 5, 5-dithiobis-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitro benzoic acid and that forms the complete reaction. The rate of production of color is measured at 412 nm in a spectrophotometer and the quantity of the enzyme as reaction is directly proportional to the quantity of the color developed. The reaction with the thiol after the addition of sufficiently rapid so as not to be the rate limiting in the measurement of the enzyme and in the concentrations that was used do not inhibit the enzyme hydrolysis. The rate of enzyme hydrolysis that can be as activity can be recorded by using a recorder (Ellman *et al.*, 1961).

Enzyme Preparation

From the fish that was sacrificed, the tissues like muscle, brain, liver, gill and kidney were quickly excised into a cold solution. The excess blood in the respective tissues is washed with a cold 0.15 M KCl solution. The tissues further were homogenised (10% w/v) in 0.1M pH8, tris HCL buffer using a potter-Elvehjam homogenizer that was fitted with Teflon pestle. The homogenates that were prepared as above were centrifuged at 5000 rpm for 10 minutes. The resultant supernatants were stored in the ice as fractions/aliquots of the respective tissues and were then used as the enzyme source for the estimation of enzyme as AChE activity which was as described above. All the enzyme preparations were carried out only at 0-40ºC and the protein content for the enzyme preparations were estimated by the method of Lowry *et al.*, (1951) using Bovine serum albumin as standard with which standard graph was plotted.

Calculation

$$\mathbf{V}=\Delta A/\text{min} \times \frac{3}{protein} \times \frac{1}{14.3} = \mu \frac{moles}{min} / mgprotein$$

ΔA/min is changes in optical density

3 is ml of solution in cuvette

14.3 is molar extinction coefficient of DTNB

Results:-

The enzyme, the neurotransmitter in the different tissues of the fish that is exposed resulted an inhibition (decreased) and are graphically represented as Fig.1 and 2.

The decrement series in sublethal concentration that is resulted is Muscle > Kidney > Gill > Brain > Liver and Brain > Liver > Gill > Muscle > Kidney respectively in technical grade and 76% EC and in lethal concentrated it is Brain > Gill > Muscle > Liver > Kidney and Brain > Gill > Liver > Muscle > Kidney respectively.

Discussion:-

The organophosphates and carbamates effect the non-target organisms, fishes especially apart from birds and mammals by inhibition (O'brien 1977). This is extensively reported by Fukuto (1990) and the way how it is going to be operated. It was recognized as a study of biomarker that can be the indices of the toxicity [Styanova *et al.* 2020; Sana Ullah *et al.* 2019; Pallavi Srivastava *et al.* 2016; and also Anilava Kaviraj and Gupta 2014].

$$\text{Acetyl choline} \xrightarrow[H_2O]{\text{AChE}} \text{choline + Acetic acid}$$

As the enzyme is present in the synoptinimal complex of the central nervous system apart from para and sympathetic system. The enzyme is present at the place that transmits the cholinergic transmission. As for the above equation the enzyme facilitates the hydrolysis and products are choline and acetic acid. The stable enzyme, phosphorylation result a coordinated action resulting unstable molecules and finding hydrolysis is no longer possible. In case of organophates, the chemical as such and the enzyme do interfere (Paraxon) resulting an inactiveness in the enzyme (Fukuto, 1990). It is an coordinated function of the brain and muscle, and in fish gills and muscle movements are absolutely necessary otherwise, the very survival is limited.

Akter *et al.* (2020) reported for organophosphate Envoy SC in the fish *Heteroneusteus fossils* and Topal *et al.* (2016) in the fish rainbow trout using chlorpyrifos as the toxicant the AChE enzyme was inhibited resulting the whole metabolism in the organs, impaired.

Doherty *et al.* (2019) reported a comparative study of fingerlings and juvenile study of the fish, *Clarias gariepinus* (African catfish) exposing them to an herbicide, Atrazine. The enzyme activity inhibition was more in juveniles than to fingerlings (due to inhibition of the activity at the synaptinimal complex in the fingerlings). The study is not related to other organs/tissues by comparison but age/size of the fish.

Natalia *et al.* (2019), in a general article mentioned that, the neurotoxicity of organophoshphates particularly, the Acetyl cholinesterase enzyme reduction of action made the fish, rendering its movement. When dietary habits of the fish taken into consideration, the present studied one is omnivorus fish and the muscle got effected rendering not to have much predatory action of movement.

Rajya Lakshmi and Subha Raju (2017) reported on the toxic action of malathion, an organophosphate to the fish *Gambusia affinis* (The mosquito control fish), the AChE activity decrement only in brain and they made a comparative study with deltamethrin a synthetic pyrethroid. The more inhibitory action of the brain result in many biochemical actions as well as its movement. The present study had an impact on muscle and movement is restricted.

Sunanda *et al.* (2016) reported in their review article some of the studies, wherein the enzyme acetyl cholinesterase (AChE) mentioned as inhibition, by chlorpyrifos in the different fish. The cross reference they made regarding Rao (2008) is noteworthy where on the study of the article, it was mentioned that the AChE activity in the fish *Oreochromis mossambicus* functionally made to have an alteration in the activity of the enzyme due to toxic action of Neurotoxic nature only that resulted, variations in the fish.

Nafisa and Siddiqui (2016) in the fish *Aphanius dispar* due to methyl parathion toxic action had an impact of the enzyme AChE. They mentioned altered behavior and profound effect on movement.

Jindal and Kaur (2014), reported in the fish, *Ctenopharyngodon idella* due to chlorpyrifos intoxication in the tissues of liver, kidney and gills due to exposure for different days of exposure (15, 30 and 40 days) at two different concentrations ($1/3^{rd}$ and $1/5^{th}$ of 96 hr LC_{50}) for 20% EC. Decrement was reported and liver showed more detoxification mechanism whereas such thing is not present in gills and kidney least recovery. Even in the present study, if we take the three tissues/organs, it showed a decrement apart from kidney and brain lesser in action with muscle and gill.

Banee *et al.* (2013) and Tilak *et al.* (2005) using chlorpyrifos as toxicants in the fish *Cyprinus carpio* and major carps respectively reported AChE inhibition.

Shailendra Kumar Sigh *et al.* (2010), reported on the toxicological and biochemical alterations of cypermethrin the synthetic pyrethroid against freshwater teleost fish *Lolisa fasciatus* at different seasons, where in inhibition of the enzyme is dose dependent manner as well as time. They opined that the muscle is effected as in the case of present study, the *Ctenopharyngodon idella*, swimming activity is severely affected.

Marigoudar *et al.* (2009) reported that cypermethrin at $1/7^{th}$ (0.57mg/l) and $1/12^{th}$ (0.33mg/l) of the lethal concentration (4mg/l) for a period of 1, 7, or 14 days and allowed recovery for another 7 days caused inhibition in acetyl cholinesterase (AChE) activity. The maximal inhibition was found in brain followed by muscle, gill and liver on day 14 at both the sublethal concentrations. Recovery showed a rise in AChE activity but significantly decreased compared to controls. They specifically emphasized on the decreased cholenergic transmission and subsequent accumulation of Acetyl choline in tissues leading to cessation of nerve impulses. Brain acetylcholinesterase activity was decreased significantly over a period of 45 days in *Labeo rohita* exposed to 0.139 ppm of cypermethrin (Adhikari *et al.,* 2004).

Kumar *et al.* (2009) observed that cypermethrin showed positive correlation between concentration and inhibition / alteration of AChE activity in *Channa punctatus* and the inhibition of AChE activity in brain were significantly higher than muscle followed by gill.

Venkataramudu *et al* (2008) reported sublethal; toxicity of deltamethrin in the fish *Channa punctatus* in both male and female sex, in Brain, kidney, liver and muscle where the inhibitory activity is observed. The same is reiterated by Golambieski *et al.* (2008). While working with Diafuvan in three fishes where muscle got more effected as in the present study.

Siroshi and Saxena (2007) reported for Cyhalothrin at 0.10, 0.15 and 0.20ppm the Cholinesterase activity was decreased by 37.17%, 43.87% and 43.80% respectively in liver and 39.88%, 42.09% and 44.30% respectively in muscles in *Channa punctatus* after exposure for 30 days. Cholinesterase activity was inhibited by 28.98%; 32.46% and 37.01% respectively in liver and 28.95%; 31.64% and 37.03% respectively in muscles of *Heterneusteus fossils* after 30 days exposure. The inhibition is too observed for alfa cypermethrin.

Golombieski *et al* (2008) reported AChE activity in carp brain and muscle after acute exposure to Difuran, widely used in Brazil, an insecticide on fingerlings of the *Cyprinus carpio, Ctenopharyngodon idella and Aristichthys nobalis.* The aim of the to know study the affect of the toxicant in brain and muscle and found as a biomarker in the three species studied. A maximum percentage of AChE for 1 mg/L respectively in brain and muscle was 28.92% and 28.89% for common carp 30.17% and 55.45% for grass carp and 55.22% and 64.5% for big head carp. The swimming activity is normal in control fish whereas in exposed it is not.

Anilava Kaviraj and Abhik Gupta (2014) reported type II synthetic pyrethroids and observed AChE as a biomarker which has a cyano group, Cypermethrin exposure, a decrease was recorded as in the present study.

Rajasekharan *et al* (2015) reported that affect of the cypermethrin on brain AChE activity in the carp *Labeo rohita* exposed 24, 48, 72, and 96 hours 10% and 20% in sublethal concentrations. The inhibitory activity was also observed.

Tiziano Verri *et al.* (2013) reported AChE inhibition in his review article for organophosphates and carbamates for different fishes.

Rajini and Revathy (2015) reported the effects of the pesticide in different combination on AChE activity in fresh water fish *Danio rerio* using organophosphate and cypermethrin and inhibition was observed.

Ahmed Topal *et al* (2016) reported the in vivo effects of Chlorpyrifos on acetylcholineasterase activity of the rainbow trout brain, inhibition was observed.

Deb and Das (2013) in their review article Chlorpyrifos mentioned that the AChE activity is a measure of the toxic action and inhibition of the enzyme was reported in different fishes.

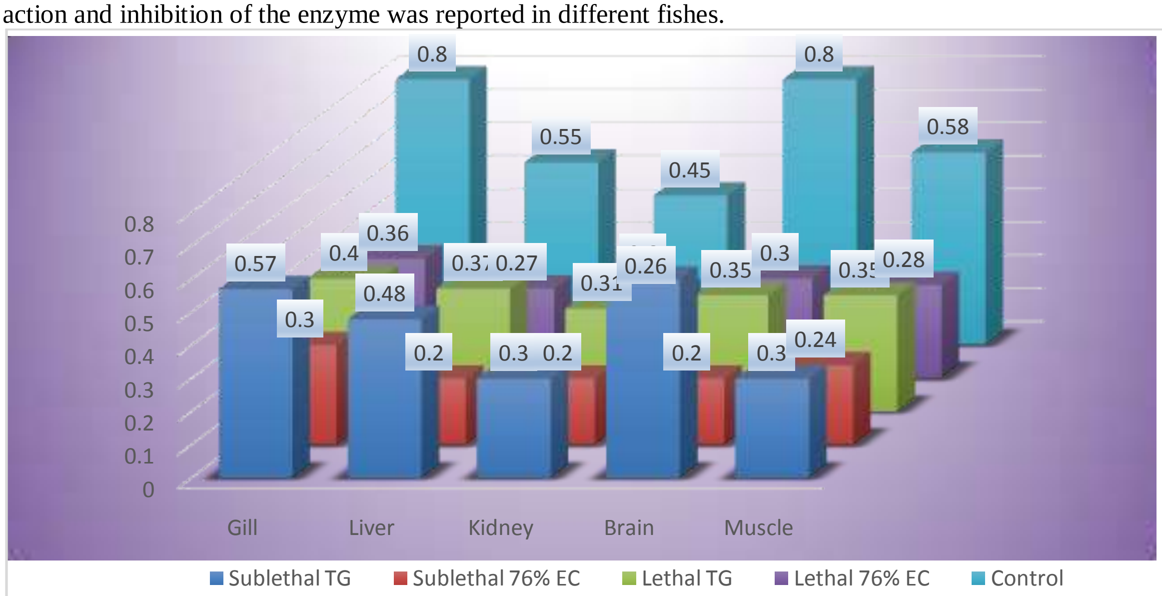

Fig. 1:- Changes in the specific activity levels of Acetyl Cholinesterase (AChE) as µ moles of acetylcholine iodide hydrolysed/gr tissue/min in the different tissues of the fish *Catla catla* exposed to both sublethal and lethal concentrations of Dichlorvos, technical grade (TG) and 76% EC (Nuvan).

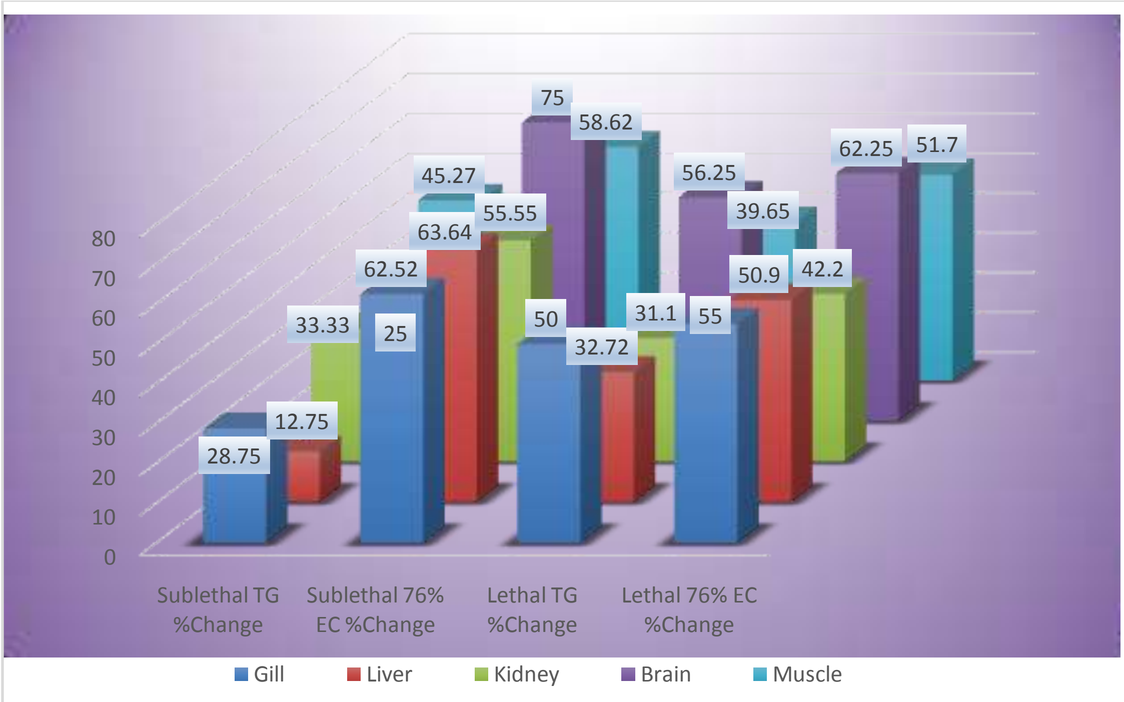

Fig. 2:- Percentage Changes in the specific activity levels of Acetyl Cholinesterase (AChE) as µ moles of acetylcholine iodide hydrolysed/gr tissue/min in the different tissues of the fish *Catla catla* exposed to both sublethal and lethal concentrations of Dichlorvos, technical grade (TG) and 76% EC (Nuvan).

Conclusion:-

Inhibition of AChE activity in functionally vital organs like gill, muscle and liver lead to impaired critical neurophysiological activity and block sodium channels of nerve filaments, thereby lengthening the depolarization phase. Further, deltamethrin like other type II synthetic pyrethroids affects the GABA receptors in the nerve filaments and other related processes. In addition, the reduction in AChE activity and ACh levels may be attributed to *in vivo* biotransformation of cypermethrin in the storage organs. The present study result is the same.

Thus, for different fishes for different chemicals of pesticides, AChE including in the present work is inhibited. The targeted enzyme is not for target species of pests and also non-target organisms like fish even in sublethal concentration, the fish especially the culturable one is not comfortable. The Permethrin is type I class of pesticide whereas Cypermethrin, Deltamethrin and Fenvalerate is of type II. The behaviour is same in both the types as the case of the AChE study and result is the death of the fish. Both organophosphates and synthetic pyrethroids inhibit the enzyme AChE and fish got so effected.

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