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ACETYLCHOLINESTERASE ENZYME INHIBITION IN THE FISH *CTENOPHARYNGODON IDELLA* (VALENCIENNES) AFTER EXPOSURE TO LETHAL AND SUBLETHAL CONCENTRATIONS OF TECHNICAL GRADE AND NUVAN 76% EC DICHLOROVOS

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ABSTRACT: Acetylcholinesterase, the potent biomarker of Aquatic ecotoxicology is tested and reported in the fish *Ctenopharyngodon idella* after exposure to lethal and sub lethal concentrations of Dichlorovos technical grade and 76% EC (Nuvan) after 10 days. The gill, liver, kidney, brain and muscle tissues/organs are selected to study the effect of the enzyme AChE in the fish. The results are as changes, 4.34%, 3.99, 3.59, 3.38 and 4.84 for technical grade in sub lethal concentration of tissues respectively and for Nuvan 76% EC 12.83%, 12.39%, 11.91%, 14.36% and 13.10% respectively. In the lethal concentrations the % changes for the same are 9.93%, 9.66% 9.43%, 9.01% and 10.24 for technical grade respectively whereas for Nuvan 76% EC, 17.18%, 16.80%, 15.50%, 11.54% and 17.95% respectively. The inhibition of the enzyme as alteration is the causative factor for death in lethal concentrations and in sub lethal concentration, showing the signs of inactivation. The commercial formulation as 76%EC showed more effect on the fish through more inhibition.
Key words: Dichlorovos, Technical grade, 76% EC, Nuvan, Static tests, Continuous flow through system, Behavioral changes.

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INTRODUCTION

Pesticides have become an integral part of world agriculture and even in aquaculture practices. The pesticide usage is increased especially due to industrialization when green revolution is thought off. Among the classes/types of pesticides organochlorines, organophosphates (OP), carbamates and synthetic pyrethroids, when transported into the aquatic environment or the usage directly in aqua culture practices of disease management, contaminate and are the most widely used, chemicals replacing the persistent organochlorines which resulted a success in controlling pests that damage the crops. Their action is by Acetyl Cholinesterase enzyme (AChE) inhibition and insect pests are eliminated.

But in aquatic environment due to same aspect of inhibition the non-target organisms are also affected even in low sublethal concentrations. It serves as a good biomarker, according to Varo *et al*., [1]. This was ably pointed out by the review articles Sana Ullah and Mohammad Jalil [2], Sankarmurthy *et al.,* [3]; Chandrasekhar Rao *et al*., [4]; Suchismitha Das [5] and individual reports of Yamin *et al*., [6]; Wang *et al*., [7]; Rao, J.V. [1]; Koul *et al*., [8]; Varo *et al*., [9,10]; Chinko [11] and Patar *et al*., [12].

Hence, an attempt is made to study the impact of Dichlorovos as contaminant, in the fish *Ctenopharyngodon idella* which is commonly used and the fish is cultured along with the other three major carps in fish farms. They also use the toxicant in the disease management of aquaculture practices which is going to be a potential for the toxic action. Hence the study of selecting the enzyme Acetyl cholinesterase (AChE) gives not only the effect but also serves as a biomarker for characterization of the toxicant.

MATERIALS AND METHODS

The fish *Ctenopharyngodon idella* measuring 3 to 5 cm in length and4 to 5 gm in weight irrespective of the sex were used in the experiment. Dichlorovs technical grade is obtained from Ram shree Chemicals, Mumbai and Nuvan is obtained from Guntur. Fish were washed with 0.1% KM_n0_4solution to avoid dermal infection. All the precautions laid down by APHA *et al.* [13,14,15] are followed, for maintaining the fish. The fish were exposed to organophosphorous pesticides Dichlorovos technical and Nuvan 76% EC to 96 hours LC_{50} Technical lethal (9.363 mg L^{-1}), technical sub lethal ($1/10^{th}$ of 96 hr LC_{50} i.e., 0.93 mg/L^{-1}), Nuvan 76% EC Lethal (11.2382 mg L^{-1}) and Nuvan 76% EC sub lethal ($1/10^{th}$ of 96 hr LC_{50} i.e., 1.1382 mg/L^{-1}) concentrations for 10 days. If mortality occurred during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish. The vital tissues of the fish, muscle, brain, liver, gill and kidney were taken for the estimation of acetyl cholinesterase (AChE).

Estimation of acetyl cholinesterase activity (AChE)

AChE enzyme assays were performed spectrophotometrically by the method of Ellman *et al.,* [16]. The principal of the method is the measurement of the rate of the production of thiocholine as acetylcholine is hydrolysed. This is accomplished by the continuous reaction of the thiol with 5, 5 - dithiobis-nitrobenzoateion to produce the yellow anion of 5-thio-2nitro benzoic acid. The rate of production of colour is measured at 412 nm ina spectrophotometer. The reaction with the thiol is sufficiently rapid so as not to be rate limiting in the measurement of the enzyme and in the concentrations used do not inhabit the enzyme hydrolysis. The rate of enzyme hydrolysis can be recorded by using a recorder [16].

ENZYME PREPARATION

The fish were sacrificed and the tissues like muscle, brain, liver, gill and kidney were quickly excised into cold solution. The excess blood is washed with 0.15 M KCl (cold) solution. The tissues were homogenized (10% w/v) in 0.1M pH 8 tris HCl buffer using potter-Elvenhjam homogenizer fitted with Teflon pestle. The homogenates were centrifuged at 5000 rpm for 10 minutes. The resultant supernatant was again centrifuged at 5000 rpm for 10 minutes. The resultant supernatants were stored in since and were used as enzyme source for the estimation of enzyme activity. All the enzyme preparations were carried out at 0-40^{o}C. Protein content for enzyme preparations were estimated by the method of Lowry *et al*., [17] using Bovine serum albumin as standard.

CALCULATION

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

ΔA/min is changes in optical density

3 is ml of solution in cuvette

14.3 is molar extinction coefficient of DTNB

ENZYME ASSAY

The reactions performed at 37^{0}C were initiated by adding small aliquots of varying concentrations of the substrate (acetyl-choline iodide) to yield a final volume of 3ml. The absorbances of 412nm were recorded continuously for 5 min. corresponding blanks lacking AChE were subtracted to yield the enzymatic activityrate. The typical runs for all experiments used were 2.7 ml buffer, 0.1 M phosphate buffer (pH 8). 50 µl (0.16mM) DTNB, 100 µl (1mg/ml) protein and 100 µl substrate.

RESULTS AND DISCUSSION

The AChE activity was estimated in different tissues like gill, liver, kidney, brain and muscle of fish *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of Dichlorvos technical grade and Nuvan 76%EC after 10 days and the values along with standard deviation and percent change over the control graphically presented. (Fig:1). Sub lethal and lethal exposure of Dichlorovos technical grade and Nuvan 76%EC, the activity of AChE were found to decrease in all the test tissues of the fish *Ctenopharyngodon idella.* The lyotropic series in decrement are:

Control	: Muscle>Gill >Liver>Kidney>Brain
Technical sub lethal	: Muscle>Gill>Liver>Kidney>Brain
Technical lethal	: Muscle>Gill> Liver>Kidney>Brain
Nuvan 76%EC sub lethal	: Brain>Muscle>Gill>Liver>Kidney
Nuvan 76%EC lethal	: Muscle>Gill>Liver>Kidney>Brain

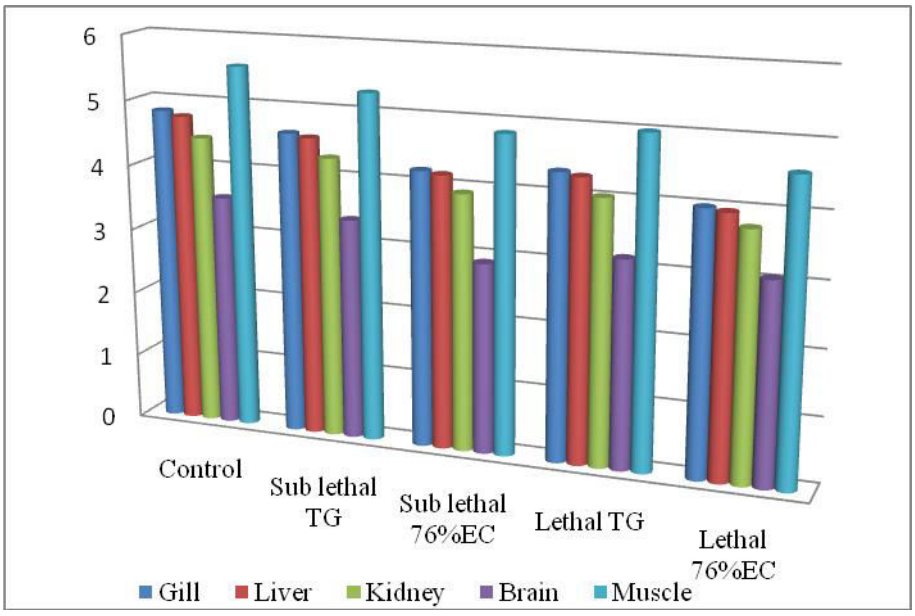

Figure 1: Change in the specific activity levels of Acetyl Cholinesterase (AChE) (µ moles of acetyl thiocholine iodide hydrolysed/gr tissue/min) in different tissues of fish exposed to sublethal concentration of dichlorvos technical grade and 76%EC nuvan

For Dichlorovos technical grade sub lethal exposure maximum percentage of elevation in AChE activity was 4.84in muscle and minimum elevation was 3.38 in brain. But in technical lethal exposure maximum percentage of elevation was 10.23 in muscle and minimum percentage of elevation was 9.01 in brain. In Nuvan 76%EC sub lethal exposure maximum percentage of elevation in AChE activity was 14.36 in brain and minimum elevation was 11.91 in kidney. But in Nuvan 76% EC lethal exposure maximum percentage of elevation was 17.95 in muscle and minimum percentage of elevation was 11.54 in brain. As per the figure 1, the AChE activity inhibition in lethal concentration is more when compared to sub lethal in both technical grade and 76% EC. The inhibition in the tissues is the causative factor finally resulting the death of the fish. It is a known fact that OP compounds inhibit the AChE [12, 18, 19, 20].

According to Patar *et al*., [12] the effects of Dichlorovos exposure on the different tissues of the fish climbing perch Aanabastestudenieus at concentrations of 0.47, 0.047 and 0.0047 for 40 days and 20 days. The toxicant has varied impact in different concentrations and the maximum is liver after 40 days and in gill after 20 days. The present study followed a different way of experimentation exposure hence cannot be compared and the decrement is the end result in both the studies.

Varo *et al*., [20] observed a significant inhibition of AChE activity in *Aphaniusiberus* exposed to 0.5, 1.2 and 4 mg/L of Dichlorovos. The fish exhibited inhibition of the AchE in brain and muscle and resisted and tolerated a high concentration. The present study resulted maximum in muscle and minimum in Brain both in lethal and sub lethal concentrations except for 76% EC Nuvan wherein Brain has maximum inhibition.

Assis *et al*., [18] also reported AChE inhibition from *Colossomamacroponum* even at the concentrations of 0.005 ppm in brain.

Varo *et al*., [9] also reported inhibition of activity ofaChE in the brain and muscles of European sea ban *Dicentricuslabrak* – both *in vivo* and *in vitro* experiments.

Dutta *et al*., [21] reported the inhibitory effects OP compounds and dependent on their binding capacity to the enzyme active site and by their phosphorylation rate in the fish behaviour and age. The toxicant is a neurotoxic due to its irreversible inhibitory effect on AChE [7].

Madhusudhana Reddy *et al*., [22] reported the acute toxicity effects of chloropyrifos on AChE activity in the field study on crabs. Significant inhibition was found in gills, muscle, hepato pancreas and nervous tissues hencethe respiration and movement is not normal.

Vineeth Kumar and David [23] also reported hepatotoxic potential of malathion an organophosphorous chemical in the freshwater teleost *Labeo rohita* (Hamilton) and reported the inhibition of the enzyme, Which resulted the behavioural changes and accumulation of acetyl choline as a result the decreased cholinergic transmission and consequent accumulation of acetyl choline and the toxicant showed a different way of inhibition different of OP compounds.

Nathaniel *et al*., [24] reported dose dependent inhibition of Chinoksolmon of mixtures of OP and Carbamates which are non-interactive whereas the pyrethroids are interactive.

The other reports on AChE were by Lakshmaiah [25], Somaiah and Sunitha [26], David *et al.,* [27] and Banaee, M *et al* [28] for different fish and for different chemicals. Thus the inhibitory action is dependent on type of pesticide whether it is OP and Carbamate or synthetic pyrethroid. The duration of exposure and concentration influence the percentage of inhibition. But such inhibitory action of the AChE enzyme is not yet reported in the fish *Ctenopharyngodon*, Dichlorovos as toxicant.

CONCLUSION

Thus it can be concluded that the pesticides belong to different groups have different mechanism of inhibition of the enzyme AChE. The tested fish showed inhibition in all the tissues which can serve as a biomarker of the pesticide toxicology. The commercial formulations have to be viewed seriously in environmental policy and planning.

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