

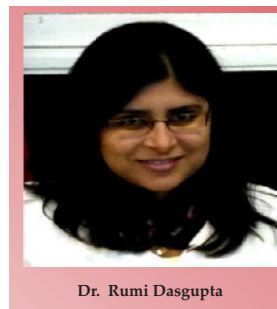
“In – Vitro Studies on Inhibition of Acetyl Cholinesterase Activities of Marine Organisms by Organophosphorus Pesticides”

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Abstract

Background: AChE activities are known to vary according to seasons with the highest values being found during summer. This is particularly true for cholinesterase activity in fish. Most enzymatic activities in poikilothermic species vary with the temperature of their environment. The level of cholinesterase activity does not directly depend on ambient temperature but on the physiological activity, which is tightly correlated with water temperature. Variations of biotic parameters such as sex, size, age, genital maturity or starvation are known to influence biological markers and make the environmental significance of markers difficult to interpret. The inhibition of AChE activities by pesticides and toxic elements like cadmium, lead and copper is well known and its use as a specific biomarker of exposure of aquatic organisms to such pollutants is widely applied in laboratory and field studies. The present work presents an evaluation of the acetyl cholinesterase activities of different species of marine organisms.

Method: Sampling was done from two Goan beaches for diversity in several physiological parameters like water quality, dissolved oxygen and contaminants disposal. Samples of *Nerita chameleon* and *Etroplus suratensis* were collected for the study. Enzymes were extracted by puncturing the forebrain region and homogenised. Protein estimation was carried out by using the Lowry Assay. The pesticides: Methyl Parathion, Ethyl Parathion, Phosphomidon, Monocrotophos and Dichlorovos were studied. Inhibition of AChE activity was determined by finding the difference in pH and % inhibition.

Results: In case of *Nerita chameleon* the order of toxicity is: DDVP > Ethyl Parathion > Phosphamidon ~ Methyl Parathion whereas in case of *Etroplus suratensis* the order of toxicity is: Methyl Parathion > Monocrotophos > Phosphamidon > Ethyl Parathion > DDVP. Out of the two samples it is found that the level of toxicity varies from species to species but the pesticides have an adverse effect on the activity of AChE leading to neurological disorders like Myasthenia gravis, glaucoma & Alzheimer's disease, etc.

Conclusion: Current sets of observations show that the study of Acetylcholinesterase enzyme can help the Eco-toxicologists to assess the impacts of various lethal & sub-lethal pollution in the marine environment. Study of different marine organisms like molluscs, fish, echinoderms, etc. can help to assess the biological effects of the neurotoxic contaminants on marine & estuarine environment.

Keywords: AChE, marine pollution, Delta pH-metric method.

Thesis Question: 1. To determine activity of acetylcholinesterase activity.

2. To evaluate the inhibitory effects of various neurotoxic contaminants on acetylcholinesterase activity.

3. To assess impact of neurotoxic contaminants on marine fishes along the Goa coast in terms of acetylcholinesterase activities

Thesis Answer: Current sets of observations show that the study of Acetylcholinesterase enzyme can help the Eco-toxicologists to assess the impacts of various lethal & sub-lethal pollution in the marine environment. Study of different marine organisms like molluscs, fish, echinoderms, etc. can help to assess the biological effects of the neurotoxic contaminants on marine & estuarine environment.

THESIS SUMMARY

Introduction

Introduction of man, directly or indirectly, of substances or energy into the marine environment resulting in toxic effects harmful to

living resources, hazard to human health, hindrance to marine activities like fishing, loss of quality for use of sea-water and reduction of amenities. The marine environment is under

continuous stress of various types of contaminants due to discharge of waste materials, industrial effluents, municipal sewage, riverine runoff, agricultural runoff etc. The various types of contaminants that are generally found in the marine environment include organic such as organochlorine pesticides, organophosphorus pesticides, polychlorinated biphenyls, polychlorinated dibenzo dioxin, dibenzo furans, polycyclic aromatic hydrocarbon, and inorganic contaminants like lead, cadmium, mercury, copper, etc. which are highly toxic. The water is most polluted in the coastal sides but as the water deepens the pollutants or chemicals gets diluted. Since the water is most polluted in the coastal side, thus, it has an adverse effect on organisms like fishes, oysters, etc. We take in those fishes which already are contaminated with deposition of metals or other toxicants which results in various abnormalities. To detect the presence of toxic pollutants various methods are there out of which biomarker is one of them. It can be done by degrading bacteria, or by using various enzymes or by means of chemicals.

Biomarker is specific in nature. Each biomarker can detect only a particular abnormality. For e.g. AChE. Acetyl cholinesterase or AChE hydrolyses Acetyl Choline during motor impulses in our body. Now if some neurotoxic contaminants are present then due to the blockage of active sites the activity of AChE gets reduced leading to neurological disorders. Thus, AChE acts as a biomarker for neurotoxic contaminants like organophosphorus pesticides, heavy metals like Pb, Cd, etc.

For decades, the measurement of ChE is a valuable tool that should be incorporated to a battery of biomarkers to maximize the confidence with which eco-toxicologists assess impacts of sub-lethal pollution in the marine and estuarine environment. As used in other organisms, like molluscs and echinoderms, and fish [1], AChE appears as a relevant means of investigating biological effects of many neurotoxic contaminants on aquatic habitats and tropic levels [2]. Quantifying brain or plasma Cholinesterase (ChE) enzyme activity is widely used method to monitor avian exposure to OP pesticides [3][4]. Enzyme cholinesterase activity related to pesticides effects has been measured in brain [5][6][7], gills [6], liver [7], heart [8], muscle [7][8][9] and erythrocyte of different fresh water species.

Studies by Sengupta R. et al. comparing the sensitivity of different species towards several pesticides revealed that invertebrate ChEs, namely insect AChE, were more sensitive to inhibition than those from vertebrates [10]. Therefore, prior to the use of an invertebrate ChE as a reliable biomarker, several features like tissue distribution, enzymatic behaviour, and optimal conditions for assessing activity and sensitivity to anti-cholinesterase pesticides should be investigated [11][12][13].

Aims & Objectives

In view of the ongoing problem of marine pollution due to neurotoxic contaminants it is indeed a prime need of the hour to assess the impact of such contamination by toxic compounds using

various biomarker techniques. To evaluate the toxicological impact of various types of contaminants, I have been prompted to undertake a systematic study on biomarker of pollution. The main aims and objective the present studies are as follows:

4. To determine activity of acetylcholinesterase activity by Delta pH-metric Method
5. To evaluate the inhibitory effects of various neurotoxic contaminants on acetylcholinesterase activity.
6. To assess impact of neurotoxic contaminants on marine fishes along the Goa coast in terms of acetylcholinesterase activities.

Materials and Methods

1. Sampling Areas:

Sampling was done from 2 beaches of Goan Coastal area. First one is the local site Dona Paula Beach few 2 kilometres away from NIO. The second sampling area is Madkai. Thus, both the sites were far enough to show diversity in several physiological parameters such as water quality, dissolved oxygen, and disposal of contaminants.

2. Animal Samples:

- a. Nerite chameleon
- b. *Etroplus suratensis*

3. Extraction of Enzymes:

- a. As soon as the organisms are collected, they are subjected to about 4°C and rest of the process is carried in the same set of conditions. The scales are removed from the surface of the fishes and the abdominal muscles are taken out. Similarly, the skull is punctured at the forebrain region and slowly the upper skull is removed and the whole brain along with the Pons is collected.
- b. As soon as the snails are collected, their weight and dimensions are measured along with their shells. Then their shells are broken & tissues are weighed.
- c. To each homogenate tube, about 4 g of tissue is added along with 4ml of extraction buffer (pH-7.4), 4 ml of Sucrose and a drop of Triton X-100. Each tube is subjected to homogenization (Homoginizer-Ultra Durax), at sheer speed of 11000 per minute, thrice for one minute with an interval of 30 seconds. The Homogenization is followed by cold centrifugation at -4°C for 30 minutes at a speed of 18500rpm. After the process of homogenization, the supernatant is pipetted out and dispensed in cryovials and stored at -20°C.

4. Estimation of Protein Content of the Extract by Hartree-Lowry Assay:

The Lowry assay (1951) is used to estimate protein content of the enzyme extract. It is the method of choice for accurate protein estimation for cell fractions [14]. The Hartree version of the Lowry assay uses fewer reagents, improves sensitivity and provides a more linear response.

5. Experimental Setup:

The reaction was carried out in small glass aliquots. To each aliquot < 7ml of Working Buffer (pH-8.02) was added followed 0.100 ml of enzyme and inhibitors if required were added. Considering the

volume of the substrate to be added later the final is made up to 7.5ml.

Finally, the reaction was commenced by addition of the substrate. First reading is taken as soon as the substrate is added, and readings were taken after every subsequent 10 minutes' interval.

The colour, which is initially dark blue gradually, changes to green and finally to golden yellow as the reaction reaches towards completion. Since the working buffer contains an indicator Bromothymol blue, which imparts a blue colour to a basic solution and gradually change its spectrum to yellow as the solution becomes acidic.

6. Organosphosphate Pesticides:

- a. Methyl Parathion
- b. Ethyl Parathion
- c. Phosphomidon
- d. Monocrotophos
- e. Dichlorovos

7. Determination of Acetylcholinesterase Activity by Delta pH-metric Method:

The change in the pH is due to the liberation of Acetic acid. The release of acetic acid is due to the hydrolysis of the substrate Acetylcholine Bromide or Butyl-Choline-thio-Iodide, by the enzyme present in the tissue homogenate in the presence of NaCl.

Acetylcholinesterase activity was measured following modified delta-pH metric method[15] in which 0.1 ml of sample enzyme was incubated at room temperature (30 °C) with 0.2 ml substrate acetylcholine bromide for an hour in a medium of phosphate buffer (0.01 M, pH-8.0 ± 0.10) indicator solution with the final volume being 7.5 ml. Bromothymol blue was used as the indicator for observation of the reaction kinetics. The changes in pH were recorded at an interval of 10 minutes. The difference in pH changes over a period of one hour corresponded to the amount of acetic acid liberated due to reaction kinetics with the acetylcholinesterase enzyme. The unit of activity of acetylcholinesterase was calibrated with respect to micromoles of acetic acid liberated by the action AChE enzyme. The protein concentration of each of the sample extract was determined as per Lowry et. al. using Bovine serum albumin as the standard[14]. The AChE activities are expressed in terms of the units per mg of protein per minute.

The amount of the acetic acid released per unit time is directly proportional to the number of substrate molecules hydrolysed (turnover number) and this release taken as the ΔpH at the end of the reaction after one hour per mg of protein is the actual measure of the activity of enzyme.

$$\text{Enzyme activity (Units/mg - protein/hour)} \\ = \left[\frac{(56.246 \times \Delta\text{pH}) \times y}{(\text{Protein content} \times 60)} \right] \times \text{Working Volume}$$

Where y = Volume of the enzyme used.

The 50% inhibition concentration (IC50) and double reciprocal plots were determined for these compounds. The aim of the present

study is to determine the relative toxicity of neurotoxic metals, pesticides and OP compounds as related to the comparative neurotoxic potential and the mechanism of AChE potential in different tissues of the fishes.

The aspects show that the measurement of 'total cholinesterase activity' under acute assay conditions in homogenates prepared with whole animals, can lead to erroneous ecotoxicological conclusions[9].

Results

To eliminate the factor concerning the effect of solvent on inhibition of AChE activity, I have taken 50ul of acetone + 100ul of Enzyme + 200ul of Substrate + 150ul of Distilled water (i.e. Control 2). The result shows that there is no change in AChE activity due to the presence of Acetone in the Enzyme-Substrate buffer medium. Measurement of AChE activity was done by measuring the pH reading every 10 minutes.

1. Inhibition of AChE activity of Nerita chamaeleon by Phosphamidon in a concentration of 1x10-6M collected from location: Dona Paula. The date of collection of the sample was 17.06.2006.

- a. The Blank pH started with a pH reading of 8.08 at 0 mins and at 60 mins the reading was 8.07. The delta pH was 0.01.
- b. The Blank + Enzyme pH started with a pH reading of 7.86 at 0 mins and at 60 mins the reading was 7.59. The delta pH was 0.27.
- c. The Blank + Substrate pH started with a pH reading of 7.96 at 0 mins and at 60 mins the reading was 6.46. The delta pH was 1.50.
- d. The Control pH started with a pH reading of 7.18 at 0 mins and at 60 mins the reading was 5.62. The delta pH was 1.56.
- e. The Sample 1 pH started with a pH reading of 7.09 at 0 mins and at 60 mins the reading was 5.71. The delta pH was 1.38 and the % Inhibition was found to be 11.53%.
- f. The Sample 2 pH started with a pH reading of 7.11 at 0 mins and at 60 mins the reading was 5.78. The delta pH was 1.33 and the % Inhibition was found to be 14.74%.
- g. The Sample 3 pH started with a pH reading of 7.03 at 0 mins and at 60 mins the reading was 5.73. The delta pH was 1.30 and the % Inhibition was found to be 16.67%.
- h. The Sample 4 pH started with a pH reading of 7.11 at 0 mins and at 60 mins the reading was 5.85. The delta pH was 1.26 and the % Inhibition was found to be 19.23%.
- i. The Sample 5 pH started with a pH reading of 7.15 at 0 mins and at 60 mins the reading was 6.05. The delta pH was 0.59 and the % Inhibition was found to be 62.17%.

2. Inhibition of AChE activity of Nerita chamaeleon by Methyl Parathion in a concentration of 1x10-8M collected from location: Dona Paula. The date of collection of the sample was 17.06.2006.

- a. The Blank pH started with a pH reading of 8.02 at 0 mins and at 60 mins the reading was 8.00. The delta pH was 0.02.

- b. The Blank + Enzyme pH started with a pH reading of 7.29 at 0 mins and at 60 mins the reading was 7.09. The delta pH was 0.20.
- c. The Blank + Substrate pH started with a pH reading of 7.36 at 0 mins and at 60 mins the reading was 6.46. The delta pH was 1.50.
- d. The Control pH started with a pH reading of 6.72 at 0 mins and at 60 mins the reading was 6.10. The delta pH was 0.62.
- e. The Sample 1 pH started with a pH reading of 6.92 at 0 mins and at 60 mins the reading was 6.32. The delta pH was 0.60 and the % Inhibition was found to be 3.22%.
- f. The Sample 2 pH started with a pH reading of 6.85 at 0 mins and at 60 mins the reading was 6.26. The delta pH was 0.59 and the % Inhibition was found to be 4.83%.
- g. The Sample 3 pH started with a pH reading of 6.79 at 0 mins and at 60 mins the reading was 6.23. The delta pH was 0.56 and the % Inhibition was found to be 9.67%.
- h. The Sample 4 pH started with a pH reading of 6.86 at 0 mins and at 60 mins the reading was 6.32. The delta pH was 0.54 and the % Inhibition was found to be 12.9%.
- i. The Sample 5 pH started with a pH reading of 6.74 at 0 mins and at 60 mins the reading was 6.32. The delta pH was 0.42 and the % Inhibition was found to be 32.25%.

3. Inhibition of AChE activity of *Eetroplus suratensis* by DDVP in a concentration of $1 \times 10^{-6} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- a. The Blank pH started with a pH reading of 7.64 at 0 mins and at 60 mins the reading was 7.63. The delta pH was 0.01.
- b. The Blank + Enzyme pH started with a pH reading of 6.43 at 0 mins and at 60 mins the reading was 6.36. The delta pH was 0.07.
- c. The Blank + Substrate pH started with a pH reading of 7.60 at 0 mins and at 60 mins the reading was 7.15. The delta pH was 0.45.
- d. The Control pH started with a pH reading of 6.35 at 0 mins and at 60 mins the reading was 5.30. The delta pH was 1.05.
- e. The Sample 1 pH started with a pH reading of 6.36 at 0 mins and at 60 mins the reading was 5.43. The delta pH was 0.93 and the % Inhibition was found to be 11.42%.
- f. The Sample 2 pH started with a pH reading of 6.38 at 0 mins and at 60 mins the reading was 5.47. The delta pH was 0.91 and the % Inhibition was found to be 13.33%.
- g. The Sample 3 pH started with a pH reading of 6.39 at 0 mins and at 60 mins the reading was 5.50. The delta pH was 0.89 and the % Inhibition was found to be 15.23%.
- h. The Sample 4 pH started with a pH reading of 6.44 at 0 mins and at 60 mins the reading was 5.82. The delta pH was 0.62 and the % Inhibition was found to be 40.95%.

4. Inhibition of AChE activity of *Eetroplus suratensis* by Ethyl Parathion in a concentration of $1 \times 10^{-6} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- a. The Blank pH started with a pH reading of 7.91 at 0 mins and at

60 mins the reading was 7.87. The delta pH was 0.04.

- b. The Blank + Enzyme pH started with a pH reading of 6.81 at 0 mins and at 60 mins the reading was 6.53. The delta pH was 0.28.
- c. The Blank + Substrate pH started with a pH reading of 7.71 at 0 mins and at 60 mins the reading was 7.22. The delta pH was 0.49.
- d. The Control pH started with a pH reading of 6.58 at 0 mins and at 60 mins the reading was 5.29. The delta pH was 1.39.
- e. The Sample 1 pH started with a pH reading of 6.60 at 0 mins and at 60 mins the reading was 5.63. The delta pH was 0.97 and the % Inhibition was found to be 30.21%.
- f. The Sample 2 pH started with a pH reading of 6.61 at 0 mins and at 60 mins the reading was 5.71. The delta pH was 0.90 and the % Inhibition was found to be 35.25%.
- g. The Sample 3 pH started with a pH reading of 6.63 at 0 mins and at 60 mins the reading was 5.84. The delta pH was 0.79 and the % Inhibition was found to be 43.16%.
- h. The Sample 4 pH started with a pH reading of 6.64 at 0 mins and at 60 mins the reading was 5.88. The delta pH was 0.76 and the % Inhibition was found to be 45.32%.
- i. The Sample 5 pH started with a pH reading of 6.66 at 0 mins and at 60 mins the reading was 6.11. The delta pH was 0.55 and the % Inhibition was found to be 60.43%.

5. Inhibition of AChE activity of *Eetroplus suratensis* by Phosphoamidon in a concentration of $1 \times 10^{-6} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- a. The Blank pH started with a pH reading of 7.57 at 0 mins and at 60 mins the reading was 7.54. The delta pH was 0.03.
- b. The Blank + Enzyme pH started with a pH reading of 6.21 at 0 mins and at 60 mins the reading was 6.09. The delta pH was 0.12.
- c. The Blank + Substrate pH started with a pH reading of 7.39 at 0 mins and at 60 mins the reading was 7.18. The delta pH was 0.21.
- d. The Control pH started with a pH reading of 6.07 at 0 mins and at 60 mins the reading was 4.61. The delta pH was 1.46.
- e. The Sample 1 pH started with a pH reading of 6.09 at 0 mins and at 60 mins the reading was 4.82. The delta pH was 1.27 and the % Inhibition was found to be 13.01%.
- f. The Sample 2 pH started with a pH reading of 6.12 at 0 mins and at 60 mins the reading was 5.07. The delta pH was 1.05 and the % Inhibition was found to be 28.08%.
- g. The Sample 3 pH started with a pH reading of 6.15 at 0 mins and at 60 mins the reading was 5.16. The delta pH was 0.99 and the % Inhibition was found to be 32.19%.
- h. The Sample 4 pH started with a pH reading of 6.17 at 0 mins and at 60 mins the reading was 5.38. The delta pH was 0.79 and the % Inhibition was found to be 45.89%.
- i. The Sample 5 pH started with a pH reading of 6.20 at 0 mins and at 60 mins the reading was 5.71. The delta pH was 0.49 and the % Inhibition was found to be 66.43%.

6. Inhibition of AChE activity of *Eetroplus suratensis* by Methyl Parathion in a concentration of $1 \times 10^{-6} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- The Blank pH started with a pH reading of 7.62 at 0 mins and at 60 mins the reading was 7.60. The delta pH was 0.02.
- The Blank + Enzyme pH started with a pH reading of 6.19 at 0 mins and at 60 mins the reading was 6.14. The delta pH was 0.05.
- The Blank + Substrate pH started with a pH reading of 7.49 at 0 mins and at 60 mins the reading was 7.17. The delta pH was 0.32.
- The Control pH started with a pH reading of 6.01 at 0 mins and at 60 mins the reading was 4.89. The delta pH was 1.12.
- The Sample 1 pH started with a pH reading of 6.09 at 0 mins and at 60 mins the reading was 5.11. The delta pH was 0.98 and the % Inhibition was found to be 12.5%.
- The Sample 2 pH started with a pH reading of 6.11 at 0 mins and at 60 mins the reading was 5.31. The delta pH was 0.80 and the % Inhibition was found to be 28.57%.
- The Sample 3 pH started with a pH reading of 6.12 at 0 mins and at 60 mins the reading was 5.58. The delta pH was 0.54 and the % Inhibition was found to be 51.78%.
- The Sample 4 pH started with a pH reading of 6.15 at 0 mins and at 60 mins the reading was 5.79. The delta pH was 0.36 and the % Inhibition was found to be 67.85%.

7. Inhibition of AChE activity of *Eetroplus suratensis* by Monocrotophos in a concentration of $1 \times 10^{-6} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- The Blank pH started with a pH reading of 7.46 at 0 mins and at 60 mins the reading was 7.42. The delta pH was 0.04.
- The Blank + Enzyme pH started with a pH reading of 6.09 at 0 mins and at 60 mins the reading was 6.05. The delta pH was 0.04.
- The Blank + Substrate pH started with a pH reading of 7.48 at 0 mins and at 60 mins the reading was 7.40. The delta pH was 0.08.
- The Control pH started with a pH reading of 6.06 at 0 mins and at 60 mins the reading was 4.31. The delta pH was 1.75.
- The Sample 1 pH started with a pH reading of 6.19 at 0 mins and at 60 mins the reading was 5.11. The delta pH was 1.08 and the % Inhibition was found to be 38.28%.
- The Sample 2 pH started with a pH reading of 6.22 at 0 mins and at 60 mins the reading was 5.19. The delta pH was 1.03 and the % Inhibition was found to be 41.14%.
- The Sample 3 pH started with a pH reading of 6.26 at 0 mins and at 60 mins the reading was 5.39. The delta pH was 0.87 and the % Inhibition was found to be 50.28%.
- The Sample 4 pH started with a pH reading of 6.33 at 0 mins and at 60 mins the reading was 5.59. The delta pH was 0.74 and the % Inhibition was found to be 57.71%.

8. Inhibition of AChE activity of *Eetroplus suratensis* by combination

of Ethyl Parathion + Methyl Parathion in (1:1) ratio, i.e. 1ml of Ethyl Parathion & 1ml of Methyl Parathion in a concentration of $1 \times 10^{-12} \text{M}$ collected from location: Madkai. The date of collection of the sample was 08.06.2006.

- The Blank pH started with a pH reading of 7.67 at 0 mins and at 60 mins the reading was 7.61. The delta pH was 0.06.
- The Blank + Enzyme pH started with a pH reading of 6.06 at 0 mins and at 60 mins the reading was 6.05. The delta pH was 0.01.
- The Blank + Substrate pH started with a pH reading of 7.47 at 0 mins and at 60 mins the reading was 7.43. The delta pH was 0.04.
- The Control pH started with a pH reading of 5.98 at 0 mins and at 60 mins the reading was 4.41. The delta pH was 1.57.
- The Sample 1 pH started with a pH reading of 6.08 at 0 mins and at 60 mins the reading was 5.21. The delta pH was 0.87 and the % Inhibition was found to be 44.58%.
- The Sample 2 pH started with a pH reading of 6.02 at 0 mins and at 60 mins the reading was 5.22. The delta pH was 0.80 and the % Inhibition was found to be 49.04%.
- The Sample 3 pH started with a pH reading of 6.09 at 0 mins and at 60 mins the reading was 5.38. The delta pH was 0.71 and the % Inhibition was found to be 54.77%.
- The Sample 4 pH started with a pH reading of 6.18 at 0 mins and at 60 mins the reading was 5.51. The delta pH was 0.67 and the % Inhibition was found to be 57.32%.

Observations

Acetylcholine is a cholinergic neurotransmitter. It is predominant at the neuromuscular junction. Now it is seen that the activity of Acetylcholinesterase is inhibited by several neurotoxic contaminants like heavy metals like Cd, Pb, Hg, etc., organophosphorous pesticides, etc.

In in-vitro conditions experiments were performed to see the effects of different neurotoxic contaminants on marine organism, i.e., a snail (*Nerita chamaeleon*) and a fish (*Eetroplus suratensis*). Result suggests that DDVP is highly toxic followed by Ethyl Parathion in case of *Nerita chamaeleon*. In comparison to DDVP & Ethyl Parathion, Phosphoamidon and Methyl Parathion is less toxic. The low toxicity of Phosphoamidon is due to the presence of phenyl group. The phenyl group adds to the stability of the compound.

In case of *Eetroplus suratensis*, Methyl Parathion is the most toxic followed by Monocrotophos, Phosphoamidon and Ethyl Parathion. Here it is seen that DDVP is the least toxic as compared to *Nerita chamaeleon*. The toxicity of Methyl parathion in this case is about 0.15 whereas in case of *Nerita chamaeleon* the toxicity of Methyl parathion is about 0.032.

Another set of observations were taken to see if there is any synergistic effect of the pesticides takes place in case of *Eetroplus suratensis* and was found that the level of toxicity goes high when two pesticides are mixed together. Compared to the combination of Ethyl parathion & Methyl parathion, the combination of DDVP & Phosphoamidon is higher.

Conclusion

- In case of *Nerita chamaeleon* the order of toxicity is
 - DDVP > Ethyl Parathion > Phosphoamidon ~ Methyl Parathion
- In order of toxicity in case of *Etroplus suratensis* is
 - Methyl Parathion > Monocrotophos > Phosphoamidon > Ethyl Parathion > DDVP
- Out of the two samples it is found that the level of toxicity varies from species to species but the pesticides have an adverse effect on the activity of AChE leading to neurological disorders like Myasthenia gravis, glaucoma & Alzheimer's disease, etc. Current sets of observations show that the study of Acetylcholinesterase enzyme can help the Eco-toxicologists to assess the impacts of various lethal & sub-lethal pollution in the marine environment. Study of different marine organisms like molluscs, fish, echinoderms, etc. can help to assess the biological effects of the neurotoxic contaminants on marine & estuarine environment.

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Conflict of Interest: Nil
Source of Support: None

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How to Cite this Article:

Dasgupta R. "In – Vitro Studies on Inhibition of Acetyl Cholinesterase Activities of marine organisms by Organophosphorus Pesticides". *Journal Medical Thesis* 2017 Jan-Apr; 5(1):21-26.