Diazinon exposed Channa striatus - implications on Enzymatic profile

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Abstract :   The present investigation was a trial to investigate the impact of various sublethal concentrations of Diazinon in the enzymatic behavior of Channa striatus. The enzyme studies is although not a direct and dependable source, but it definitely gives an idea about the impact of external abiotic factors on the living status of the aquatic inhabitants. The results revealed a significant increase in AST (451 ± 18.0 to 591 ± 56.0; V = 8.06), and ALT (15.2 ± 3.2 to 20.5 ± 6.9; V = 1.02), however, a significant decrease was observed in CK (639.05 ± 208.95 to 613.91 ± 297.23; V = 88344.32), ALP (0.73 ± 0.16 to 0.72 ± 0.22; V = 0.05); ACP (0.09 ± 0.04 to 0.06 ± 0.04) and AchE (7686.0 ± 105.0 to 2896 ± 162.0; V = 26.6).

IndexTerms - **Investigation, Channa striatus, Enzyme, inhabitants.**

I. INTRODUCTION

Pesticides are an economical means to control growth of unwanted pests. Excessive use of these chemicals results in environmental pollution and toxicity to non target organisms. Thus, the use of pesticides has gained worldwide concern (Rao, 2004). The injuries caused by insecticides to aquatic environment are clear and fish are found to bioaccumulate due to the direct exposure to chemicals and ingestion of contaminated preys and food (Livingstone, 2001; Matsumoto et al., 2006). Many of these compounds or their metabolites have shown toxic effects related to oxidative stress (Winston and Di Giulio, 1991).

Diazinon is an organophosphorous insecticide (OPI) and acaricide developed in the early 1950s. It is also used throughout the world in the control measure in public health services especially applied to control ectoparasites in veterinary medicine (Watterson, 1999; EPA, 2005). The major source of Diazinon residues in edible crops are from its use as agricultural pesticide while those in meat, offal and other animal products arise from its use as a veterinary drug containing active ingredient. It is mobile and moderately persistent in the environment and does not bioaccumulate (Pehkonen and Zhang, 2002). Due to its chemical properties and widespread use, diazinon is frequently found in point sources and non-point sources in urban and agricultural areas (EPA, 2003).

Although Diazinon is well known to have nuerotoxic, hematotoxic, hepatotoxic, genotoxic and renal effects and influence the reproductive, developmental, respiratory, cardiovascular systems little is known of how it contributes to the oxidative stress on higher animals. OPIs are shown to exert their action by inhibiting activity of acetyl cholinesterase (AChE) which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Kwong, 2002) resulting in accumulation of acetylcholine (Fulton and Key, 2001). This leads to tremors, convulsions and finally the death of the aquatic organism. Several factors seem to be involved in affecting the AChE activity caused by OPIs such as length of time and exposure concentration. Diazinon is commonly used for pest control in the agricultural fields surrounding freshwater reservoirs and contaminating aquatic ecosystems. The toxicity of diazinon depends on the inhibition of acetylcholine esterase activity (AChE, EC 3.1.1.7) like other OPIs (Chambers and Carr, 1995). Therefore, measurement of AChE activity in the fish has been described as a method for diagnosing anticholinesterase pesticides in aquatic solutions (Dellali et al., 2001). The knowledge of the major factors responsible for the species selective toxicity of this compound among fish may help to improve the classification of OP compounds according to the regulations devoted to the environmental protection (Keizer et al., 2001).

The mechanism of a toxic effect of diazinon is the same as of other organophosphorous substances. There is an inhibition of a whole series of enzymes and mainly of acetylcholinesterase (Goodman et al. 1979; Sastry and Sharma 1980; Ansari et al. 1987; Hamm et al. 1998). Oh et al. (1991) present three factors causing the selective toxicity of diazinon for various fish species: different inhibition of acetylcholinesterase, different detoxification and absorption.

II. MATERIALS AND METHODS

Collection and maintenance of fish:

Fish of particular size (22-24 cm) and weight (25-30 g) range were used for the experiment. The test solutions were renewed every 24 hr to maintain the optimum dissolved oxygen level. While conducting the experiments, care was taken not to deviate from the modified main principles of bioassay techniques outlined by Sprague (1973) and recommended by APHA (1989). Fish were fed with 2% of body weight fish feed once a day.

Acute Toxicity:

To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by

American Public Health Association (1960). The toxicant concentration used in the present series of tests were approximately the wide range of concentrations viz., Control, LC10, LC20, LC30, LC40, LC50, LC60, LC70, LC80, LC90, LC99 aqueous solutions were prepared. In each group twenty one fishes were introduced with three replications.

Enzyme Assays

a) Estimation of aminotransferase activity (AST)

Aspartate (AST, 1-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) aminotransferase activity levels were estimated by the method of Reitman and Frankel (1957). 2.5 per cent homogenates of tissue was prepared in Na-K-phosphate buffer (0.1 M, pH 7.4) at 0ºC and centrifuged at 3000 rpm for 15 min. The reaction mixture in a final volume of 1.0 mL contained 0.5 mL Na-K-phosphate buffer (0.1 M, pH 7.4), 0.1 mL (0.4 M) 1-aspartate or (0.4 M) d-alanine, 0.1 mL of 0.02 M α-ketoglutarate and 0.2 mL of supernatant. The reaction mixture was incubated at 37ºC for an hour. Adding 1.0 mL of 2,4-dinitrophenylhydrazine in 0.1 N HCl stopped the reaction and the solutions were allowed to stand for one hour. 10 mL of sodium hydroxide (0.4 N) was added and the contents were mixed by inversion. The intensity of the color was read at 530 nm in a linear read out grating Spectrophotometer (Cecil, Model CE 373) against zero time control. The activity is expressed as mg of pyruvate/g wet weight of tissue/hr.

b) Estimation of Alanine Aminotransferase (ALT)

Alanine (ALT, 1-alanine; 2-oxoglutarate aminotransferase, EC 2.6.1.2) aminotransferase activity levels was estimated by the method of Reitman and Frankel (1957). 5 per cent homogenates of the tissues viz., gill, liver, kidney, brain and muscle 2.5 per cent homogenates of tissues were prepared in Na-K-phosphate buffer (0.1 M, pH 7.4) at 0ºC and centrifuged at 3000 rpm for 15 min. The reaction mixture in a final volume of 1.0 mL contained 0.5 mL Na-K-phosphate buffer (0.1 M, pH 7.4), 0.1 mL (0.4 M) 1-aspartate or (0.4 M) d-alanine, 0.1 mL of 0.02 M α-ketoglutarate and 0.2 mL of supernatant. The reaction mixture was incubated at 37ºC for an hour. Adding 1.0 mL of 2,4-dinitrophenylhydrazine in 0.1 N HCl stopped the reaction and the solutions were allowed to stand for one hour. 10 mL of sodium hydroxide (0.4 N) was added and the contents were mixed by inversion. The intensity of the color was read at 530 nm in a linear read out grating Spectrophotometer (Cecil, Model CE 373) against zero time control. The activity is expressed as mg of pyruvate/g wet weight of tissue/hr.

c) Estimation of Creatine Kinase (CK):

The tissues were then blotted dry and weighed. Immediately, a 10% tissue homogenates (w/v) of the tissue was prepared by using a Potter Elvehjem homogenizer fitted with a teflon plunger in a O.25M sucrose, 0.02M triethanolamine hydrochloride buffer of pH 7.4 containing 0.12mM dithiothreitol (DTT). Homogenates were then centrifuged at 1000g for 10 minutes to remove nuclei and cell debris. The supernatants were further centrifuged at 12000g for 40 minutes and the supernatants were used for the determination of creatine kinase.

d) Estimation of Tissue Acid Phosphatase:

Hundred mg of wet tissue was weighed and homogenized in a glass homogenizer using 10 mL distilled water. To test tube 0.5 mL of substrate solution (p-nitrophenyl phosphate) and 0.5 mL of 0.1 N citrate buffers was added. The test tube with above solution was kept in water bath maintained at 37ºC for 5 min. Then, l mL of the tissue extract was added to the test tube. The test tube with the tissue extract was then kept in water bath at 37ºC for 30 min. After completion of 30 min the reaction was arrested in the extract by adding 3.8 mL of 0.1 N sodium hydroxide. The colour formed at the end was read at 415 nm Grating Spectrophotometer and the values expressed in % mole of PNP/mg protein/hr.

e) Estimation of Tissue Alkaline Phosphatase:

Hundred mg of wet tissues from gill, liver, kidney, brain and muscle were weighed and homogenized using 10 mL distilled water. To each test tube 0.5 mL of substrate solution (p-nitrophenyl phosphate) and 0.5 mL of glycine buffer was added. The test tube with above solutions was then kept in water bath and maintained at 37ºC for 30 min. After completion of 30 min the reaction was arrested in the extract by adding 10 mL of 0.02 N sodium hydroxide. The colour formed at the end was read at 415 nm in Grating Spectrophotometer (Cecil Model CE-373). The values are expressed in % mole of PNP/mg protein/hr.

f) Estimation of AChE:

Acetylcholinesterase activity levels in the selected tissues of Channa striatus was estimated following the method of Metcalf (1951). A 2% (w/v) homogenate of the tissues was prepared in 0.25 M cold sucrose solution (8.577 g of sucrose in 100 mL of distilled water). The uncentrifuged homogenate was used for the enzyme assay. Incubation mixture in a final volume of 2 mL, contained 1 mL buffer substrate (4 mL of 0.1 M Na-K-phosphate buffer + 1 mL of 0.008 M acetylcholine chloride) and 1 mL of homogenate. The reaction was stopped after 30 min of incubation at 37ºC by the addition of 2 mL of alkalinehydroxylamine hydrochloride + 1 mL of 3.5 m sodium hydroxide) which was followed by the addition of 1 mL of hydrochloric acid (1:1 water: HCl). The contents were shaken thoroughly and filtered. 2.5 mL of aliquots of the filtrate were taken and 0.5 mL of clear ferric chloride solution was added to each alqiuot. The intensity of the colour developed was measured at 545 nm in a double-beam spectrophotometer against a reagent blank. The enzyme activity was calculated from a previously drawn standard graph. The enzyme activity was expressed in % moles of acetylcholine hydrolyzed mg protein/hr.

III. RESULT

The present research thrusted upon the observation on the changes in the enzyme profile of Channa striatus on exposure to sub lethal concentrations of Diazinon. Varied changes were observed, which suggested the quantum of damage to the metabolic profile of fish.

a) Aspartate aminotransferase (AST):

AST is an enzyme found in high levels in the liver, heart, and muscles. It is also found in lesser amounts in other tissues. AST levels are often compared with results of other tests, such as alkaline phosphatase (ALP), total protein, and bilirubin to help determine which form of liver disease is present. AST is often measured to monitor the liver damage. The mean±SD value of AST expressed in IU/L, 451±18.0 during first replica (N = 21). The AST showed a increase after the exposure with a mean±SD of 591±56.0. The effect of 96 h LC50 (24.16 ppm) of diazinon on AST of Channa striatus (Bloch) showed variance of 8.06 as compared to control group (3.75). The probability value was calculated as 0.35, with significance levels of 2.8E-17 (I tier) and 5.0E-15 (II tier). The lower and upper bound values at 95% confidence interval were 0.83 and 1.0 respectively. In second replica, expressed in IU/L, AST was 433.0±0.01 (N = 21). The AST showed an increase after the exposure with a mean±SD of 568±0.00. The effect of 96 h LC50 (24.16 ppm) of diazinon on AST of Channa striatus (Bloch) showed variance of 0.00 as compared to control group (0.001). The probability value was calculated as 0.99, with significance levels of 0.00 (I tier) and 0.00 (II tier). The lower and upper bound values at 95% confidence interval were 0.00 and 6.13 respectively. The concentration dependent response of AST in Channa striatus (Bloch) is graphically represented in figure 01, revealing regression equation of $y = -0.66\ln(x) + 7.268$. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 178.04 for 96 h LC50.

b) Alanine aminotransferase (ALT):

Alanine transaminase (ALT) is a transaminase enzyme. It is also called alanine aminotransferase (ALAT) and was formerly called serum glutamate-pyruvate transaminase (SGPT) or serum glutamic-pyruvic transaminase (SGPT). ALT is found in plasma and in various body tissues, but is most common in the liver. ALT is an enzyme found in a high level in the liver. Injury to the liver results in release of ALT into the blood. This test is used to determine if the test animal has liver damage. An increased ALT level is usually a sign of liver damage. The mean±SD value of ALT expressed in µkat.l-1, was 15.2±3.2 during first replica (N = 21). The ALT showed an increase after the exposure with a mean±SD of 20.5±6.9. The effect of 96 h LC50 (24.16 ppm) of diazinon on ALT of Channa striatus (Bloch) showed variance of 1.02 as compared to control group (0.35). The probability value was calculated as 0.52, with significance levels of 0.00 (I tier) and 0.00 (II tier). The lower and upper bound values at 95% confidence interval were 0.2 and 1.0 respectively. In second replica, expressed in µkat.l-, ALT was 17.3±2.4 (N = 21). The ALT showed an increase after the exposure with a mean±SD of 38.9±8.2. The effect of 96 h LC50 (24.16 ppm) of diazinon on ALT of Channa striatus (Bloch) showed variance of 0.003 as compared to control group (0.004). The probability value was calculated as 0.92, with significance levels of 7.0E-16 (I tier) and 8.2E-15 (II tier). The lower and upper bound values at 95% confidence interval were 2.88 and 3.00 respectively. The concentration dependent response of ALT in Channa striatus (Bloch) is graphically represented in figure 2, revealing regression equation of $y = -0.189\ln(x) + 1.469$. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 269.58 for 96 h LC50.

c) Creatinine Kinase (CK):

Creatine kinase (CK) — also known as creatine phosphokinase (CPK) or phospho-creatine kinase — is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and utilizes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP. In tissues and cells that consume ATP rapidly, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus creatine kinase is an important enzyme in such tissues. Clinically, creatine kinase is assayed in blood tests as a marker of damage of CK-rich tissue such as in rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and the autoimmune myositides and in acute renal failure. The mean±SD value of CK expressed in µkat.l-, was 639.05±208.95 during first replica (N = 21). The CK showed a decrease after the exposure with a mean±SD of 613.91±297.23. The effect of 96 h LC50 (24.16 ppm) of diazinon on CK of Channa striatus (Bloch) showed variance of 88344.32 as compared to control group (43658.62). The probability value was calculated as 0.79, with significance levels of 2.6E-14 (I tier) and 5.9E-14 (II tier). The lower and upper bound values at 95% confidence interval were 0.53 and 2.69 respectively. In second replica, expressed in IU/L, CK was 645.0±2.5 (N = 21). The CK showed a decrease after the exposure with a mean±SD of 622.00±3.0. The effect of 96 h LC50 (24.16 ppm) of diazinon on CK of Channa striatus (Bloch) showed variance of 9.0 as compared to control group (6.25). The probability value was calculated as 0.94, with significance levels of 0.00 (I tier) and 0.00 (II tier). The lower and upper bound values at 95% confidence interval were 1.20 and 1.52 respectively. The concentration dependent response of CK in Channa striatus (Bloch) is graphically represented in figure 3, revealing regression equation of $y = -24.1\ln(x) + 655.7$. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 178204.50 for 96 h LC50.

d) Alkaline Phosphate (ALP):

The mean±SD value of ALP expressed in µkat.l-1, was 0.73±0.16 during first replica (N = 21). The ALP showed a decrease after the exposure with a mean±SD of 0.72±0.22. The effect of 96 h LC50 (24.16 ppm) of diazinon on ALP of Channa striatus (Bloch) showed variance of 0.05 as compared to control group (0.02). The probability value was calculated as 0.89, with significance levels of 0.00 (I tier) and 0.00 (II tier). The lower and upper bound values at 95% confidence interval were 0.00 and 0.72 respectively. In second replica, expressed in µkat.l-1, was 0.68±0.01 (N = 21). The ALP showed an increase after the exposure with a mean±SD of

0.70±0.01. The effect of 96 h LC50 (24.16 ppm) of diazinon on ALP of Channa striatus (Bloch) showed variance of 0.001 as compared to control group (0.0001). The probability value was calculated as 0.46, with significance levels of 2.1E-15 (I tier) and 9.5E-15 (II tier). The lower and upper bound values at 95% confidence interval were 0.02 and 1.0 respectively. The concentration dependent response of ALP in Channa striatus (Bloch) is graphically represented in figure 4, revealing regression equation of y = 0.002ln(x) + 0.698. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 295.49 for 96 h LC50.

e) Acid Phosphate (ACP):
The mean±SD value of ACP expressed in μkat.l-1, was 0.09±0.04 during first replica (N = 21). The ACP showed a decrease after the exposure with a mean±SD of 0.06±0.04. The effect of 96 h LC50 (24.16 ppm) of diazinon on ACP of Channa striatus (Bloch) showed variance of 0.00 as compared to control group (0.00). The probability value was calculated as 0.98, with significance levels of 1.00 (I tier) and 0.00 (II tier). The lower and upper bound values at 95% confidence interval were 0.00 and 0.06 respectively. In second replica ACP expressed in μkat.l-1, was 0.09±0.00 (N = 21). The ACP showed a decrease after the exposure with a mean±SD of 0.07±0.002. The effect of 96 h LC50 (24.16 ppm) of diazinon on ACP of Channa striatus (Bloch) showed variance of 0.00 as compared to control group (0.00). The probability value was calculated as 0.98, with significance levels of 0.00 (I tier) and 1.00 (II tier). The lower and upper bound values at 95% confidence interval were 0.00 and 0.07 respectively. The concentration dependent response of ACP in Channa striatus (Bloch) is graphically represented in figure 5, revealing regression equation of y = -0.01ln(x) + 0.099. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 310.75 for 96 h LC50.

f) Activities of Enzyme Acetylcholinesterase (AchE):
The mean±SD value of AchE expressed in IU/L, was 7686.0±105.0 during first replica (N = 21). The AchE showed a decrease after the exposure with a mean±SD of 2896±162.0. The effect of 96 h LC50 (24.16 ppm) of diazinon on AchE of Channa striatus (Bloch) showed variance of 26.6 as compared to control group (8.41). The probability value was calculated as 0.99, with significance levels of 1.8E-14 (I tier) and 1.2E-14 (II tier). The lower and upper bound values at 95% confidence interval were 1.24 and 1.78 respectively. In second replica,   AchE expressed in IU/L, was 7513.0±121.0 (N = 21). The AchE showed a decrease after the exposure with a mean±SD of 2532.0±159.0. The effect of 96 h LC50 (24.16 ppm) of diazinon on AchE of Channa striatus (Bloch) showed variance of 45.56 as compared to control group (292.4). The probability value was calculated as 0.99, with significance levels of 2.4E-15 (I tier) and 6.9E-15 (II tier). The lower and upper bound values at 95% confidence interval were 0.39 and 3.6 respectively. The concentration dependent response of AchE in Channa striatus (Bloch) is graphically represented in figure 6, revealing regression equation of y = -119.ln(x) + 588.2. Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 60031.13 for 96 h LC50.

IV. DISCUSSION

In order to evaluate the effect of diazinon [0,0-diethyl-0-(2-isopropyl-6- methylpyrimidin-4yl) phosphorothioate] on common carp (Cyprinus carpio L.), Luskova et al. (2002) assessed the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide Basudin 600 EW (containing 600 g.l-1 diazinon as the toxic substance). The authors observed a significant decrease of cholinesterase (p < 0.01) and lactate dehydrogenase (p < 0.05) in the experimental group. The values of alanine and aspartate aminotransferases, creatine kinase, alkaline and acide phosphatases were comparable in the experimental and control groups. Banaee (2008) revealed low levels of alkaline phosphatases and significantly higher (p<0.05) values of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, compared to the control group. AchE is sensitive to organophosphate and carbamates pesticides that are well known as potent (AchE) inhibitors (Van der Oost et al., 2003). So, plasma (AchE) activity could be used as a biomarker of exposure to pesticides, as it decreased in fish collected from sites contaminated by pesticides (Dorval et al., 2004). Serum (AchE) revealed a significant inhibition of (AchE) in the exposed fish during the acute and chronic exposures compared to the control group. Also, the inhibition of (AchE) is correlated with exposure concentration, but not with exposure time as reviewed by Roex et al. (2003), Rao (2006b); David et al., 2007; Guimarães et al. (2007) and Cong et al. (2009).

In order to investigate the effects of organophosphorus (OP) pesticide diazinon on acetylcholinesterase (AChE: EC 3.1.1.7) activity in the brain of a freshwater fish, Oreochromis niloticus. The influence of organophosphorus (OP) Diazinon on gluatathione (GSH) content, acetylecholinesterase (AChE) in Tilapia (Orechromis niloticus) was studied by Nagwa et al. (2007). Results revealed that the in vivo chronic exposure of Tilapia to sub-lethal concentrations of Diazinon for 30 days caused a reduction in total GSH and AChE activity. Both total GSH and AChE activity were increased at the end of the recovery intervals. It was concluded that GSH could be a valuable biomarker to mirror pollutant status in the aquatic systems. The difference of inhibition level between treatments was significant, indicating that the inhibition in AChE was correlated with the concentration of the toxicants. Reduced AChE activity was mostly caused by Diazinon as neurotoxins. Nguyen et al. (2009) reported that diazinon caused long term inhibition of brain ChE activity and a significant 30% growth inhibition.

Diazinon toxicity was evaluated through acute and chronic exposures of fish to 1/2 and 1/10 the calculated LC50 for 1w and 6 w respectively by Safinaz et al. (2009). Results showed that significant increase in AST and ALT activities. Furthermore, diazinon exposures were associated with an inhibition of cholinesterase activity. Concerning serum ALT and AST, results revealed a significant increase in the diazinon exposed fish during the acute and chronic exposures compared with the control group. These results agreed with those demonstrated by Radwan and El – Said (2006) and Agrahari et al. (2007). As it is known, liver of vertebrates generally and fish particularly is the principle organ of detoxification as reviewed by Freeman et al. (1983). So, the increase in ALT

and AST transaminase might be attributed to tissue damage, particularly liver (Palanivelu et al., 2005).The elevation of these enzymes in the extracellular fluid or plasma is a sensitive indicator of even cellular damage (Van der Oost et al., 2003; Palanivelu et al., 2005). Thus, the measurement of transaminases and phosphatase activities in blood plasma of fish can be used as indicator for pesticide toxicity (Agrahari et al., 2007).

The acute effect of diazinon on the African catfish (Clarias gariepinus) was assessed by Adedeji (2010). The activities of selected enzymes showed a significant decrease of cholinesterase (p < 0.05) lactate dehydrogenase (p < 0.05) alkaline phosphatase and acid phosphatase in the experimental group. The values of alanine and aspartate, aminotransferases, creatine kinase, were comparable in the experimental and control groups. Diazinon alone is no inhibitor of cholinesterase. However, in animal bodies, it is converted to diazoxon, which is a strong inhibitor of ACHE enzyme (Gallo and Lawryk, 1991). There was a significant decrease (p < 0.05) in the plasma concentration of alkaline and acid phospatases in the test treatment following the acute effect of diazinon. Sastry and Sharma (1980) reported a decrease of activities in alkaline and acid phosphatases in the brain of Channa punctatus following the effect of diazinon. According to these authors, the alkaline phosphatase activity is inhibited after 96 h of the effect and then it resumes its normal values or even an increased activity is observed. Goel et al. (1982) reported serum alkaline and acid phosphatases decreased by 15% in Heteropneustes fossilis, resulting from the effect of the organophosphate malathion.

The activities of alkaline and acid phosphatases in blood plasma of Cyprinus carpio were almost identical in the control and test treatment following exposure to acute effect of diazinon (Luskova et al., 2002). The resulting activity values of alkaline and acid phosphatase support the assumption that the liver tissue of the experimental fish was markedly affected. Similarly, the practically identical activity of alanine and aspartate aminotransferases and creatine kinase, observed in the control and experimental groups, indicate that diazinon damages neither parenchymatous tissues nor skeletal musculature nor disturb the permeability and integrity of cell membranes. Luskova et al. (2002) in their examinations of the biochemical blood plasma profile of carp Cyprinus carpio indicate a marked neurotoxic effect of diazinon in fishes.

Diazinon-induced changes in the total protein and transaminase activities of Clarias gariepinus were assessed by Inyang et al. (2010). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in plasma, muscle, liver, gills and kidney. In addition to significant differences in ALT and AST in liver, kidney, gills and muscle, concentration dependent activities in ALT was observed in the liver and gills. Acetylcholinesterase activity responded positively in a concentration dependent pattern in experiments of Khalid (2014). OP pesticides have several toxic properties, the most prominent effect of which is AChE inhibition. AChE activity is therefore widely used in biomonitoring studies as a biomarker of OP pesticide exposure. The reduction of AChE activity is assumed to result from the direct action of diazinon exposure on the active site of this enzyme. Rath and Misra (1981) reported a positive correlation with insecticide concentration and the time of exposure associated with the degree of AChE inhibition of Tilapia mossambica in relation to the interacting effects of sublethal concentrations of dichlorvos. Similar results have also been reported in sunfish, Lepomis gibbosus (Benke and Murphy, 1974); in Danio rerio (Ansari and Kumar, 1987), Seriola dumerilli (Jebali et al., 2006), freshwater catfish Heteropneutes fossilis (Chandra, 2008) after malathion exposure and in Poecilia reticulata (Archana et al., 2011). A decrease in AChE activity by diazinon intoxication has been reported in different animals and fish as Oreochromis niloticus (Tridico et al., 2010). However, most of the AChE studies are done in fish brains, because the most pronounced effects are observed in nervous tissue.

The activities of selected enzymes were measured in 15 specimens of controls exposed for 96 h to the effects of Basudin 600 EW at a concentration of 32.5 mg.l-1 (containing 600 g.l-1 diazinon as the toxic substance) by Luskova et al. (2002). Compared with the control group, a significant decrease of cholinesterase (p < 0.01) and lactate dehydrogenase (p < 0.05) was ascertained in the experimental group by the authors. The values of alanine and aspartate aminotransferases, creatine kinase, alkaline and acid phosphatases were comparable in the experimental and control groups. The findings of the above authors lends complete support to our findings, which revealed significant (P<0.05) decrease AST, CK and ACHE. No significant (P>0.05) differences were observed in ALP, while as increase was observed in ALT in experimented group as compared to control.

In order to investigate the effects of organophosphorus (OP) pesticide diazinon on acetylcholinesterase (AChE: EC 3.1.1.7) activity and its relationship to lipid peroxidation (LPO) in the brain of a freshwater fish, Oreochromis niloticus, Nevin et al. (2006) used Malondialdehyde (MDA) content as biomarker for LPO. Fish were exposed to 1 and 2 mg/L sublethal concentrations of diazinon for 1, 7, 15 and 30 days. The authors reported that during the entire experimental group, AChE activity in brain significantly decreased (up to 93% of control), which showed that diazinon inhibited AChE activity in fish. The inhibition of AChE activity in the brain of O. niloticus correlated with increased MDA levels after 7 and 15 days diazinon exposures (r = −0.661, P < 0.019; r = −0.652, P < 0.022, respectively). The observations are in coherence with present findings which revealed significant decrease in ACHE activity from 7686.0 to 2896.0 IU/L, with a regression values of y = -119.ln(x) + 588.2.

In yet another trial by Nagwa et al. (2007), the influence of organophosphorus (OP) Diazinon on gluathione (GSH) content, acetylecholinesterase (AChE) in Tilapia (Orechromis niloticus) was studied. The authors experimented on uniform (40 g) male tilapia, divided in 3 replicates for two concentrations (LC33.5 and LC10) for 30 days. Their results revealed reduction in total GSH and AChE activity. Banaee et al. (2008), on the other hand, determined the chronic toxicity of Diazinon and its effects on some hematological parameters and biochemical blood plasma profiles of common carp, Cyprinus carpio. The authors reported that the experimental groups showed significantly lower values (p < 0.05) in alkaline phosphatases and significantly higher (p<0.05) values of plasma glucose, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase.

Juvenile snakehead fish Channa striata were exposed twice to 4-day pulses of 0.016, 0.079 or 0.35 mg/L of diazinon, separated by a 2 week interval to imitate the exposure conditions in the field by Nguyen et al. (2009). The authors reported inhibition in brain AChE activity and reduction in growth. Other works documented a significant increase in cortisol, glucose, urea, uric acid, creatinine and serum Ca++ levels by Safinaz et al. (2009) who investigated the clinical and biochemical alterations associated with dizinon toxicity in Clarias gariepinus. Authors reported a significant increase in AST and ALT activities of the exposed fish. Furthermore, diazinon exposures were associated with an inhibition of cholinesterase activity.

The acute effect of diazinon on the African catfish (Clarias gariepinus) was assessed by comparing the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide DiazintolR (162 mg/ml of diazinon as the active substance) by Adedeji (2010). Their results showed a significant decrease in cholinesterase ($p < 0.05$), lactate dehydrogenase ($p < 0.05$), alkaline phosphatase and acid phosphatase in the experimental group. The values of alanine and aspartate, aminotransferases, creatine kinase, were comparable in the experimental and control groups. Diazinon-induced changes in the transaminase activities of Clarias gariepinus, were assessed by Inyang et al. (2010) who exposed the fish to varying sub-lethal concentrations of diazinon (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) in semi-static bioassays for 30 days. The authors reported significant differences in ALT and AST in liver, kidney, gills and muscle when compared with the control, which completely supports our findings.

Indian carp (Cirrhinus mrigala) exposed to two sub-lethal concentrations (0.815 mg/L and 1.63 mg/L) of diazinon for 30 days showed a significant decrease in activity of enzyme acetylcholinesterase when compared with controlled fish (P<0.05), as evidenced by Haider and Rauf (2014). Compared to the controlled fish, the plasma activities of enzyme asparate aminotransferase and alanine aminotransferase increased significantly after 20 and 30 days in fish exposed to 0.815 mg/L of diazinon, while the activities of these enzymes in fish exposed to 1.63 mg/L of diazinon increased significantly at all sampling periods (P<0.05). On the other hand, the plasma activity of the enzyme lactate dehydrogenase decreased significantly in both diazinon treated fish groups after 10 days of exposure (P<0.05) and returned to the normal value after 20 and 30 days of exposure.

A research was conducted to study the influence of diazinon on acetylcholinestrase (AChE) and lipidperoxidation (LPO) on freshwater common carp (Cyprinus carpio L.) by Khalid (2014). Adult fish (mean body weight 42.6±3.86 g and 19.1±2.66 cm mean length) were divided into five groups with 3 replicates and subjected to 5 concentrations (100, 200, 300, 400 and 500 µg L-1) of diazinon for 30 days and compared with control (untreated) fish. Induction of oxidative stress in various tissues was evidence of increased lipid peroxidation levels, which seems to be associated with the concentration of diazinon. Acetylcholinesterase activity responded positively in a concentration dependent pattern. It was concluded that LPO and AChE could be a valuable biomarker as an indicator of water pollution on aquatic ecosystems. The findings of the above authors lends complete support to our findings which showed significant (P<0.05) decrease in AST ($y = -0.66\ln(x) + 7.268$), CK ($y = -24.1\ln(x) + 655.7$), ACP ($y = -0.01\ln(x) + 0.099$), and AChE ($y = -119.\ln(x) + 588.2$), while as significant (P<0.05) increase and moderate change was observed in ALT ($y = 0.189\ln(x) + 1.469$), and ALP ($y = 0.002\ln(x) + 0.698$) respectively.
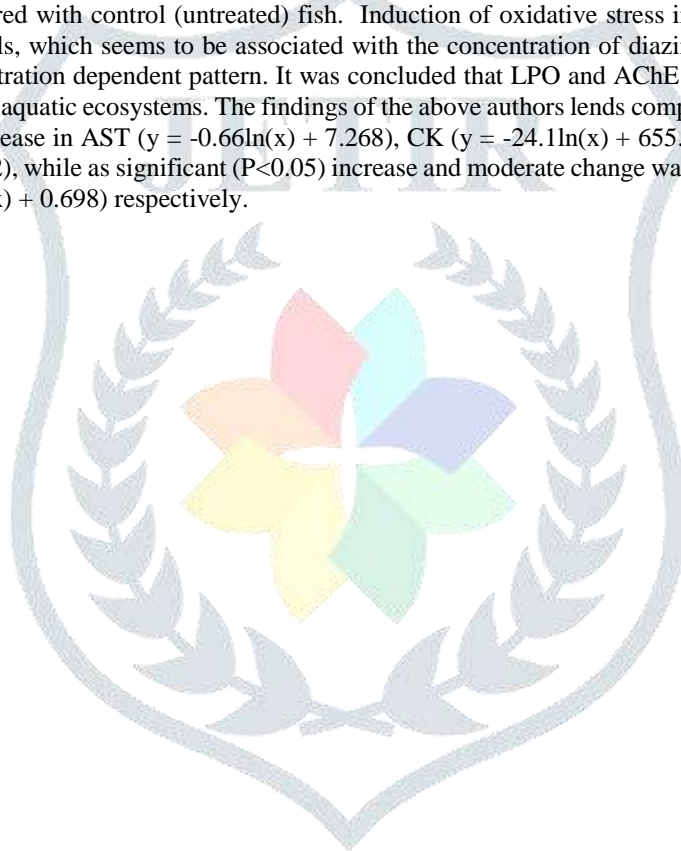
Table 01: **Values for first replica of mean enzyme parameters of *Channa striatus* (Bloch) affected by sublethal exposure of Diazinon**

Indices	Units	Groups	N	Means	SD	Variance	Probability	Sig. 1	Sig. 2	95% Confidence Interval	
										Lower Bound	Upper Bound
AST	IU/L	Control	10	451	18.0	3.75	0.35	2.8E-17	5.0E-15	0.83	1.0
		Treated	21	591	56.0	8.06					
ALT	IU/L	Control	10	15.2	3.2	0.35	0.52	0.00	0.00	0.2	1.0
		Treated	21	20.5	6.9	1.02					
CK	µkat.l^{-1}	Control	10	639.05	208.95	43658.62	0.79	2.6E-14	5.9E-14	0.53	2.69
		Treated	21	613.91	297.23	88344.32					
ALP (Liver)	µkat.l^{-1}	Control	10	0.73	0.16	0.02	0.89	0.00	0.00	0.00	0.72
		Treated	21	0.72	0.22	0.05					
ALP (Kidney)	µkat.l^{-1}	Control	10	0.68	0.31	0.03	0.86	1.2E-14	2.2E-13	0.19	0.36
		Treated	21	0.52	0.19	0.05					
ACP (Liver)	µkat.l^{-1}	Control	10	0.09	0.04	0.00	0.98	1.00	0.00	0.00	0.06
		Treated	21	0.06	0.04	0.00					
ACP (Kidney)	µkat.l^{-1}	Control	10	0.08	0.01	0.01	0.96	2.5E-15	2.0E-14	0.03	0.06
		Treated	21	0.05	0.02	0.00					
AChE	IU/L	Control	10	7686.0	105.0	8.41	0.99	1.8E-14	1.2E-14	1.24	1.78
		Treated	21	2896.0	162.0	26.6					

AST - aspartate aminotransferase; ALT - alanine aminotransferase; CK - creatine kinase; ALP - alkaline phosphatase; ACP - acid phosphatase; AChE=activities of enzyme acetylcholinesterase

Table 02: Values for second replica of mean enzyme parameters of *Channa striatus* (Bloch) affected by sublethal exposure of Diazinon

| Indices	Units	Groups	N	Means	SD	Variance	Probability	Sig. 1	Sig. 2	95% Confidence Interval	
										Lower Bound	Upper Bound
AST	IU/L	Control	10	433.0	0.01	0.001	0.99	0.00	0.00	0.00	6.13
		Treated	21	568	0.00	0.00					
ALT	IU/L	Control	10	17.3	2.4	0.004	0.92	7.0E-16	8.2E-15	2.88	3.0
		Treated	21	38.9	8.2	0.003					
CK	$\mu kat.l^{-1}$	Control	10	645.0	2.5	6.25	0.94	0.00	0.00	1.20	1.52
		Treated	21	622.0	3.0	9.0					
ALP (Liver)	$\mu kat.l^{-1}$	Control	10	0.68	0.01	0.0001	0.46	2.1E-15	9.5E-15	0.02	1.0
		Treated	21	0.85	0.01	0.001					
ALP (Kidney)	$\mu kat.l^{-1}$	Control	10	0.62	0.20	0.21	0.32	0.003	2.1E-10	0.11	1.1
		Treated	21	0.42	0.11	0.02					
ACP (Liver)	$\mu kat.l^{-1}$	Control	10	0.09	0.000	0.00	0.98	0.00	1.00	0.00	0.07
		Treated	21	0.07	0.002	0.00					
ACP (Kidney)	$\mu kat.l^{-1}$	Control	10	0.07	0.00	0.02	0.98	0.09	0.03	0.02	0.10
		Treated	21	0.06	0.001	0.03					
AChE	IU/L	Control	10	7513.0	121.0	292.4	0.99	2.4E-15	6.9E-15	0.39	3.6
		Treated	21	2532.0	159.0	45.56					

AST - aspartate aminotransferase; ALT - alanine aminotransferase; CK - creatine kinase; ALP - alkaline phosphatase; ACP - acid phosphatase; AChE=activities of enzyme acetylcholinesterase
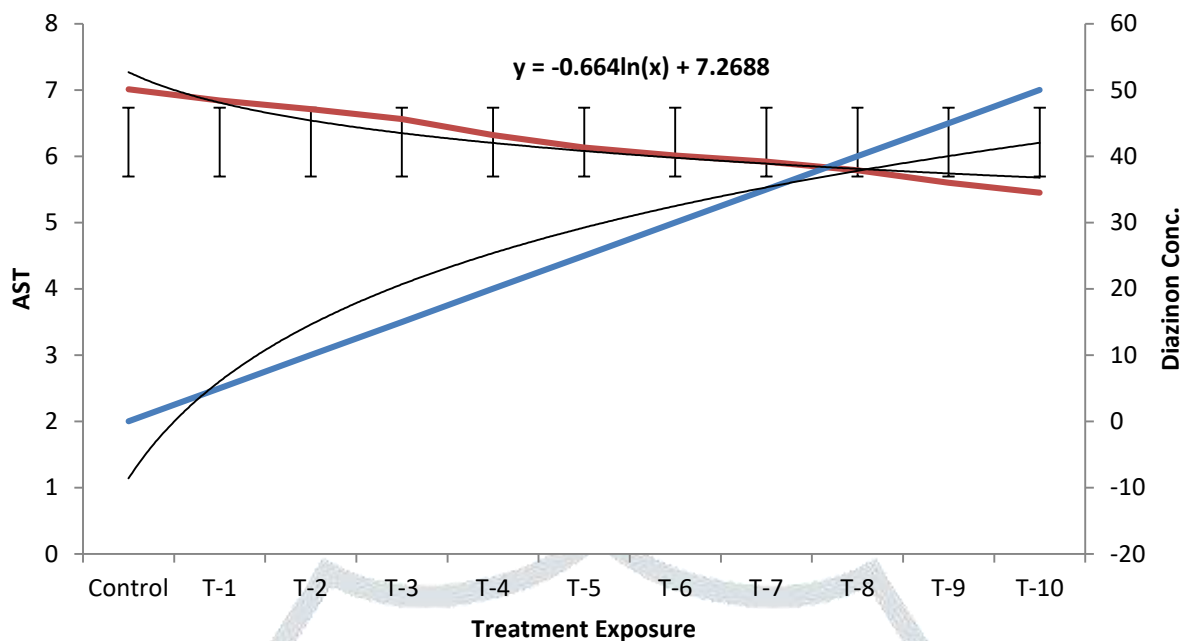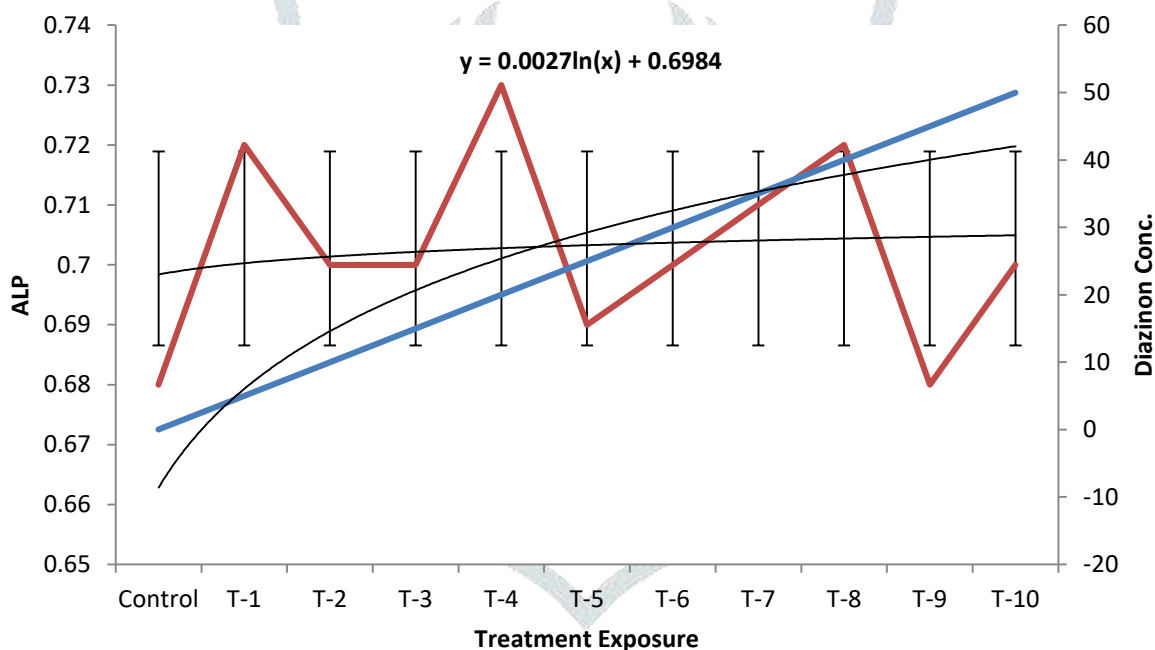
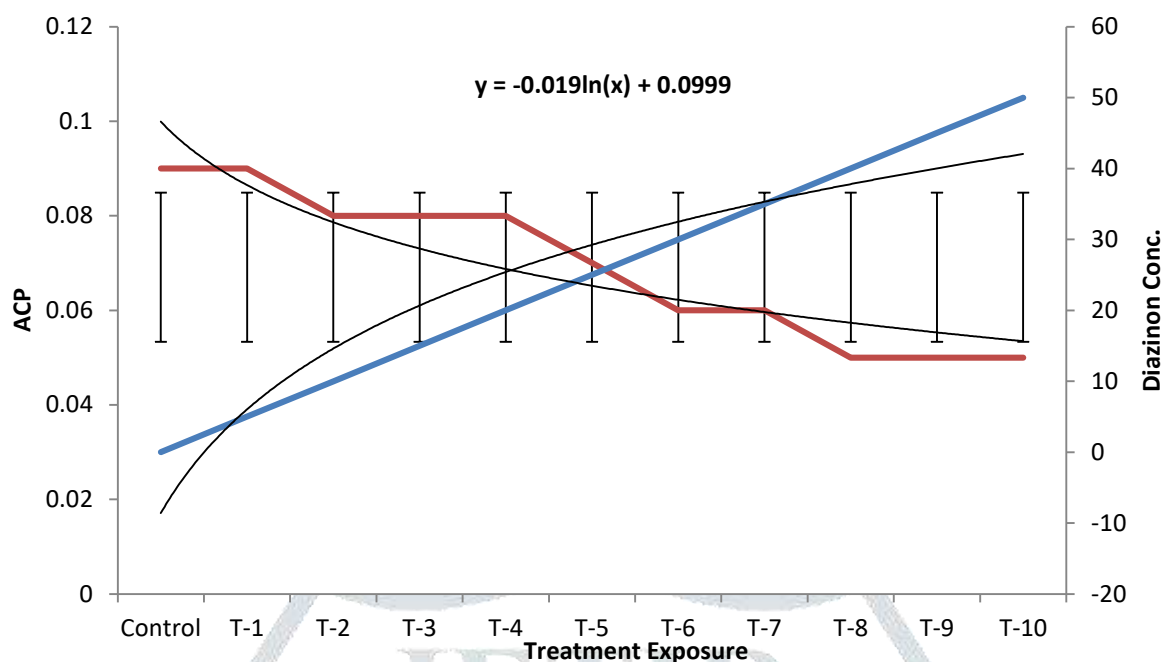**Graph 01: Effect of various concentrations of Diazinon on AST in *Channa striatus* (Bloch)**

$y = -0.664\ln(x) + 7.2688$
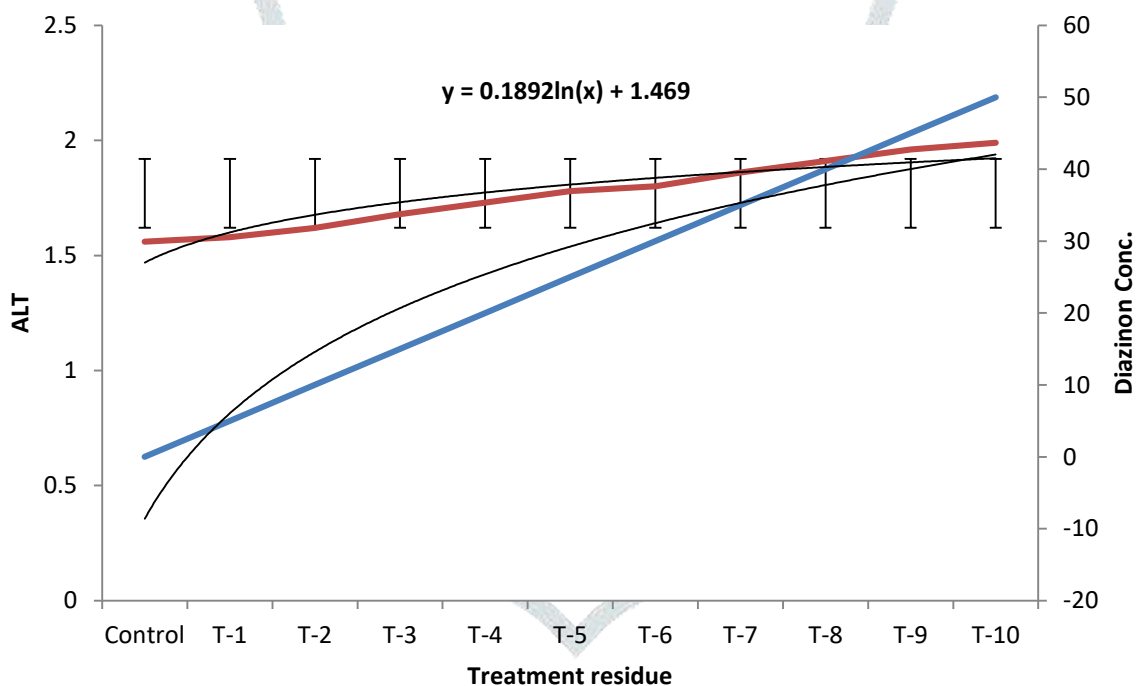
Graph 02: Effect of various concentrations of Diazinon on ALT in *Channa striatus* (Bloch)

$y = 0.1892\ln(x) + 1.469$

$$y = -24.13\ln(x) + 655.73$$
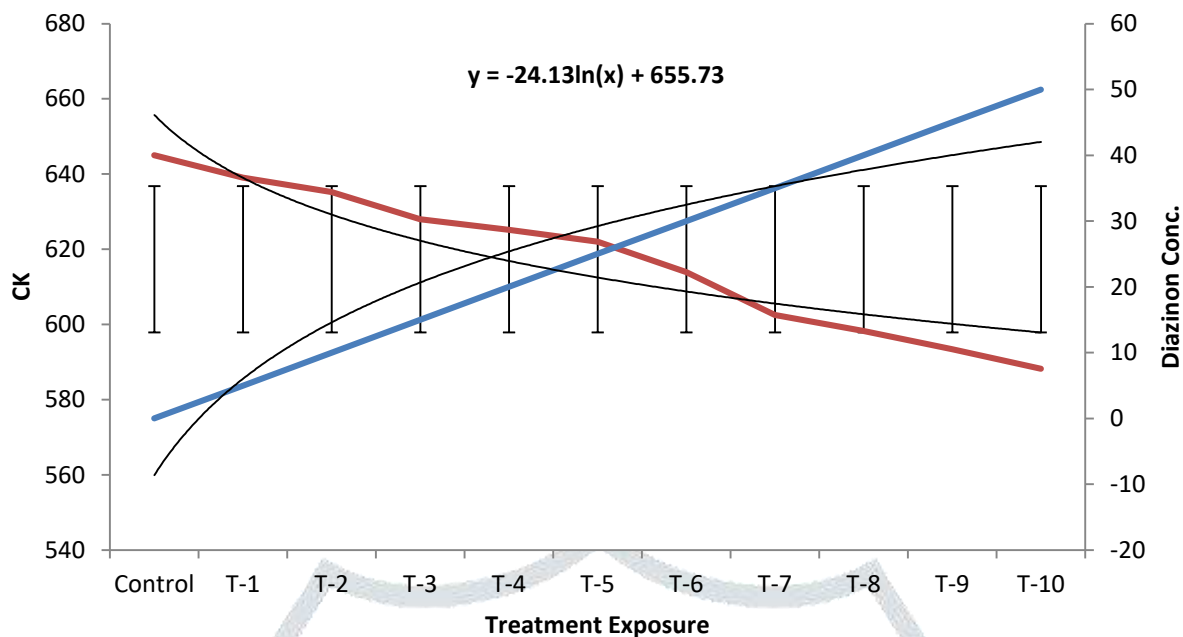
Graph 03: Effect of various concentrations of Diazinon on CK in *Channa striatus* (Bloch)

$$y = 0.0027\ln(x) + 0.6984$$

Graph 04: Effect of various concentrations of Diazinon on ALP in *Channa striatus* (Bloch)

$$y = -0.019\ln(x) + 0.0999$$

Graph 05: Effect of various concentrations of Diazinon on ACP in *Channa striatus* (Bloch)

$$y = -119.7\ln(x) + 588.28$$
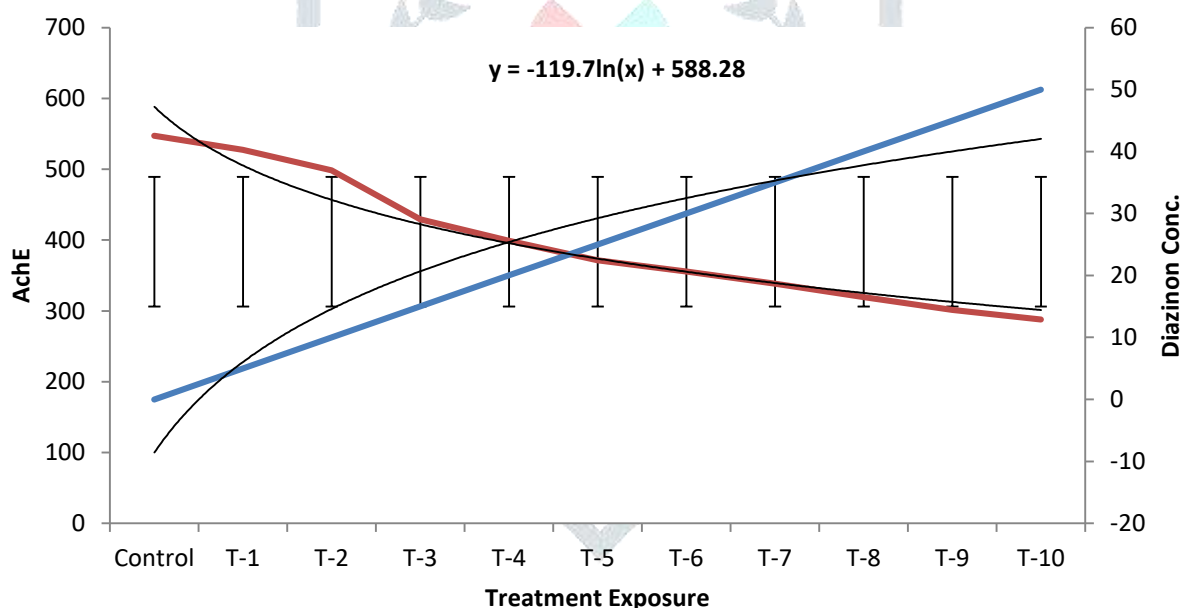
Graph 06: Effect of various concentrations of Diazinon on AchE in *Channa striatus* (Bloch)

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