



Effects of alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch)

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Abstract: The fresh water fish *Channa punctatus* (Bloch) were exposed to lethal and sublethal concentrations of a chloroacetanilide herbicide Alachlor and its commercial formulation Lasso 50% Emulsifiable Concentrate EC to study the impacts on some biochemical parameters - the energy dependent sources: such as glycogen, total proteins and metabolic enzymes: Aspartate Amino Transferase (AAT), Alanine Amino Transferase (ALAT), Lactate Dehydrogenase (LDH), Deoxyribonucleic acid (DNA) and Ribonucleic Acid (RNA). The glycogen, total proteins, DNA, RNA were all decreased but the activity of the enzymes AAT, ALAT and LDH were all increased which is due to the toxic stress. The percentage decrease being more pronounced at lethal concentrations than at sublethal concentrations.

Key words: Alachlor, Lasso 50% EC, Biochemical parameters.

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Introduction

Alachlor is an acetanilide herbicide is used to control annual grasses and weeds in fields like corn, soyabeans and peanuts. It is a selective systemic herbicide absorbed by germinating plants and by roots. Being anthropogenic, the acetanilide herbicides are transported into aquatic environment and apparently interfere with several physiological processes including biosynthesis of lipids, proteins and flavonoids (Lee *et al.*, 2004; Tilak *et al.*, 2007; Peebua *et al.*, 2008).

These herbicides are widely used in agriculture and are commonly detected in surface water and ground water and have relatively low acute toxicity. However, repeated exposure has been reported to cause hepato-toxicity, irreversible degeneration and tumour formation in some animals (USEPA, 1997). Besides these, it is strongly suspected endocrine disrupting chemical by the USEPA (2002). Many mammalian and aquatic toxicological studies with alachlor were performed under the conditions of acute, subacute and chronic exposures to the non-target organisms. However, not many studies using fish have been carried out (Yi *et al.*, 2007). Therefore in the present study, an attempt has been made to explore the effect of alachlor technical grade and its commercial formulation lasso 50% EC on the biochemical parameters of the fresh water fish, *Channa punctatus* (Bloch) which is an available edible fish in the local area.

Materials and Methods

The test fishes *Channa punctatus* (Bloch) were brought from a fish farms at Nandivelugu. The size of the experimental

fishes was 6 to 9 cm in length and 6.5 to 7.5 g in weight. The fish were acclimatized at room temperature (28±2°C) in the laboratory conditions for 10 days. A batch of ten fishes was exposed for 10 days to sublethal and lethal concentrations of alachlor technical and lasso 50% EC purchased from Rallis India Limited, Bangalore. The sublethal (1/5th of static 96 hr LC₅₀) concentration is 1.2064 mg l⁻¹ for alachlor technical grade and 1.5386 mg l⁻¹ for lasso 50% EC formulation. The lethal (96 hr LC₅₀) concentration is 6.032 mg l⁻¹ for alachlor technical grade and 7.693 mg l⁻¹ for lasso 50% EC formulation. The batch of fish with out toxicant served as controls (APHA, 1998). Then tissues like gill, liver, kidney, brain and muscle were taken out from the control and exposed fish after the exposure period for the estimation of glycogen, total proteins, lactate dehydrogenase (LDH), aspartate aminotransferase (AAT), alanine aminotransferase (ALAT), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The total glycogen was estimated by the method of Kemp *et al.* (1954). The total protein content was estimated by the modified method of Lowry *et al.* (1951). The lactate dehydrogenase activity was estimated by the method of Srikanthan and Krishnamurthi (1955) as modified by Govindappa and Swami (1965). The activity of aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT) was determined by the method of Reitman and Frankel (1957) as described by Berg Meyer and Bernt (1965). The DNA and RNA were estimated by the methods of Searchy and MacLnnis (1970 a, b).

Statistical analysis: Values were expressed as mean + SE (n = 5). For statistical analysis of the data analysis of



Table - 1: The amount of glycogen content (mg g⁻¹) in the tissues of the fish, *Channa punctatus* (Bloch) exposed to alachlor technical and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	22.45 ± 0.75	10.88 ± 0.31 (-48.46)**	14.26 ± 0.00 (-36.49)*	4.28 ± 0.00 (-80.94)**	5.14 ± 0.04 (-77.11)**
Liver	82.88 ± 0.05	35.06 ± 0.00 (-33.57)**	60.54 ± 0.04 (-29.96)*	30.26 ± 0.04 (-63.49)*	56.54 ± 0.01 (-31.79)*
Kidney	20.16 ± 0.13	10.26 ± 0.00 (-49.11)*	11.42 ± 0.07 (-43.76)**	2.82 ± 0.31 (-86.02)**	3.72 ± 0.07 (-81.55)**
Brain	42.78 ± 0.08	26.42 ± 0.07 (-38.25)*	28.18 ± 0.01 (-34.15)*	10.99 ± 0.14 (-74.32)**	12.68 ± 0.08 (-70.36)**
Muscle	36.92 ± 0.02	22.16 ± 0.31 (-39.97)*	27.16 ± 0.08 (-26.44)*	16.96 ± 0.07 (-54.06)*	17.24 ± 0.04 (-53.31)*

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

Table - 2: The amount of total protein (mg g⁻¹) in the tissues of the fish *Channa punctatus* (Bloch) exposed to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	71.28 ± 0.11	60.04 ± 0.04 (-15.97)*	63.04 ± 0.00 (-11.57)*	54.48 ± 0.05 (-23.57)*	55.14 ± 0.04 (-22.64)*
Liver	84.40 ± 0.10	60.64 ± 0.78 (-28.15)*	61.60 ± 0.04 (-27.08)*	50.40 ± 0.04 (-40.30)**	51.68 ± 0.00 (-38.87)*
Kidney	75.24 ± 0.07	59.30 ± 0.02 (-21.19)*	60.30 ± 0.08 (-19.86)*	51.12 ± 0.06 (-32.06)*	52.12 ± 0.02 (-30.73)*
Brain	77.78 ± 0.42	59.10 ± 0.10 (-36.04)*	55.20 ± 0.03 (-29.12)**	38.28 ± 0.02 (-50.85)**	40.60 ± 0.07 (-47.87)**
Muscle	92.40 ± 0.14	55.32 ± 0.04 (-40.13)**	56.76 ± 0.02 (-38.58)**	40.74 ± 0.00 (-55.91)**	41.76 ± 0.04 (-54.81)**

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

Table - 3: Lactate dehydrogenase (LDH) levels (mg of lactic acid g⁻¹ wet weight of tissue) in the different tissues of *Channa punctatus* (Bloch) on exposure to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	26.52 ± 0.1019	30.52 ± 0.1732 (+15.08)*	27.52 ± 0.0748 (+3.770)*	37.70 ± 0.2738 (+42.15)*	28.95 ± 0.1019 (9.162)*
Liver	5.36 ± 0.0748	10.06 ± 0.1496 (+87.68)*	11.04 ± 0.1469 (+105.97)**	12.96 ± 0.1414 (+141.79)**	13.26 ± 0.1414 (+147.38)*
Kidney	67.17 ± 0.1019	67.25 ± 0.1224 (+0.1191)	67.56 ± 0.1140 (+0.580)	67.66 ± 0.1414 (+0.7294)	67.74 ± 0.1116 (+0.848)
Brain	26.94 ± 0.6480	27.96 ± 0.1732 (+3.786)*	27.94 ± 0.1414 (+3.711)*	28.84 ± 0.1224 (+7.052)*	28.52 ± 0.1326 (+5.86)*
Muscle	51.16 ± 0.1166	73.44 ± 0.1414 (+43.54)**	73.80 ± 0.1019 (+44.25)**	75.45 ± 0.1166 (+47.47)**	75.65 ± 0.1183 (+47.86)**

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

Table - 4: Aspartate aminotransferase activity (AAT) (μ moles of pyruvate formed mg protein⁻¹ hr⁻¹) in the tissues of *Channa punctatus* (Bloch) exposed to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	72.16 ± 0.31	126.16 ± 0.76 (+74.83)**	130.92 ± 0.32 (+81.43)**	146.45 ± 0.70 (+102.95)**	148.28 ± 0.77 (+105.48)**
Liver	206.45 ± 0.9	285.48 ± 0.18 (+38.28)*	226.78 ± 0.12 (+19.84)*	340.27 ± 0.37 (+64.81)**	349.28 ± 0.10 (+69.18)*
Kidney	148.28 ± 0.77	179.27 ± 0.21 (+20.81)*	182.92 ± 0.78 (+23.36)*	285.03 ± 0.63 (+92.22)**	298.04 ± 0.88 (+100.99)**
Brain	133.40 ± 0.02	156.71 ± 0.63 (+17.47)*	158.88 ± 0.77 (+25.48)*	199.45 ± 0.64 (+66.05)**	201.48 ± 0.41 (+51.03)*
Muscle	59.02 ± 0.82	86.77 ± 0.31 (+47.01)**	88.72 ± 0.42 (+50.32)**	110.00 ± 0.36 (+50.98)*	109.71 ± 0.44 (+85.88)**

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**



Table - 5: Alanine aminotransferase (ALAT) activity (μ moles of pyruvate formed protein⁻¹hr⁻¹) in the tissues of *Channa punctatus* (Bloch) exposed to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	62.30 ± 0.04	83.23 ± 0.11(+ 33.59)*	82.60 ± 0.22 (+ 32.58)*	122.62 ± 0.38 (+ 96.82)**	124.42 ± 0.66 (+ 99.71)**
Liver	217.97 ± 0.16	286.00 ± 0.16(+ 31.21)*	280.40 ± 0.77 (+ 28.64)*	420.00 ± 0.82 (+ 92.68)**	423.72 ± 0.44 (+ 94.39)**
Kidney	198.50 ± 0.18	217.97 ± 0.16(+ 4.94)	221.86 ± 0.88 (+ 11.76)*	315.73 ± 0.17 (+ 59.05)*	312.40 ± 0.11 (+ 57.38)*
Brain	71.33 ± 0.13	94.32 ± 0.48(+ 32.20)*	101.40 ± 0.02 (+ 42.20)*	142.00 ± 0.35 (+ 99.5)**	139.02 ± 0.32 (+ 94.8)**
Muscle	52.75 ± 0.01	98.23 ± 0.42(+ 86.21)**	86.44 ± 0.33 (+ 63.86)*	110.00 ± 0.36 (+ 108.53)**	100.44 ± 0.08 (+ 90.40)**

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

Table - 6: DNA (mg g⁻¹ wet weight of tissue) of the fish *Channa punctatus* (Bloch) exposed to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	520.88 ± 0.1414	370.88 ± 0.6151 (-28.76)*	380.44 ± 0.6149 (-26.96)*	295.48 ± 0.6084 (-43.27)*	299.36 ± 0.5994 (-42.52)*
Liver	505.83 ± 0.9921	387.81 ± 0.6016 (-23.33)**	394.20 ± 0.2817 (-22.06)*	306.44 ± 0.1428 (-39.41)**	312.98 ± 0.6044 (-38.12)*
Kidney	57.32 ± 0.6009	294.36 ± 0.6151 (-61.13)**	262.30 ± 0.2134 (-65.32)**	285.02 ± 0.6162 (-62.36)*	252.27 ± 0.6023 (-66.68)**
Brain	330.48 ± 0.5836	314.93 ± 0.9825 (-4.70)	295.48 ± 0.6030 (-10.59)*	272.61 ± 0.2348 (-17.51)*	280.36 ± 0.6076 (-15.16)*
Muscle	140.59 ± 0.6113	92.56 ± 0.5870 (-34.16)*	86.98 ± 0.6044 (-38.13)*	78.26 ± 0.2678 (-44.38)*	72.46 ± 0.3847 (-48.46)*

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

Table - 7: RNA (mg g⁻¹ wet weight of tissue) of fish *Channa punctatus* (Bloch) exposed to alachlor technical grade and lasso 50% EC

Tissue	Control	Sub-lethal		Lethal	
		Alachlor TG	Lasso 50% EC	Alachlor TG	Lasso 50% EC
Gill	120.36 ± 0.5904	102.46 ± 0.2643 (-14.87)*	110.32 ± 0.4764 (-8.34)	86.38 ± 0.2430 (-28.23)*	80.46 ± 0.6978 (-33.15)*
Liver	140.62 ± 0.6019	122.84 ± 0.6184 (-12.64)*	119.36 ± 0.6076 (-15.11)*	79.42 ± 0.5911 (-43.52)**	73.22 ± 0.1446 (-47.93)**
Kidney	154.02 ± 0.6140	136.42 ± 0.4284 (-11.42)*	142.36 ± 0.2673 (-7.57)	98.02 ± 0.4839 (-36.35)*	87.36 ± 0.3486 (-43.28)*
Brain	98.36 ± 0.2846	66.36 ± 0.2486 (-32.53)*	64.82 ± 0.2830 (-34.09)*	51.91 ± 0.6145 (-47.22)**	48.43 ± 0.5298 (-50.76)**
Muscle	117.48 ± 0.6846	94.24 ± 0.6060 (-19.78)*	91.36 ± 0.5909 (-22.23)*	74.14 ± 0.6184 (-36.89)*	69.52 ± 0.6065 (-40.82)**

Each value is mean of 5 individual observations, (±) indicates standard error (SE), Values in the brackets indicate percent decrease over control values are significant at p<0.05*, p<0.01**

variance and Student's 't' test was employed (Bruning and Kintz, 1977).

Results and Discussion

There was no mortality at sublethal concentrations but at lethal concentrations the mortality was 30%. The calculated values along with standard deviation and per cent change over control for various biochemical parameters like glycogen, proteins, LDH, AAT, ALAT, DNA and RNA are given in Table 1 to 7.

Glycogen, proteins, DNA and RNA content of various tissues were found to be decreased whereas the activity of different enzymes like LDH, AAT and ALAT were found to be increased compared to that of the controls. The percentage change being greater at lethal concentration than at sublethal concentration.

According to Sastry and Shukla (1990), the muscle glycogen content was decrease due to cadmium toxicity on *Channa punctatus*. Similar findings were reported by Srinivasula Reddy and Bhagya Lakshmi (1994), Kaviraj and Das (1994). Sapna *et al.* (2002) reported a significant decrease in the brain glucose levels of *Heteropneustes fossilis*, exposed to sublethal concentration of carbaryl (0.04 ppm) for a period of one month and the glucose content was decreased from 67.3 to 80.8 for a period of 30 days. Geeta Bhaskaran (1996) studied the effects of starvation on biochemical constituents in *Anabas testudineus*, after 11, 40, 80, 120 and 300 days. Hepatic lipids showed 10-56% decrease and serum glucose exhibited 5-46% depletion between 11-300 days. The fish utilized carbohydrates and proteins mostly between 120-300 days of starvation.



Bhamre *et al.* (2001) reported that the average glycogen content of the whole body decreased significantly in the muscle of *Parreysia favidens* when exposed to mercuric chloride (3.24 mg^l⁻¹) for 72 hr. Glycogen depletion is more prevalent under hypoxic conditions and it is quite likely that a situation similar to hypoxia might be occurring in the tissues of fish exposed to alachlor technical and lasso 50% EC formulation. Further, lowered whole animal oxygen consumption may stimulate phosphorylase activity bringing about a drop in glycogen level. In general, glycogen depletion is due to stress and strain and the reserves being used as substitution of metabolic requirement (Tilak *et al.*, 2005). Total depletion of glycogen would result in the disruption of enzymes associated with carbohydrate metabolism (Tilak *et al.*, 2001a).

A significant decrease in protein was observed in all the tissues under lethal and sublethal concentrations of both the technical grade of alachlor and lasso 50% EC formulation over the controls. The variation in distribution suggests difference in metabolic calibers of various tissues. Pandi Bhaskaran (1991) reported depletion in the protein content in muscle and liver of *Tilapia mossambica*, *Mystus vittatus* and *Channa striatus* exposed to fenvalerate. Jeba Kumar *et al.* (1990) reported decrease in protein content of *Lipidocephalichthys thermalis* exposed to sublethal concentration of fenvalerate. Tilak *et al.* (2003) reported a decrease in protein content in *Channa punctatus* exposed to sublethal concentration of fenvalerate. The similar decreasing trend in total proteins was also reported in the liver, brain and gill tissues of *Catla catla* under sublethal and lethal concentrations of fenvalerate by Anita Susan *et al.* (1999). A significant decrease was reported in the protein content in almost all tissues in *Ctenopharyngodon idellus* (Valenciennes) by Tilak and Yacobu (2002). Tilak *et al.* (2001b) reported that when the fresh water fish, *Labeo rohita* (Hamilton) was exposed to sublethal concentrations of pesticide mixture of monocrotophos and fenvalerate (1:4), the protein content was decreased.

Gill is an important organ because of its direct contact with water, which allows the pesticide to enter through it and get accumulated in the body of the fish. It has been suggested that water borne pollutants damage the fish gill, presumably by causing breakdown of the gas exchange mechanism with consequent tissue hypoxic conditions. According to Tilak and Yacobu (2002), the glycogen and protein values are significantly decreased due to fenvalerate exposure in the fish *Ctenopharyngodon idellus*. It suggests that fenvalerate alters the process of protein metabolism by decreasing protein content due to the process of proteolysis.

Lee *et al.* (2004) expressed that the alachlor apparently interferes with physiological processes including biosynthesis of lipids, proteins and flavanoids. It also causes hepato-toxicity, tumour

formation in animals. Similar observations were also reported by USEPA (1986).

The LDH activity in all the tissues of *Channa punctatus* (Bloch) showed a very significant increase over the control. The increasing trend was more significant in lethal concentrations than in sublethal concentrations.

Tilak *et al.* (2003) reported a decrease in pyruvate levels and increase in lactate levels in *Channa punctatus* (Bloch) exposed to sublethal and lethal concentrations of fenvalerate. This can be attributed to toxic stress resulting in the inhibition of pyruvate oxidation under hypoxic conditions which indicates the shifting of aerobic respiration to anaerobic respiration. LDH levels are increased in the tissues due to stress when exposed to toxicant.

In the present study, increase in lactate levels can be attributed to toxic stress resulting in the inhibition of pyruvate oxidation under hypoxic conditions which indicates the shifting of aerobic respiration to anaerobic respiration.

The increased trend in both AAT and ALAT activities indicates that there is more conversion of aminoacids into ketoacids than that utilized for energy synthesis (Siva Prasada Rao and Ramana Rao, 1984; Samuel and Sastry, 1989; Malla Reddy *et al.*, 1991; Tilak *et al.*, 2003).

The present work revealed that the variations in biochemical parameters serve as an indication in monitoring the pathological status of the pesticide treated fish. Transaminase activity is reported to increase in serum during pathological conditions (Latner, 1975). These variations were tissue specific and species specific, hence can be used as meaningful indicators of pesticides pollution. Such differential behaviour with regard to tissues can be further examined to develop a more meaningful indicators or markers to assess or to characterize the particular pollutant and its potential toxicity to fish.

Deoxyribonucleic acid (DNA): A decrease in the nucleic acid content was also observed in all the tissues that are exposed to toxicants. Nucleic acids have an important role in all biological activities and also regulate the biological synthesis of proteins which are structural and functional. Any alteration in nucleic acid content leads to variations in protein profile (Durai Raj and Selvarajan, 1992; Abou Donia *et al.*, 1988).

Gautam *et al.* (2002) reported the histo-chemical observations on nucleic acids (RNA and DNA) in the stomach and intestine of *Channa punctatus* (Bloch) after the treatment with endosulfan and diazinon pesticides. A significant decrease in nucleic acids of gastrointestinal tract was reported. However, the decrease in nucleic acids content after diazinon treatment

was not significant. The treatment with endosulfan in mucosa and submucosal tissues show very little impact on nucleic acid content. However, in diazinon treatment the DNA was completely decreased.

The nucleic acids play a major role in all biological activities and are regulators of all biological synthesis. All the enzyme activities are controlled by the process of transcription. When the transcription process is curtailed, no mRNA and no protein synthesis occur. As a result, metabolism is impaired. The effect of fenvalerate on DNA content of the gill but not on DNA content of kidney may be due to efficient uptake of the toxicant across the gill. This tissue specific difference may be further assigned to the differential effects of fenvalerate or its metabolites on the synthesis or degradation of DNA in gill and kidney cells of the fish (Bradbury *et al.*, 1987).

In conclusion, the present work indicates that alachlor causes considerable changes in the intermediary metabolism of the fish, *Channa punctatus*. The cause for these alterations appears to be the result of high energy demands. The liver showed a metabolic profile consistent with this demand, with partial depletion of carbohydrate reserves and probable protein consumption. In the aquaculture context, it should be pointed out that consumption of muscle protein as energy source can reduce productivity even in the absence of mortality.

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