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Specific activity levels of Proteases, Aminases and GDH in freshwater fish, *Channa punctatus* (Bloach) on exposure to Imidacloprid

V Anil Kumar

Department of Zoology, Kakatiya Government College, Hanamkonda, Telangana State, India
E-mail: anilveludandi@yahoo.com

ABSTRACT

The decreased protein content and enhanced free amino acids level might suggest that the reduced synthesis or increased proteolytic activity or at times both can exist in pesticides exposed fish. The elevation of neutral proteases may indicate the damage caused to the tissues due to impairment of energy supply. The elevated alkaline protease activity may indicate higher protein degradation, since alkaline protease prefer to act on structural proteins of tissues. Therefore, the proteins are denatured leading to more activation of proteases. The activities of AAT and ALAT are enhanced with the following toxic exposure of OC compound resulted in incorporation of amino acid into TCA cycle for energy production. This is further confirmed by the enhanced levels of GDH which is responsible for the incorporation of amino acids into TCA cycle. The overall decrease in protein content and enhanced levels of amino acids transaminases and GDH might suggest the utilization of proteins under stress conditions of the fish.

Keywords: *Chann Punctatus* , proteases, Aminases, GDH, Imidacloprid

INTRODUCTION

The indiscriminate and extensive use of insecticides to protect crops poses a serious threat to humans and the surrounding environment. The pesticides which are liberated into aquatic environment have a deterious effect on fish and subsequently to man. Kilgore and Mingyuli (1975) emphasized that the concentration of pesticide residues was found to be more in aquatic ecosystem rather than the terrestrial ecosystem.

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The freshwater fishes constitute an important link in food chain and their pollution by insecticides unbalances the aquatic ecosystem Imidacloprid is an organo Chloride compound being widely used in India to control agricultural pests. However, OC compounds not only affect the respiratory system but also interfere with the cellular level respiration (Betoulle et al, 2000). The sublethal exposure of OC compounds have produced several changes in energy metabolism of fish. The freshwater teleost Channa Punctatus is an edible fish and is considered to be economically important in pisiculture industry. The present study was under taken to identify the different shifts involved in metabolism in liver, brain, gill, muscle and kidney tissues of the insecticide exposed fish.

MATERIALS AND METHODS

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Freshwater fish *Channa Punctatus* is a edible fish weighing average of 82-120gms and 25.5 + 1.21cm in length, here procured from a local market, Warangal

Table-1: Activities of proteases, Aminases and GDH in tissue of control and Imidacloprid exposed fish *Channa Punctuatus* (Bloach)

Parameters	Tissue	Control	Imidacloprid			
			24 Hrs	48 Hrs	72 Hrs	96 Hrs
Alkaline Protease	LIVER	1.36±0.15	1.39*±0.16 PC=2.20	1.47*±0.31 PC=8.08	1.51*±0.39 PC=11.02	1.57*±0.29 PC=15.44
	BRAIN	1.21±0.50	1.27*±0.36 PC=4.95	1.31*±0.14 PC=8.26	1.42±0.56 PC=17.35	1.53±0.66 PC=26.44
	GILL	1.29±0.68	1.34*±0.45 PC=3.87	1.39*±0.019 PC=7.75	1.44*±0.21 PC=11.62	1.57±0.32 PC=21.70
	MUSCLE	1.24±0.10	1.29*±0.46 PC=4.03	1.39*±0.019 PC=11.29	1.42*±0.017 PC=14.51	1.48±0.63 PC=19.35
	KIDNEY	1.08±0.10	1.16*±0.32 PC=7.40	1.24*±0.14 PC=14.81	1.29±0.61 PC=19.44	1.34±0.41 PC=24.07
Neutral Proteases	LIVER	1.36±0.14	1.49*±0.65 PC=9.55	1.69±0.63 PC=4.26	1.82±0.039 PC=33.82	0.01±0.20 PC=47.79
	BRAIN	1.23±0.33	1.29*±0.011 PC=4.87	1.37*±0.023 PC=11.38	1.42*±0.019 PC=15.44	1.53±0.33 PC=24.39
	GILL	1.29±0.11	1.36*±0.076 PC=5.42	1.42*±0.020 PC=10.07	1.68±0.065 PC=30.23	1.79±0.32 PC=38.75
	MUSCLE	1.32±0.29	1.39*±0.080 PC=13.63	1.41*±0.65 PC=6.81	1.49*±0.023 PC=12.87	1.63±0.66 PC=23.48
	KIDNEY	0.96±0.01	1.20±0.30 PC=25	1.29±0.051 PC=34.37	1.31±0.15 PC=36.45	1.42±0.14 PC=47.91
AAT	LIVER	3.04±0.54	3.19*±0.29 PC=4.934	3.27*±0.52 PC=7.565	3.39*±0.46 PC=11.513	3.48*±0.46 PC=14.47
	BRAIN	2.04±0.62	2.18*±0.13 PC=6.862	2.24*±0.63 PC=4.805	2.38±0.72 PC=16.66	2.46±0.84 PC=20.54
	GILL	2.18±0.66	2.24*±0.26 PC=2.752	2.32±0.63 PC=6.422	2.49±0.28 PC=27.32	2.42±0.43 PC=20.64
	MUSCLE	1.72±0.29	1.93*±0.68 PC=12.209	2.06±0.48 PC=19.76	2.19±0.28 PC=27.32	2.42±0.43 PC=40.69
	KIDNEY	1.09±0.14	1.42±0.24 PC=30.27	1.68±0.17 PC=54.12	1.85±0.66 PC=69.72	1.95±0.40 PC=78.89
ALAT	LIVER	3.04±0.54	3.19*±0.29 PC=4.934	3.27*±0.52 PC=7.565	3.39*±0.46 PC=11.513	3.48*±0.46 PC=14.47
	BRAIN	2.04±0.62	2.18*±0.13 PC=6.862	2.24*±0.63 PC=4.805	2.38±0.72 PC=16.66	2.46±0.84 PC=20.54
	GILL	2.18±0.66	2.24*±0.26 PC=2.752	2.32*±0.63 PC=6.422	2.49±0.28 PC=27.32	2.42±0.43 PC=20.64
	MUSCLE	1.72±0.29	1.93*±0.65 PC=12.209	2.06±0.48 PC=19.76	2.19±0.28 PC=27.32	2.42±0.43 PC=40.69
	KIDNEY	1.09±0.14	1.42±0.24 PC=30.27	1.68±0.17 PC=54.12	1.85±0.66 PC=69.72	1.95±0.40 PC=78.89
GDH	LIVER	0.37±0.01	0.41*±0.03 PC=10.81	0.47±0.015 PC=27.02	0.52±0.06 PC=40.54	0.58±0.037 PC=56.75
	BRAIN	0.28±0.016	0.32*±0.013 PC=14.28	0.39±0.015 PC=39.28	0.47±0.021 PC=67.85	0.53±0.025 PC=89.28
	GILL	0.19±0.018	0.24±0.015 PC=42.10	0.27±0.041 PC=42.10	0.31±0.021 PC=40.90	0.39±0.024 PC=105.26
	MUSCLE	0.22±0.013	0.24±0.032 PC=19.09	0.29±0.025 PC=31.81	0.31±0.021 PC=40.90	0.35±0.017 PC=59.09
	KIDNEY	0.15±0.010	0.17*±0.019 PC=13.33	0.24±0.0361 PC=60	0.27±0.014 PC=80	0.31±0.012 PC=106.66

Each value is mean ± S.D of Six individual observations. All value are statistically significant from control at 1% level ($P<0.01$). PC denotes percent change over control not significant.

(A.P.). The collected fish were kept in a cement tank 180 x 90 x 90 cm (6x3x3 feet) at least for one month for acclimatization under laboratory conditions to continuous water flow. The average temperature of water was 22 - 24^{0}C. The fish were fed *ad libitum* with ground nut – cake along with the commercial pellets (1 – 1.5% body weight). They were starved one day before experiment (Butler worth 1972). Without discrimination of Sexes, both the Sexes of fish were used for the experiment. The LC 50 of commercial grade imidacloprid (0.58 ppm) was determined for 48 hours by the method of Bayna et al (1977). Batches of six (6) fish were exposed to 24, 48, 72 and 96 hours for sublethal concentration (0.19 ppm) along with control fish in separate tanks consisting six liters of water, at the room temperature.

After removing the fish at stipulated time interval liver brain, mucle gill and kidney were quickly isolated and kept in ice – jacked petri dishes for biochemical estimations.

The total protein content was determined by Folin Phenol method (Lowry et al: 1951) free amino acids by ninhydrin method (More and stein 1954) ammonia by nesslerization (Bergmeyer 1965) glutamine by acid hydrolysis method described by Colowick and kalpan (1967). The different rest conditions of the enzyme assays are given in Table-1.

For assaying neutral and alkaline proteases, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. A clear cell free supernatant was used for the away of proteases by the method of Davis and Smith (1955). Neutral protease activity was assayed at PH-7.0 using phosphate buffer and alkaline protease activity at PH 9.0 with carbonate bicarbonate buffer and 10mg of denatured hemoglobin protein was used as substrate. The assay glutamate dehydrogenase (GDH), 10% tissue homogenates were prepared in ice cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. A clear cell free supernatant was used for the assay of GDH by the method of Lee and Lardy (1965).

In addition to substrate, buffer and enzyme, 0.1 micromoles of NAD+ and 2 micromoles of INT were added to the reaction mixture.

For assaying AAT and ALAT activity by the method of Reitman and Frankel (1957) 10% tissue homogenates were prepared in cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes the supernatant was used for the enzyme source.

RESULTS AND DISCUSSION

Table 1 shows the enzyme activities of neutral and alkaline proteases, glutamate dehydrogenase, AAT and ALAT which were found to be increased in liver, brain, gill, muscle and kidney tissues of the fish exposed to imidacloprid for 24, 48, 72 and 96 hours. The increased activity of neutral and alkaline proteases indicates increased protein degradation to yield excess energy to overcome the toxic impact. The enhancement in the activity of GDH due to imidacloprid toxicity was observed in liver, brain, gill, muscle and kidney tissues. This indicates higher oxidation of amino acids to combat the toxic effect of OC compound. The higher activity of GDH may result in efficient operation of oxidative deamination under toxic impact of imidacloprid. The activities of AAT and ALAT are enhanced with the following toxic exposure of OC compound resulted in incorporation of amino acids into TCA cycle for energy production. This is further confirmed by the enhanced levels of GDH which is responsible for the incorporation of amino acids into TCA cycle. The overall decrease in protein content and enhanced levels of amino acids transminases and GDH might suggest the utilization of proteins under stress conditions of the fish.

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Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Betoulle, C.; Duchiron, C., De Schaux, P., 2000. Lindane increase invitro respiratory burst activity and intracellular calcium levels in rainbow trout (oncorhynchus mykiss) head kidney phagocytes. J. Aquatic. Toxicology 48:211-221.

[2] Butlerworth, K.L., 1972. River pollution: cause and effects Academic Press, New York.

[3] Bayne, B.L.; Widdows, L.; Worral, C., 1977. Physiological responses of marine biota to pollutants J. Academic press, New York.
[4] Bergmeyer, H.U. 1965. Methods of Enzymatic analysis, academic press, New York, P 837.
[5] Colowick S.P. and Kaplan, N.O. 1967. Determination of amide residues by chemical methods in Hirs CHW (ed) Methods in enzymology Vol.2 Academic press, New York, pp 63-65.
[6] Davis, N.C. and Smith, E.L. 1955. Methods of biochemical analysis Glick D(ed) Interscience publishers, New York, p 247.
[7] Kilgore W.W. and Mingguli 1975. Environmental toxicology in insecticide biochemistry and physiology plenum press, New York p 669.
[8] Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.T. 1951. Protein measurement with the folin phenol reagent. J.Biol. Chem., 193: 265-275.
[9] Lee, Y.L., and Lardy, H.A., 1965. Influence of thyroid hormones on L. Glycerophosphate dehydrogenase in various organs of the rat. J. Biol. Chem. 240: 1427.
[10] Moore, S., and Stein, W.H., 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem 211: 907-913.
[11] Reitmam. S., and Frankel, S., 1957. Colorimetric method for the determination of Serum glutamic oxylaectic and glutamic pyruvic transaminases. J. Am. J.Clin. Pathol. 28: 56-63.
[12] Sastry K.V. and Sarma S.K. 1978. The effect of in vivo exposure of endrin on the activities of acid, alkaline and glucose-6 – phasphatases in liver and kidney of ophiocephalus punctatus Bull. Environ contam Toxicol., 20: 456.