ORIGINAL RESEARCH PAPER

Zoology

BIOCHEMICAL STUDY OF CHANNA GACHUA IN TISSUE LIKE LIVER AND GONAD AFTER TOXIC EXPOSURE

KEY WORDS: Confidor, Bavistin, Acute Toxicity, Cholesterol

Savita Y. Waghmare

P.G. and Research Department of Zoology, Dhanaji Nana Mahavidyalaya, Faizpur.Tal.Yawal, Dist. Jalgaon. India (Maharashtra)

ABSTRACT

In the present study, Static bioassay were conducted to determine the toxicity of imidacloprid Confidor and Carbendazim Bavistin on the freshwater fish, Channa gachua. Effects of toxicants Confidor and Bavistin on total cholesterol content in tissues like liver and gonad of freshwater teleost fish, Channa gachua were studied after acute (24 to 96hrs) exposure in laboratory condition. Fishes were exposed to various concentrations of Confidor and Bavistin then percent mortality was recorded. The Lc50 value of Confidor and Bavistin for 96 hrs were 0.9ppm and 0.10ppm, respectively. Behavioural changes like, rapid opercular movement, large mucous covering over body, erratic swimming and jerky movements were observed in the experimental fishes. For biochemical analysis, fishes were exposed to lethal concentration of Confidor and Bavistin. After this, tissues like liver and gonad were taken from the control and experimental groups and estimation of cholesterol was done by Knobil method. It has been observed that, cholesterol concentration were decreased in experimental liver and gonad.

Introduction:

Fishes are the bioindicator species of the aquatic environment, because they are sensitive to any change in the water quality. Water pollution has become a serious problem throughout the world, it is unfortunate that the rivers are being increasingly used as natural dustbin for discharge of all sort of community and industrial wastes (Veeraiah and Durgaprasad, 2002; Binukumari et al, 2015). Various types of pesticides including insecticides, herbicides, fungicides etc are used for agricultural production, but this agricultural runoff finally reached in the water bodies and pollute the aquatic biota. Pesticides are not highly selective but are generally toxic to many macrophytes, non-target organisms such as fish (AyOola, 2008; Franklin et al, 2010; Gijare and Tantarpale, 2014). Some agrochemicals can indirectly affects fish by interfering with their food supply or altering the aquatic habitat, even when the concentrations are too low to affect the fish directly (Khan and Francis, 2005). Bioaccumulation of these pesticides threat the long term survival of fishes by disrupting the ecological relationship between organisms and loss of biodiversity (Xie et al., 1996; Marel et al., 1998; Abedi et al., 2013; Pandey et al., 2016).

The indiscriminate dumping of untreated wastes into aquatic environments brings about physical, chemical and biological deterioration of water bodies (Benjamin and Thatheyus, 2012). The pervasive use of pesticides in agriculture, public health and forestry ultimately leads to the contamination of aquatic biotopes posing a great threat to the environment (Visweswaraiah et al, 1975). Continues overuse of the pesticide causes reduction in the gene pool of the fish and other aquatic organisms. (Ishi and Patil, 2017)

Insecticide Confidor and fungicide Bavistin are widely used in agricultural fields to protect the crops from various pests and to increase the agricultural production which is helpful for economic upliftment of peoples. Pesticides mixed in freshwater bodies through agricultural runoff during rainy season and produce the harmful physiological effects on the non- target aquatic organisms like fish which indirectly affect to the human beings. Bioaccumulation of these pesticides threats the long term survival of fishes by disrupting the ecological relationship between organisms and loss of biodiversity (Morel et al., 1998; Abedi et al., 2013; Srivastava et al., 2016).

Channa gachua is a popular food fish fish in all over the India including Khandesh region of north Maharashtra. Economically this fish has great importance because of its high nutritive value. Confidor acts as systemic insecticide and Bavistin acts as Broad Spectrum systemic fungicide hence widely used in agricultural crops. Confidor contains imidacloprid 17.80% SL. Imidacloprid is highly toxic to an acute basis to aquatic animals. Bavistin contains Carbendazim 50%WP. Therefore, in the present work freshwater teleost fish, *Channa gachua* was selected to study the toxic effects of Confidor and Bavistin on behaviour and cholesterol content in tissues like liver and gonad.

Materials and Methods: Fishes were collected from the Girna river dam area, near Chalisgaon city Taluka, Jalgaon District, Maharashtra, in India. They were collected from their natural habitat and brought to the laboratory. Healthy fishes weighing between 30-50 gms and size 8-9 cm. were selected for experiment. These fishes were kept in the glass aquarium previously washed with potassium permanganate and water. The fishes were acclimatized to laboratory conditions for 10 to 15 days prior to experimentation. The water was analysed for different parameters like temperature, pH, dissolved oxygen, total alkalinity, hardness and bicarbonates by standard APHA, (1985) method. Active, healthy and same sized fishes were selected for present work. The stock solution of Confidor was prepared. From these stock solutions, different concentrations were prepared. In toxicity bioassay test, fishes were divided into several groups, each group comprising of 10 fishes. One group was considered as experimental group exposed to reagent grade of Confidor for 24, 48, 72, and 96 hours for acute exposure. Another group was without pollutants and was considered as control. Proper aeriation was maintained in test and control aquaria by air pump throughout experimentation. Fishes were fed regularly with fish food and water was changed every day in the control as well as treated group. Mortality was recorded during 96 hours.

The toxicity tests were carried out under static bioassay conditions up to 96 hours. The data collected was then analyzed statistically by means of Probit analysis method on transforming toxicity curve (percentage mortality versus concentration) into regression line. Mortality in probit /log of concentration by Finney, (1971) method simplified by Busvine, (1971) which allows the average medium lethal concentration, LC50 was calculated for 96 hrs. During the experiment the behavioural changes were critically observed. To count the mortality, the fishes were examined individually and those, which have lost the ability of opercular movement and did not respond to the tactile stimulus, were considered as dead. Each biochemical parameter was assessed in five individual animals. The fishes were starved for one day prior to experimentation in order to avoid the metabolic differences, if any due to differential feeding and food reserves. The cholesterol was estimated by using Knobil

method (Knobil et.al.1954) from liver and gonad in control and experimental fishes. The amount of cholesterol content was calculated by referring to standard graph value and it was expressed in terms of percentage of wet tissue. Pure dry ash free cholesterol was used as a standard.

Results and Discussion:

The acute toxicity of Confidor and Bavistin as percent mortality in different concentrations are shown in Table 1. The changes in biochemical composition of liver and gonad of fresh water fish *Channa gachua*, exposed to acute treatment of Confidor and Bavistin were studied along with experimental animals with respect to the percentage of cholesterol in wet tissue. The cholesterol content of the liver and gonad was found to be decreased after acute treatment by Confidor and Bavistin. The percent decrease of cholesterol in liver, after 96hrs treatment with Confidor and Bavistin was 4.856 to 1.453 % and 4.998 to 3.2509 % respectively. The average cholesterol content of gonad was decreased from 5.099 to 2.7882% and 5.998 to 4.0409 % respectively. The percent depletion in cholesterol content was found in the liver and gonad after pesticidal stress as a period of exposure increased. The animals treated with Confidor are more pronounced as compared to the Bavistin. Results of cholesterol alteration is summerized in Table 2 and figure 1 and 2.

The mortality of the experimental fish is depend on dose. Behavioural changes are seen during stress in the experimental organism as compared to the control. Experimental fishes shows hyper excitation, restlessness, swimming with jerky and irregular movements and tried to come out of aquarium. Activities were reduced, the fish settled to the bottom, increased mucous secretion with slimy white layer, increased and decreased opercular movements were seen. Fishes showed rapid zigzag movement with sudden jerks and avoidance of response. It seems that fish loses balance, as a result of influence of toxicant. Before death, the body colour of the fish turned faint due to loss of skin pigmentation, and fish died with open mouth. Fishes exposed to Bavistin shows dark coloration; less excitations, aerial excursion also few, opercula movements were increased, mucous secretion was copious. The examination of dead fish exhibited open opercula, bent body, which gill and copious mucous with signs of bleeding.

Acute toxicity tests are useful in providing rapid estimates of the concentrations of toxicants that cause direct irreversible harm to the organism (Parrish; 1985). Behavioural characteristics are obviously sensitive indicators of toxicants effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system (Marigoudar et al., 2009). Jerky movements of fish were due to the nervous imbalance whereas increased opercular movements were done for clear respiration. According to Arillo and Melodia(1990), the mucous secretion all over the body on exposure to pollutants has an ameliorative effect against the toxicants. A profuse mucous secretion could cause a hardship for aerial breathing in the medium because the lamellar fusion will reduce the surface area for gaseous exchange (Parithabhanu, 2013). Similar results were recorded previously by Pathan et al., 2009; Marigoudar et al., 2009; Benjamin and Thatheyus, 2012.

Pesticidal toxication produce biochemical changes in organs of fish and for curing the stress an organism need sufficient energy which is obtained from reserve materials like glycogen, protein and lipid. When the stress is mild then only stored glycogen is used as source of energy, but when the stress is strong at that time the stored energy in protein and lipid may be used. Hence, lipid is an important constituent of animal tissue which plays active role in energy metabolism. Lipids are important dietary constituents and it serves as condensed reserve of energy. Depletion in cholesterol content suggest that organic reserves seems to have utilized at longer exposure because the animals have no longer adopt to pesticidal stress conditions. Lipid content of fish reduce with increasing concentration of pollutants and this reduction may have been due to the utilization of lipid energy demand under stress condition Gijare and Tantarpale, (2014), found declined trend of cholesterol in the liver and muscle of freshwater fish, *Ophiocephalus orientalis* after exposure of the sublethal concentration of cypermethrin. They states that, the reduced cholesterol level is due to the inhibition of cholesterol biosynthesis in the liver and reduced absorption of dietary cholesterol. Binukumari et al., (2015), studied the effects of Ekalux on the biochemical parameters of the freshwater fish, *Labeo rohita* and they recorded low level of total cholesterol in tissues like liver, kidney, gill and muscles. They suggested that lipid might have been channeled for energy production for other metabolic functions in which these products play a vital role during stress.

Similar results were also recorded earlier by various scientists. Govindan et al., (1994), reported decrease in lipid content in liver, muscle and brain of *Gambusia affinis* exposed to a pesticide phosphamidon. Das and Mukherji, (2003), recorded decrease in lipid content in liver and muscle tissues of *Labeo rohita*. Abiram et al., (2012), recorded decline in the level of lipid in *Terapon jarbua* after toxicity of endosulfan. Waghmare and Wani, (2014), recorded cholesterol depletion in liver and gonads of freshwater fish, *Labeo rohita* after exposure to an insecticide polo. Sivandan and Binukumari, (2021), recorded lipid depletion in *L. rohita* after malathion intoxication. The present investigation reveals that insecticide Confidor and Bavistin induce the number of abnormal behaviour and alteration in cholesterol content in liver and gonad of freshwater teleost fish, *Channa gachua*.

Table 1: The acute toxicity of Confidor and Bavistin as percent mortality in different concentrations

Toxicant	Conc. in ppm	Log of Conc.	No. of fish alive out of ten	Mortality (%)	Probit Kill	Lc50 value in ppm
Confidor	5	0.6990	08	20	4.15	0.9
	6	0.7782	07	30	4.47	
	7	0.8451	04	60	5.25	
	8	0.9031	03	70	5.52	
	10	1	00	100	8.09	
Bavistin	5	0.6990	09	10	3.71	0.10
	6	0.7782	07	30	4.47	
	7	0.8451	06	40	4.74	
	8	0.9031	05	50	5	
	10	1	03	70	5.52	

Table 2. Effects of Confidor and Bavistin on Cholestrol content in liver and gonad of Channa gachua during period of acute exposure

Tissue	Treatment	Acute Exposure			
		24h	48 h	72 h	96 h
Liver	Control	5.9850 +1.84388	5.974 +1.79801	5.853 +1.27677	5.782 +1.57954
Gonad	Control	5.2870 +1.79904	5.2069 +1.76167	5.1043 +1.55228	5.002 +2.08443
Liver	Confidor	4.856 +1.71362	3.725 +1.38714	2.591 +1.3768	1.453 +1.1877
Gonad	Confidor	5.099 +1.27527	4.2541 +1.42003	3.1142 +1.36395	2.7882 +1.63875
Liver	Bavistin	4.998 +1.76254	4.0018 +2.37682	3.8120 +2.1457	3.2509 +1.9650

Gonad	Bavistin	5.998 +1.934846	5.1640 +2.25618	4.1023 +1.71818	4.0409 +1.94056

Values expressed as % of wet wt. of tissue; + indicates S.D. of five observation;Values are significiant at P < 0.05**

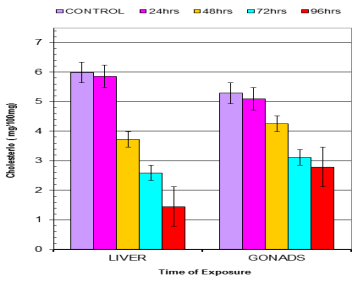

Fig 1. Variations in Cholesterol content of liver and Gonad of Channa gachua after acute exposure to Confidor

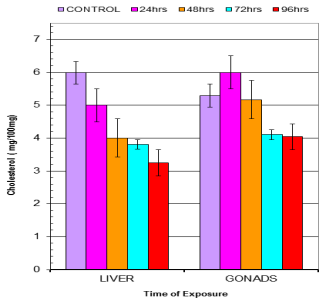

Fig 2. Variations in Cholesterol content of Liver and Gonad of Channa gachua after acute exposure to Bavistin

Conclusion:

From the present study, it was concluded that, the Confidor and Bavistin which are widely used in agroecosystem is not safe to non-target organisms like fish because it produces the behavioural changes and decrease the level of cholesterol content in the tissues of freshwater fish, Channa gachua. It has been suggested that, these toxicants are harmful to the aquatic animals, hence the random use of pesticides must be avoided and help to save aquatic life

REFERENCES:

1. Abedi, Z., Hasantabar, F., Khalesi, M. K., Babaei, S. (2013):"Enzymatic activities in common carp, Cyprinus carpio influenced by sublethal concentrations of cadmium, lead and chromium". W.J. Fish Mar. Sci., 5 :144-151.
2. Abirami, J., Bharathi, M., Kumaresan, S.(2012): "Studies on toxicity effects of endosulfan on Terapon jarbua and some aspects of histopathology and biochemical compositions". J. Pharm. Res., 5(2) :754-759.
3. APHA (1985):"Standard Methods of Examination of Water and Waste Water" (16th Edn.) Washington DC.
4. Arillo, A. and Melodia, F. (1990): "Protective effect of fish mucous against Cr(VI) pollution". Chem., 20 :397-402.
5. Ayoola, S.O. (2008): "Toxicity of glyphosate herbicide on Nile tilapia (Oreochromis niloticus) juvenile". Afri. J. Agri. Res., 3(12) :825-834.
6. Binukumari, S., Vijaya Kumar, K., Mohan kumar, M. (2015): "The effects of Ekalux on the biochemical parameters of the freshwater fish, Labeo rohita". IOSR- J. Environ. Sci. Toxicol. Food Tech., ((5) I :58-60.
7. Busvine, J.R. (1971): "A Critical Review of the Techniques for Testing Insecticides". Commonwealth Institute of Entomology, London.
8. Das, B.K. and Mukherjee, S.C. (2003): "Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences". Comp. Biochem. Physio. C Toxicol. Pharmacol., 134 :109-121.
9. Dorey Benjamin and Thatheyus, A. J. (2012) : "Acute toxicity of Nickel and Cadmium to the Cichlid fish, Oreochromis mossambicus (Peters)". Res, Zoo., 2(4) :19-22.
10. Finney, D.T. (1971): " Probit Analysis". Third Edition. Cambridge Uni. Press. London.
11. Franklin, R.K., Loo, H. S. and Osuanu, H.A. (2010):"Incorporation of Bentazone in freshwater fish, Puntius ticto (Ham)". J. Sci. Res. Rep., 2(1) :26-29.
12. Gijare, S.S. and Tantarpale, V.T. (2014):"Effects of cypermethrin on lipid and cholesterol contents of freshwater fish, Channa orientalis (Bloch)". Ind. J. Res., 3(8) :200-201.
13. Govindan, V.S., Jacob, L. and Devika, R. (1994): "Toxicity and metabolic changes in Gambusia affinis exposed to phosphamidon". J. Ecotoxicol. Environ. Mon., 4(1) :16.
14. Khan Zaheer, M. and Francis, C.P. Law (2005): "Adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish, amphibians, reptiles)". Proc, Pak. Acad. Sci., 42(4) :315-323.
15. Knobil, E., Hagney, M. G., Wilder, A. G.and Briggs, F. N. (1954): "Simplified Methods for Determination of Total Adrenal Cholesterol". Proceeding of the Society for Experimental Biology and Medicine, 87:48-50.
16. Marigoudar, S.R., Nazeer Ahmed, R.and David, M. (2009): "Impact of Cypermethrin on behavioural Responses in the freshwater teleost, Labeo rohita (Hamilton)". W.J. Zoo., 4(1) :19-23.
17. Morel, F. M. M., Kraepiel, A.M.L. and Amyot, M. (1998): "The chemical cycle and bioaccumulation of mercury". A. R. Eco. Evol. Sys., 29 :543-566
18. Parithabhanu, A. (2013): "Pesticide toxicity and Behavioural responses in the fish Oreochromis mossambicus". Int. J. Fish Aqua. Sci., 3(2) :161-164.
19. Parrish, P.R. (1985): "Acute Toxicity Tests, Fundamentals of Aquatic Toxicology", edited by Rand G.M. and Petrocelli S.R., Hemisphere publishing Corporation, New York, pp 31-57.
20. Pathan, T.S., Sonwane, D.L. and Khillare, Y.K. (2009): "Toxicity and behavioural changes in freshwater fish, Rasbora daniconius exposed to paper mill effluent". Bot. Res. Int., 2(4) :263-266.
21. Sivanandan J.M. and Binukumari (2021): "Acute and sublethal intoxication of malathion in an Indian major carp, Labeo rohita : haematological and biochemical responses". EAHT, 36(3): 11.
22. Srivastava, P., Singh, A. and Pandey, A.K. (2016): "Pesticides toxicity in fishes: biochemical, physiological and genotoxic aspects". Biochem. Cell. Arch., 16(2) : 199-218.
23. Veeraih, K. and Durgaprsad, M.K. (2002): "Study on the toxic effect of cypermethrin onorganic constituents of freshwater fish, Labeo rohita". Pro. Acad. Environ. Biol., 7(2) : 143-148.
24. Visweswaraiah, K., Jayram, M. and Mujumdar, S. K. (1975): " Environmental pollution with pesticides". J. Food. Sci. Tech., 2:53-60.
25. Waghmare, S.Y. and Wani, G.P. (2014): " Cholesterol alteration in a freshwater fish, Labeo rohita after exposure to an insecticide Polo". Int. J. Curr. Microbiol. Appli. Sci., 3(11) :221-225.