Biochemical Communication

Biosci. Biotech. Res. Comm. 6(2): 214-219 (2013)

BBRC
Bioscience Biotechnology Research Communications

Biochemical alterations in liver of *Clarias batrachus* exposed to a Neem based biopesticide

Anisuddin Siddiqui[1], Prerna Pahariya[2], Rajendra Chauhan[3] and MM Prakash Shrivastava[4]

[1,3]*Department of Zoology, Government Holkar Science College, Indore*
[2]*Department of Zoology, MLB Government College, Bhopal India*

ABSTRACT

Presence of pesticides in stream and lakes is largely due to the runoff from agricultural fields and outfall from manufacturing factories. Pesticides are not highly selective but are generally toxic to many non-target organisms. The aquatic environment is also polluted by pesticides and leads to many changes in organism physiology. In the present investigation an attempt has been made to observe the biochemical changes in the liver of fish *Clarias batrachus* exposed to lethal concentration of agroneem. Neem (*Azadirachta indica* A. Juss) is perhaps the most useful traditional medicinal plant in India. Biologically active ingredients of this plant have diverse applications.

KEY WORDS: FISH, CLARIAS BATRACHUS, AZADIRACHTA INDICA, PESTICIDES, AQUATIC ENVIRONMENT.

INTRODUCTION

Utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 2-5% per annum Usage of Organophosphorus (OP) pesticides is found to be increasing in recent years since they are biodegradable and therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies (Maharajan *et al*., 2013).

Recent emphasis is on the use of natural pesticides, which are usually of plant origin to replace deleterious chemical pesticides. Although synthetic pesticides are target specific and effective, their effect on environment is mostly deleterious. Plant based pesticides contain active principles with low half-life period and their effects on the environment are not too detrimental (Ahmad *et al*., 2012)

In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by substances from plants. One of the most promising natural compounds is azadirachtin, an active compound extracted from the neem tree (*Azadirachta indica*), whose antiviral, antibacterial and antifungal properties have been known for several years. It is generally considered less harmful to the environment than other more commonly used pesticides. Therefore, neem-based insecticides are being investigated as alternatives to synthetic insecticides for the control of agricultural insect pests. Neem has also been used successfully in aquaculture systems to control fish predators. The aqueous extract of neem leaves and other neem-based products have been extensively used in fish-farms as alternative for the control of fish parasites and fish fry predators such as dragon-fly larvae (Reza *et al*., 2012).

ARTICLE INFORMATION:

**Corresponding Author*
Received 30th October, 2013
Accepted after revision 31st December, 2013
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007

Online Contents Available at: http//www.bbrc.in/

Neem (*Azadirachta indica A*. Juss) is perhaps the most useful traditional medicinal plant in India. Biologically active ingredients of this plant have diverse applications. Each part of the neem tree has some medicinal property and is thus commercially exploitable. These compounds belong to the natural products called triterpenoids (Limonoids) (Kumar *et al*., 2010).

Azadirachta indica is a fast growing evergreen popular tree found commonly in India, Africa and America. It has been used in ayuervedic medicine for more than 4000 years due to its medicinal properties. Neem is called 'arista' in Sanskrit a word that means a perfect, complete and imperishable Arishtha is the Sanskrit name of the neem tree meaning reliver of sickness and is considered as 'Sarbarogaribarini'. The tree is regarded as village 'dispensory' in India. The importance of the Neem tree has been recognized by the US National Academy of Sciences, which publish a report in 1992 entitled 'Neem- a tree for solving global problems (Sharma *et al*., 2011).

Agroneem can be used in place of chemical pesticides, as it is environment friendly, which dose not toxify the air we breath, food we eat, soil we cultivate nor water we use for irrigation. In short, these bio-pesticides are equally effective, far cheaper and fully eco-friendly. Azadirachtin obtained from neem was subjected to toxicological testing to document its safe use as pesticide so that it becomes necessary to use it as ecologically safe and biologically active botanical substance which is metabolized and not passed on to the next trophic level.

MATERIALS AND METHODS

Adult fresh water fish *Clarias batrachus* of uniform size and (length 15±2cm, width 3.8±59 cm, 120±20g) were collected from local water bodies of the city, and kept in glass aquaria containing 59 l of de-chlorinated tap water for 7 days to acclimatize to laboratory conditions. Water quality was measured according to the method of APHA (1995) water was changed every day. Dead fish were removed to avoid water fouling fishes were fed daily on commercial fish food.

In this study, tap water free from chlorine was used and the water had the following physico-chemical characteristics given in Table-1. Before start of the experiment, fish were randomly divided into two groups which were housed in 200 liter glass aquarium with tap water which was continuously aerated.

Biological pesticide or insecticides: (Agroneem/ Bioneem) was used in the present study. It is a neem based products widely used as a pesticidal or insecticidal agent of natural origin. Azadirachtin biologically obtained insecticide is drived from the neem tree (*Azadirachta indica*) which can disturb number of vital physiological processes so that their activity is strongly affected.

Bioneem/Agroneem containing Azadirachtin - 1500 ppm was used.

TABLE 1: Physico-chemical parameters of the aquaria with experimental fishes.

Temperature	27.4°C
PH	8
Dissolved oxygen	8mg/l
Total alkalinity (Bicarbonate)	118mg/l
Total hardness	183mg/l
Choride concentration	44.98mg/l

Chemical Composition of the biopesticide was:

Azadirachtin	5.00
Neem Kernal extract in solvent	60.00
Emulsifer (Polysorbate 20)	35.00
Treated Neem oil	100.00

Dose and Duration:

Dose 0.3ml/l or 30ppm. Duration short term, 12hours, 24 hours, 48 hours, 72 hours, and 96 hours.

Experimental Design:

In the present study experimental fishes were divided into two major groups viz.

Control group:

The control was run with distilled water as vehicle. Water used in the experiment was dechlorinated and was having pH 8 and temperature 25-30^0 C.

Experimental group:

Set A: In this group, 10 fishes were used. The test fishes were exposed to sub-lethal dose of Agroneem 30 ppm

Set B: In this group 10 fishes were used, and test fishes were treated with agroneem (biochemical pesticide) of 30 ppm concentration.

RESULTS AND DISCUSSION

Fishes of control and treated groups were sacrificed at 0 hours, 12hours, 24hours, 48hours, 72 hours and 98 hours. The liver lobes were removed, blotted and weighed and then processed for various biochemical tests. Effects of biopesticide (Agroneem) 30 ppm on liver biochemistry of *Clarias batrachus* are as follows:

Deoxy Ribose Nucleic Acid content of the liver of experimental fishes was observed for short period i.e. 96 hours, obtained data showed no change in DNA quantity as the duration of exposure of the test chemical (Agroneem) was increased. Whatever change was observed in the 96 hours experiment (0.20%) was insignificant and negligible and may be due to experimental error (Table-2).

Ribose Nucleic Acid content of liver of experimental fishes was observed. The result obtained showed decreasing trend as the duration of exposure of test chemical (Agroneem) was increased. The decrease in RNA content after 96 hours of exposure of Agroneem (30 ppm on liver) was 1.23%, which was again very low. Thus result obtained showed that experimental

TABLE 2: Efect on DNA of *Clarias batrachus*.

S. No.	Exposure In (Hours)	DNA Content in (µg/mg) wt. of tissue		Difference	% Alter
		Control	Experimental		
1	0	0.498	0.498	0.000	0.000
2	12	0.498	0.498	0.000	0.000
3	24	0.498	0.498	0.000	0.000
4	48	0.498	0.498	0.000	0.000
5	72	0.498	0.498	0.000	0.000
6	96	0.498	0.497	0.001	-0.201

pesticide was unable to show the remarkable change in the quantity of RNA content (Table-3, Fig.-1).

The quantity of total protein in the liver of experimental fish was found decreased order. The value of total protein after 12 hours, 24 hours, 48 hours, 72 hours, and 96 hours exposure of Agroneem were found decreased in the order of 4.38%, 53.72%, 54.53%, 76.26%, 78.57% respectively. The important observation in this experiment was that the protein value decreases upto 78.57% just within 96 hours. This shows that initially toxicant exposure induces the utilization of stored protein and further stimulates protein synthesis to cope additional demand of protein to fight against Bioneem toxicity (Table-4, Fig-2).

The Glutamate Pyruvate Transaminase activity was found affected in the exposure of Agroneem (Biochemical pesticide) in *Clarias batrachus*. The decrease in GPT after 12 hours, 24

TABLE 3: Effect on RNA of *Clarias batrachus*.

S. No.	Duration (Hours)	RNA (µg/mg)		Difference	% Alter
		Control	Experimental		
1	0	0.568	0.568	0.000	0.00
2	12	0.568	0.567	0.001	-0.18
3	24	0.568	0.566	0.002	-0.35
4	24	0.568	0.565	0.003	-0.53
5	72	0.564	0.564	0.004	-0.70
6	96	0.568	0.561	0.007	-1.23

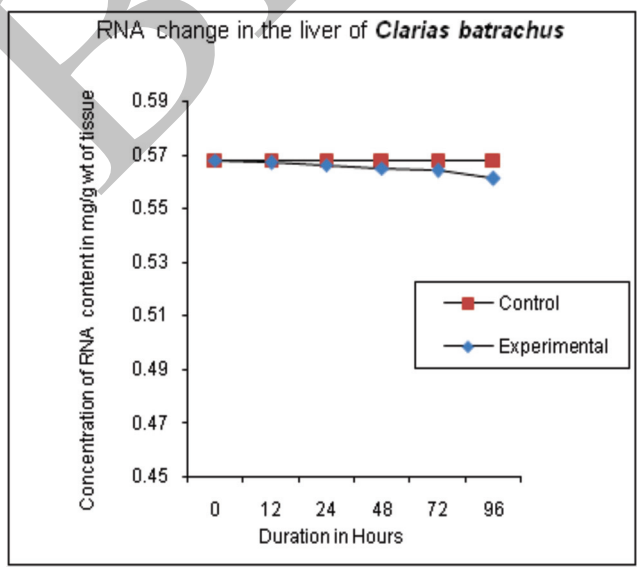

FIGURE 1: Effect on RNA of *Clarias batrachus*.

TABLE 4: Effect on Total Protein of *Clarias batrachus*.

S. No.	Duration (Hours)	Protein (µg/mg)		Difference	% Alter
		Control	Experimental		
1	0	4.726	4.726	0.000	0.00
2	12	4.726	4.519	0.207	-4.38
3	24	4.726	2.187	2.539	-53.72
4	24	4.726	2.149	2.577	-54.53
5	72	4.726	1.122	3.604	-76.26
6	96	4.726	1.013	3.713	-78.57

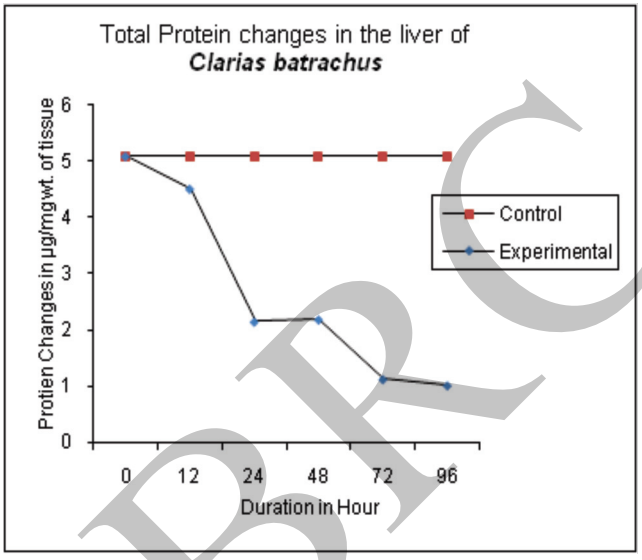

FIGURE 2: Effect on Total Protein of *Clarias batrachus*.

hours, 48 hours, 72 hours and 96 hours were found 11.85%, 29.61%, 39.17%, 43.81% and 72.57% respectively. The inhibition in GPT activity of liver was very high and reaches upto 72.57% (Table-5, Fig-3).

The Glutamate Oxaloacetate Transaminase activity in the liver of *Clarias batrachus*. After 12 hours, 24 hours, 48 hours, 72 hours and 96 hours exposure of test chemicals the reduction in enzymatic activity (GOT) were found 6.62%, 19.56%, 23.7%, 29.29% and 30.49% respectively. This showed Glutamate Oxaloacetate Transaminase activity inhibited very less in comparison to Glutamate Pyruvate Transaminase (Table-6, Fig-4).

Various biochemical parameters like Deoxyribonucleic acid, Ribonucleic acid, total protein, Glutamate oxaloacetate Transminase, Glutamate Pyruvate Transminase have been worked out in normal as well as in experimental fishes i.e. *Clarias batrachus*.

In the experimental study the RNA activity in 96 hours treated with (Agroneem 30ppm) fish *Clarias batrachus*, the value was 0.56/µm/mg of tissue, which was 1.23% less the control value. In author opinion this reduction was may be due to experimental error. Thus there was almost no change in the quantity of RNA. Sahana *et. al.*, (1986) reported decrease in RNA activities in liver, Kidney intestine, stomach, muscles, testes and ovary of a fresh water air breathing fish *Clarias batrachus*.

Depletion of tissue proteins in the fish exposed to various pesticides has been reported by several investigators. When experimental fishes *Clarias batrachus* were exposed to biopesticides Agroneem for 96 hours the depletion in the total protein was 78.57percent in comparison to control (Shaikh, 1997) observed the effect of *Azadirachata indica* and stated that biochemically, there was decrease in total protein and acid phosphatase activity. Thus our results support the observation of previous authors.

In the present study the control value of total protein content in the liver of *Clarias batrachus* was 0.56µg/mg of tissue. Value of the total protein content in the liver of experimental fishes after 96 hours duration was 1.013µg/mg of tissue 30 ppm of Agroneem. Views of (Sarvanan and Harikrishna,

TABLE 5: Effect on GPT of *Clarias batrachus.*

S. No.	Duration (Hours)	GPT Activity in Mg Mole/L/Min)		Difference	% Alter
		Control	Experimental		
1	0	0.302	0.302	0.000	0.00
2	12	0.302	0.27	0.032	-11.85
3	24	0.302	0.233	0.069	-29.61
4	24	0.302	0.217	0.085	-39.17
5	72	0.302	0.21	0.092	-43.81
6	96	0.302	0.175	0.127	-72.57

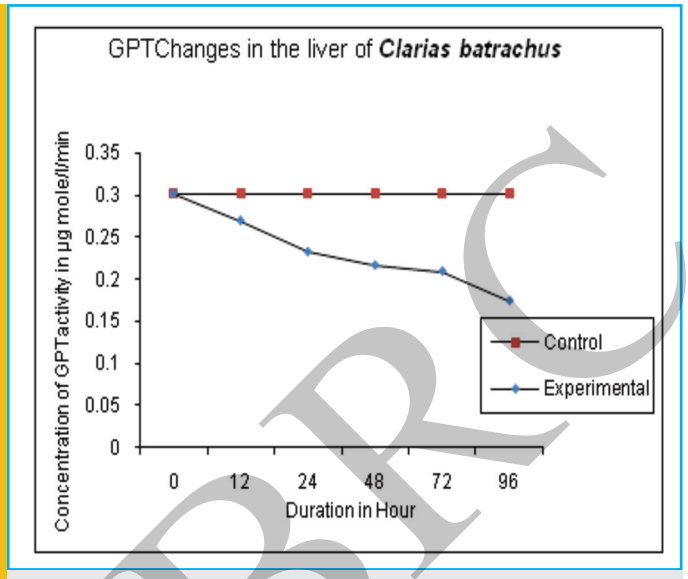

FIGURE 3: Effect on GPT of *Clarias batrachus.*

1998) also suggested that deplection of protein could be due to the mobilization of proteins to meet the impending energy demands when the animal was under stress. The author and above researcher finding thus support the view Alkaline Phosphatase is known to be widely distributed in animal tissues. In the present investigation alkaline Phosphatase activity in Clarias batrachus was found decreased as the duration of the exposure of 30ppm Agroneem (biopesticide) increases.

In the present investigation, the 30ppm exposure of Agroneem (Bioneem) to *Clarias batrachus* altered the concentrations of Glutamate oxaloacetate Transminase (GOT) and Glutamate Pyruvate Transminase (GPT) enzymes in the liver.

The author indicated that leaf extract of *A. indica* has caused significant alterations in glycogen and protein content of liver and muscles of fish *L. rohita* which might be helpful to establish the safer usage of aqueous extracts of *A. indica* in agriculture farms (Saravanan *et al.*, 2010). Activities of both these enzymes were found inhibited upto 30.49% and 72.57% respectively. Inhibition in the Glutamate Pyruvate Transminase was more pronounced than Glutamate oxaloacetate Transminase. Some authors described significantly inhibition in Glutamate Pyruvate Transminase due to chronic herbicide intoxication in the liver of fresh water fish *Nemachelius denisonii.* Thus the author finding show that Glutamate Pyruvate Transminase is more susceptible to toxicants Bioneem than Glutamate oxaloacetate transminase.

Authors recommended that more and more plant products should be developed with proper and targeted action and this eventually helps in keeping the environment free from hazardous chemicals Stalin *et al.*, 2008).

CONCLUSION

From the above investigation of the present work revealed that the variations in biochemical parameters serve as indices in monitoring the pollution of aquatic environment. The variations observed during experiment can be used as meaningful indicators of pesticide pollution. Such differential behavior with regards to tissues and fish of the above said parameters can be further examined to develop more meaningful indicators or markers to assess or to characterize the particular pollutant and its potential for toxicity. It is concluded that neem based product are safer to use as they have greater margin of safety to fishes as compared to other synthetic chemical pesticides.

TABLE 6: Effect on GOT of *Clarias batrachus.*

S. No.	Duration (Hours)	GOT Concentration in KA Units/100ml		Difference	% Alter
		Control	Experimental		
1	0	0.997	0.997	0.000	0.00
2	12	0.997	0.931	0.066	-6.62
3	24	0.997	0.802	0.195	-19.56
4	24	0.997	0.762	0.235	-23.57
5	72	0.997	0.705	0.292	-29.29
6	96	0.997	0.693	0.304	-30.49

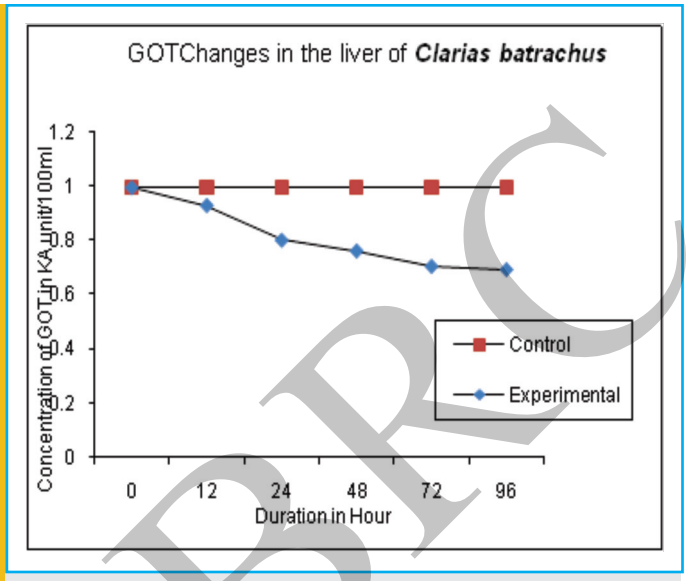

FIGURE 4: Effect on GOT of *Clarias batrachus.*

REFERENCES

Bhat Bilal Ahmad, Vishwakarma Santosh (2012). A comparative study on toxicity of a synthetic pesticide dichlorvos and a Neem based pesticide, Neem-On to *Labeo rohita* (Hamilton). Current World Environment, Vol.7(1), pp 157-161.

Davoodi Reza and Abdi Gholamreza (2012). Comparative study on the acute toxicity of synthetic pesticides, permethrin 25% and Monocrotophos 36%, and Neem based Pesticide, Neem Gold EC 0.03%, to juvenile. J. Biol. Environ. Sci., Vol. 6. No. 16, pp 105-108.

Kasturi M., Ahamed R. N., Pathan K.M., Shaikh P.D. and Manivannan B. (1997). Effect of *Azadirachta indica* leaves on the seminal vesicles and ventral prostate in Albino rats. Indian J. Physiol. Pharmacol., 41(3): 234-240.

Kumar Sudhir P., Mishra Debasis Ghosh Goutamand Panda S. Chandra (2010). Biological action and medicinal properties of various constituents of *Azadirachta indica* (Meliaceae) Overview. Scholars Research Library Annals of Biological Research, Vol. 1, No.3, pp 24-34.

Maharajan A., Usha R., Ruckmani Paru P.S., Vijay kumar B.S., Ganapiriya V. and Kumarasamy P (2013). Sublethal Effect of Profenofos on Oxygen Conssumption and Gill Histopathology of the Indian Major Carp, *Catla catla* (Hamilton). International Journal of pure and applied Zoology. Vol.1, No.2, pp 196-204.

Sahana SS, Jana S, Choudhuri MA. (1986). HG (II)-Induced changes in some biochemical parameters in the freshwater fish *Clarias batrachus*. Physiol Bohemoslov 35(1); 81-85.

Saravanan M., Vasantha Kumar, Malarvizhi A. and Ramesh M. (2010). Biosafety of *Azadirachta indica* (A. Juss) leaves extracts on certain biochemical parameters of *Labeo rohita*. Journal of Biopesticides. Vol.3, No.1, pp 227-231.

Saravanan T. S. and Harikrishnan R. (1998). Tissue sugar as an indicator of metallic stress in *Anabas testudineus* (Bloch) Bio . Sci. Res. Bull., 14,103-108.

Sharma Pankaj Lokeshwar Tomar, Bachwani Mukesh, Vishnu Bansal (2011). Review on Neem (*Azadirachta indica*): Thousand problems one solution. International Research Journal of Pharmacy.Vol. 2, No.12, pp 97-102.

Stalin Israel S., Kiruba S, Das Manohar Sam S. (2008). A comparative study on the toxicity of a synthetic pyrethroid, Deltamethrin and a Neem based pesticide, Azadirachtin to *Poecilia reticulate* Peters (1859) (Cyprinodontiformes: poeciliidae). Turkish Journal of fisheries and aquatic sciences vol. 8, No.1, pp 1-5.