Journal of the Indian Fisheries Association
31, 2004, 97-100

EFFECT OF NEEMTA 2100 TOXICITY ON ACETYLCHOLINESTERASE AND SERUM GLUTAMATE OXALOACETATE TRANSAMINASE ENZYMES IN SERUM OF FISH, *OREOCHROMIS MOSSAMBICUS*

Mahira Parveen*, Ritu Sharma and Santosh Kumar

Department of Biosciences, Barkatullah University, Bhopal 462026 (M.P.) India.

ABSTRACT

Acetylcholinesterase and Serum glutamate oxaloacetate transaminase enzymes have been used as marker monitoring the effect of neem seed based pesticide Neemta 2100 on the fish, *Oreochromis mossambicus*. Fishes exposed to sublethal concentrations of neemta 2100 for acute periods of 24 and 48 hours were sacrificed to determine enzyme activities in serum affected due to toxicity. Laboratory studies of *in vivo* exposure of this pesticide showed synergistic inhibitory effect during acute period of toxicity. Acetylcholinesterase was noticed as 6.25 µm substrate hydrolyzed / mg protein / hour and Serum glutamate oxaloacetate transaminase was noticed as 36.71 µm substrate hydrolyzed / mg protein / hour in control fish serum. Significant decrease in GOT level in Neemta 2100 treated fishes after short term exposure indicated its severe toxicity to fish.

Key words: Serum, Neemta 2100, Enzymes, Fish

INTRODUCTION

There are various products of neem plant which are widely in practice nowadays. The pesticides based on neem parts such as seeds, leaves and stem bark etc. have showed a great pharmaceutical value and work has been performed on their toxicity testing in vertebrates (Gupta *et al.* 1999; Tripathi, 2000). Neem tree and its pure individual active constituents have various biocidal and medicinal properties (Ascher, 1993 and Tripathi, 2000). Toxic effects of neem pesticides on fishes and other aquatic animals has been reported previously (Osuala and Okwuosa, 1993). Azadirachtin (neem) not only affects the fish but also prevents fishes by acting as an effective antibacterial for fishes (Das *et al.*, 1999). However, the LC_{50} of neem based pesticides to fishes have been evaluated for acute periods.

The evaluation of AChE and GOT in fishes is helpful in determining the tolerance level of azadirachtin in fishes. One of the neem pesticides Neemta 2100, a commercially used biopesticide has been reported to cause 90% mortality in *Corcyra cephalonica* and nematodes (Gupta *et al.* 1999). In the present investigation, the effect of azadirachtin based pesticide Neemta 2100 has been studied on enzymes acetylcholinesterase (AChE) and serum

* email : mahira_pp@yahoo.co.in

glutamate oxaloacetate transaminase (SGOT) in serum of fishes after acute poisoning.

MATERIAL AND METHODS

The fishes, Oreochromis mossambicus of 10-12 cm were procured from upper lake of Bhopal. The fishes were acclimatized in aquaria in laboratory conditions for 10 days and were fed with fish food of mixture of rice bran and groundnut oil cake in equal proportion. The neem seed pesticide Neemta 2100 manufactured by A.J. Chemicals, Ahmedabad was used as a toxicant.

Fishes were exposed to Neemta 2100 with sublethal concentration of 0.005 ml/ 1 for 24 and 48 hours. The blood was taken from heart after sacrificing control and treated fishes. The AChE enzyme activity was estimated according to the method of Metcalf (1951). SGOT enzyme activities were determined according to the method of Reitman and Frankel (1957). Both the enzyme specific activities were expressed as µm substrate hydrolyzed/ mg protein / hour units in serum of fishes. The data were subjected to 't' test for knowing the statistical significance level at $P<0.05$.

RESULTS AND DISCUSSION

Fishes exposed to Neemta 2100 showed lethargic behavior and movement towards surface. This lethargic behavior appeared immediately after the addition of toxicant into aquarium. In control fish serum the AChE activity was observed as 6.25 µm substrate hydrolyzed / mg protein / hour. The serum AChE activity was reduced after 24 and 48 hours to 4.32 and 3.76 µm substrate hydrolyzed / mg protein / hour respectively. The significant decline ($P<0.05$) in AChE was observed after 48 hours. SGOT activity decreased from 36.71 µm substrate hydrolyzed / mg protein / hour (control) to 35.21 µm (24 hours exposure). There was a further decline of 40% in animals treated for 48 hours (Table 1).

Table 1 : Acetylcholinesterase and serum glutamate oxaloacetate transaminase activities in serum of fish, Oreochromis mossambicus exposed to Neemta 2100 for 24 and 48 hours. All values are mean ± S.E. expressing significance level at *P<0.05.

Neemta 2100 exposure	AChE activity (µm substrate hydrolyzed/mg protein/hour)	SGOT activity (µm substrate hydrolyzed/mg protein/hour)
Control (untreated)	6.25 ± 00.36	36.71 ± 2.94
24 hours	4.32 * ± .23	35.21 ± 3.76
48 hours	3.76 * ± .20	32.35 * ± 2.88

The sublethal concentration of Neemta 2100 showed its inhibitory property towards AChE and SGOT enzymes of serum after short term exposure of 24 and 48 hours. These biochemical changes associated with azadirachtin, the major constituent of Neemta 2100 toxicity were externally reflected in lethargic behavior of fishes. AChE and GOT enzymes are the target enzymes for azadirachtin based pesticides. The acetylcholinesterase enzyme activity if inhibited by azadirachtin, which appears to block the release of neurosecretory materials by interacting with acetylcholine. Their mode of interaction may be reversible in nature. The deterrence of GOT enzyme results from a variety of compounds working in concert with one another, producing different behavioral responses, which vary in magnitude between the different periods of exposure. Other neem based pesticides have also been reported to be toxic and even caused mortality during acute toxicity in fishes (Anjaneyulu and Mishra 1999; Logambal and Michael 2000; Shazly and Sharnoubi, 2000).

Biochemical alterations depicted by the GOT and AChE are almost similar as esterases and transaminases are major target enzymes involving blood, heart, liver, kidney and other tissues. Interaction of acetylcholine and oxaloacetate with azadirachtin is a complex phenomenon involving interplay between several substrates and various metabolic pathways. Reduced enzymatic levels caused by azadirachtin and other associated compounds has been attributed to decreased neurosecretory and hepatosecretory activities. This decreased biosynthesis and release of acetylcholine and oxaloacetate in serum promoting the conversion of respective substrates decreasing enzyme activities support the reports of Anjaneyulu and Mishra (1999).

All biologically active neem compounds are suspected to be derived from one parent compound the tetracyclic triterpenoid tirucallol, which is considered responsible for the majority of effects inside the body of organisms exposed to neem compounds (Mordue & Blackwall 1993; Martin *et al.* 1997). Neem today is regarded as nature's own answer to effective insect control and offers promise in the fight against pesticide resistance (Mitra, 1963). Its main constituent azadirachtin with other bitter compounds called meliacins produced from neem such as nimbin, salannin, meliantriol etc have diverse modes of action. Therefore, knowing the level of tolerance of these pesticides to fishes should be helpful in preventing them from lethal exposure.

The authors are of the opinion that these enzymatic decreases may be due to hypo disruptive action of the azadirachtin on the erythropoietic tissues. The present study indicates that exposure of Neemta 2100 induces biochemical abnormalities in enzymatic systems of *Orechromis mossambica* (Jacobson, 1989). Further investigations are needed to elucidate the possible biochemical modes of action associated with azadirachtin and derived compounds. The promotion of biological pest control would greatly reduce dependence on pesticides and alleviate environmental pollution, thereby to manage pests in an ecofriendly and sustainable manner.

REFERENCES

Anjaneyulu, G.S.V.R. and Mishra, K.D., 1999. Acute toxicity of Neemax (neem seed powder) to the freshwater fish, *Puntius ticto* (Ham.) *Poll. Res.,* **18:** 391-394.

Ascher, K.R.S., 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica. Arch. Insect Biochem. physiol.,* **22:** 433-449.

Das, B.K., Mukherjee, S.C., Sahu, B.B. and Murjani, G., 1993. Neem *(Azadirachta Indica)* extract as an antibacterial agent against fish pathogenic bacteria. *Ind. J. Exp. Biol.,* **37:** 1097-1100.

Gupta, P., Siddiqui, M.R. and Joseph, S., 1999. Compatibility of the entomopathogenic nematode *Stenernema corpocapsae* with some commercial neem formulations. XIVth International Plant Protection Congress (IPCC) 3-4.

Jacobson, M., 1989. *Focus on phytochemical pesticides,* volume 1 : The neem tree. CRC Press, Boca Raton, FL. 178.

Logambal, S.M. and Michael, R.D., 2000. Immunostimulatory effect of azadirachtin in *Oreochromis mossambicus* (Peters). *Ind. J. Exp. Biol.,* **38:** 1092-1096.

Martin, P. Wilson, I.D. Morgan, E.D. Jones, G.R. and Jones, K., 1997. Evaluation of a molecular imprinted polymer for use in the solid phase extraction of propanolol from biological fluids. *Analytical Communications.* **34:** 45-47.

Metealf, R.L., 1951. Methods in biochemical analysis In : D. Glick (Ed.) Interscience Publishers Inc : New York, 5-9.

Mitra, C. R., 1963. *Indian Central Oilseeds Committee,* Himayatnagar, Hyderabad, pp. 35-43.

Mordue, A.J. and Blackwell, A., 1993. Azadirachtin : an update. *J. Insect Phys.,* **39:** 903-924.

Osuala, F.O. and Okwuosa, V.N., 1993 Toxicity of *Azadirachta indica* to freshwater snails and fish, with reference to the physicochemical factor effect on potency. *Appl. Parasitol.,* **34:** 63-68.

Reitman, S. and Frankel, D. A., 1957 Colorimetric method for the determination of serum oxaloacetic and glutamate pyruvic transaminase. *Amer. J. Pathol.,* **28:** 53-56.

Shazly, el M.M. and Sharnoubi, el F.D., 2000. Toxicity of a neem *(Azadirachta indica)* insecticide to certain aquatic organizms. *J. Egypt, Soc. Parasitol.,* **30:** 221-231.

Tripathi, Y. C., 2000. Potential of neem constituents in pest management. *Everyman's Science.* **35:** 78-83.