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Effect of Bio-Pesticide (Agroneem) on the biochemistry of kidney of *Clarias batrachus*

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Abstract

In the present study authors studied the effect of bio-pesticide (Agroneem) on the biochemistry of kidney of *Clarias batrachus*. The biochemical parameters studied were RNA, total protein, acid phosphatase, alkaline phosphatase, GOT and GPT. These parameters in the experimental animal were found decline. The order of toxicity of Agroneem to biochemical parameters from higher to lower were- acid phosphatase (-78.623%)< total protein (-78.078%)< alkaline phosphatase (-70.594%)< RNA (-44.049%)< GPT (-39.618%)< GOT (-29.948%). Thus on basis of obtained results in the present investigation it can be concluded that 96 hrs. exposure of 30 ppm of Agroneem aqueous solution has toxic effect and alter the biochemistry of kidney. Therefore, it is recommended to the user of this bio-pesticide that they should be careful about the dose they are using.

Keywords:- *Bio-pesticide, Biochemistry, Toxicity, Clarius batrachus*

Introduction

Biological pesticides vary in their toxicity and in their potential ecological impact. They are relatively non-toxic to people with few side effects. Agroneem (Azadiractin) is a Neem seed based bio-pesticide. It is very complex tetranotriterpenoid obtained from the seed. It is considered relatively non-toxic and it is not likely to accumulate or cause long-term effects (Miller and Uetz, 1998).

Very few workers have studies toxicity of neem products to fish species. Attri and Prasad (1980) have reported toxic concentration of neem extract to fish and frog tadpoles. Deshmukh and Periyal (1992) observed acute toxicity of Neemark to fish *Tilapia mossembica*. Wan *et al.* (1996) have described 96 hrs. LC_{50} value of Azadiractin to juvenile salmon. Consulted literature showed that Azadiractin (Agroneem) effects the biochemistry of various organs of fishes, were not extensively investigated. Therefore, present study was under taken to investigate the Azadiractin induced change in the biochemistry of kidney of *Clarias batrachus*.

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Materials and Method

Experimental animal

Healthy *Clarias batrachus* were purchased from local fish market and acclimatized to the laboratory conditions for one week, during which they were regularly fed with Prawn powder and Soya meal.

Experimental design

In the present investigation experimental fishes were divided into two groups.

(1) Control group: - In this group 15 fishes were kept and exposed to normal distilled water.
(2) Experimental group: - In this group 15 fishes were exposed to 30.00 ppm Agroneem solution.

Experimental duration: In both control and experiment group fishes were exposed to maximum 96 hrs.

Autopsy: Fishes of control and experimental groups were scarified at 0 hrs., 12 hrs., 24 hrs., 48 hrs., 72 hrs., and 96 hrs. The kidney were removed, blotted, weighed and then processed for various biochemical assay.

Biochemical analysis: Following standard biochemical methods were used, which are described in the laboratory manual in biochemistry (Jayaraman, 2000).

(1) Extraction and estimation of RNA.
(2) Estimation of total protein (TP) by Biuret method.
(3) Determination of alkaline phosphatase (ALP) by King and King method.
(4) Determination of acid phosphatase (ACP) by King and King method.
(5) Estimation of activity of glutamate oxalo-acetate transminase enzyme (GOT).
(6) Estimation of activity of glutamate pyruvate transminase enzyme (GPT).

Results and Discussion

RNA content: The total content of RNA in the kidney of *Clarias batrachus* was found to be decreased gradually after the exposure of 30 ppm Agroneem. The normal value of RNA content in animal was observed 0.563 mg/gm wt. of kidney which after Agroneem exposure of 12, 24, 48, 72 and 96 hours reduced to 0.481, 0.427, 0.414, 0.386 and 0.315 mg/gm of tissue wt. respectively. In the experiment total 44.049 per cent inhibition in RNA activity was observed after 96 hrs.

Table 1: RNA changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	RNA content in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	0.563	0.563	0.00	0.00
12	0.563	0.418	0.082	14.065
24	0.563	0.427	0.136	24.156
48	0.563	0.414	0.149	26.465
72	0.563	0.326	0.177	31.438
96	0.563	0.315	0.248	44.049

Total protein (TP): The total protein in the kidney of *Clarias batrachus* was found decreased gradually in experiment group. The normal value of total protein content in control animal was observed 4.726 mg/gm wt. of kidney which after 12, 24, 48, 72 and 96 hours exposure of biopesticide reduced to 4.519, 2.187, 2.147, 1.122 and 1.036 mg/gm wt. of tissue respectively. The kidney of experiment animals showed decrease in TP activity, which was gradual and exposure duration dependent. The total protein content decrease 78.078 per cent after 96 hrs.

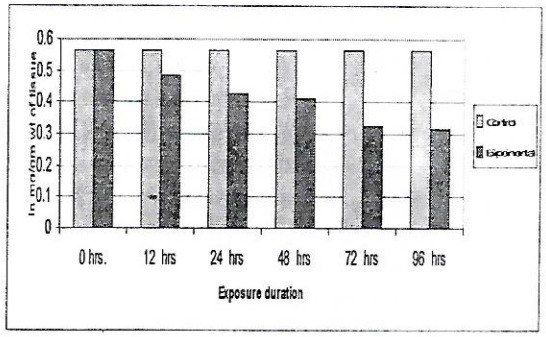

Fig. 1: RNA changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

Alkaline Phosphatase (ALP): The content of ALP in the kidney of *Clarias batrachus* was also observed decreased gradually after the exposure of 30 ppm Agroneem. The normal value of ALP content in control animal was observed 7.230 KA units/100 ml of kidney, which after 12, 24, 48, 72 and 96 hours of exposure reduced to 7.142, 5.763, 4.213, 2.376 and 2.126 KA units/100 ml respectively. This experiment showed the inhibition ALP upto 70.564 per cent in just 96 hrs of duration. The inhibition in the enzyme activity was gradual and exposure duration dependent.

(4) Acid phosphatase (ACP): The content of Acid phosphatase in the kidney of *Clarias batrachus* was found decreased gradually upto 96 hrs after the exposure of 30 ppm Agroneem. The control value of Acid phosphatase was observed 6.437 KA units/100 ml in kidney, which after 12, 24, 48, 72 and 96 hours exposure of test solution, reduced to 6.125, 5.629, 4.326, 2.479 and 1.376 KA units/100 ml respectively. The inhibition of Acid phosphatase activity in 96 hrs was 78.623 per cent.

Table 2: Total protein change in kidney *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	Total protein in mg/gm wt. of tissue		Difference	% altera
	Control	Experimental		
0	4.726	4.726	0.00	0.00
12	4.726	4.519	0.207	4.380
24	4.726	2.187	2.539	53.724
48	4.726	2.147	2.577	54.528
72	4.726	1.122	3.604	76.258
96	4.726	1.036	3.690	78.078

Table 3: Alkaline PO_4^{2-} change in kidney *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	Alkaline phosphatase content in KA unit/100 ml		Difference	% alter
	Control	Experimental		
0	7.230	7.230	0.00	0.00
12	7.230	7.142	0.088	1.217
24	7.230	5.763	1.467	20.290
48	7.230	4.213	3.017	41.728
72	7.230	2.376	4.854	67.136
96	7.230	2.126	5.104	70.564

Table 4: Acid PO_4^{2-} changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	RNA content in mg/gm wt. of tissue		Difference	% alter
	Control	Experimental		
0	6.437	6.437	0.00	0.00
12	6.437	6.125	0.312	4.847
24	6.437	5.629	0.808	12552
48	6.437	4.326	2.111	37.794
72	6.437	2.479	3.958	61.488
96	6.437	1.376	5.061	78.623

GOT: The content of GOT in the kidney of *Clarias batrachus* was decreased very slowly and gradually after the exposure of 30 ppm Agroneem. The control value of GOT was observed 0.975 mg/gm in the kidney of experimental fish, which after 12, 24, 48, 72 and 96 hours of exposure decreased to 0.927, 0.856, 0.804, 0.742 and 0.683 mg/gm respectively. This experiment showed that inhibition of GOT was very low in comparison to RNA, Protein, Acid and Alkaline phosphatase as the value was found decrease only 29.948% in 96 hrs of exposure of studied biopesticide chemical.

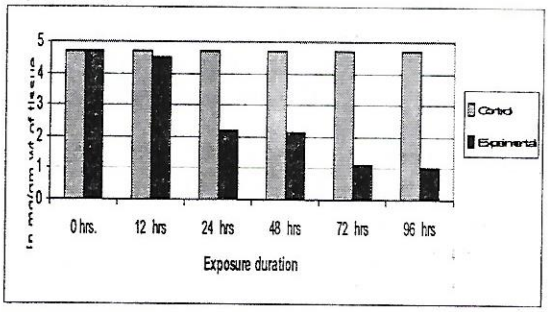

Fig. 2: Total protein changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

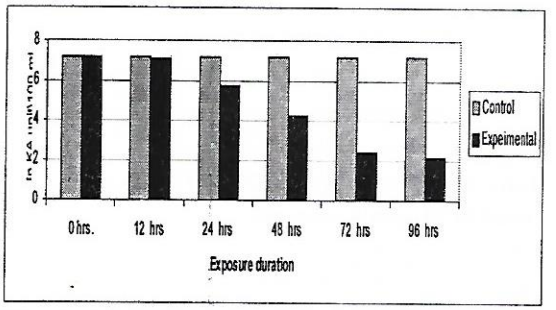

Fig. 3: Alkaline PO_4^{2-} changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

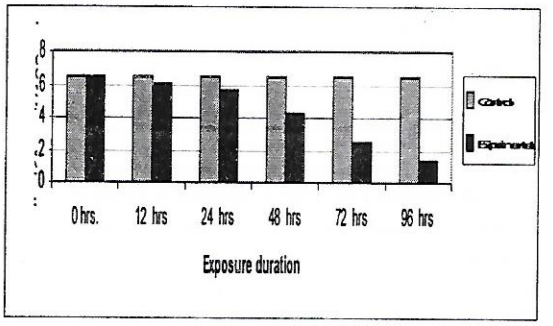

Fig. 4: Acid PO_4^{2-} changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

GPT : The content of GPT in the kidney of *Clarias batrachus* was also decreased gradually after the exposure of 30 ppm Agroneem. The control value of GPT content was observed 0.313 KA units/100 ml of kidney, which after 12, 24, 48, 72 and 96 hours exposure reduced to 0.287, 0.284, 0.239, 0.211 and 0.189 KA units/100 ml respectively. GPT also showed very less reduction in 96 hrs in term of per cent i.e. 39.616%. This finding was closer to GOT finding in contrast to RNA, Protein, Acid and Alkaline phosphatase.

Table 5: GOT changes in kidney *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	Total protein in mg/gm wt. of tissue		Difference	% altera
	Control	Experimental		
0	0.975	0.975	0.00	0.00
12	0.975	0.927	0.048	4.923
24	0.975	0.856	0.119	12.205
48	0.975	0.804	0.171	17.538
72	0.975	0.742	0.233	23.897
96	0.975	0.683	0.292	29.948

Table 6: GPT change in kidney *C. batrachus* exposed to 30 ppm Agroneem

Exposure duration in hrs.	Alkaline phosphatase content in KA unit/100 ml		Difference	% alter
	Control	Experimental		
0	0.313	0.313	0.00	0.00
12	0.313	0.287	0.026	8.306
24	0.313	0.284	0.029	9.265
48	0.313	0.239	0.074	23.642
72	0.313	0.211	0.102	32.587
96	0.313	0.189	0.124	39.616

Attri and Prasad (1980) have reported toxicity of neem extracts to fish and frog tadpoles. Deshmukh and Periyal (1992) have observed acute toxicity of neemark to fish, *Tilapia mossambica*. Wan *et al.* (1996) have studied 96 hrs LC_{50} values of Azadirachtin to juvenile of *Salmon*. Farah *et al.* (2006) described that neem extract exhibit strong antimutagenic activity in fish, *Channa punctatus*. Gandhi *et al.* (1988) documented acute toxicity of neem oil in rats and rabbits and described dose related pharmacotoxic symptoms with a number of changes in the biochemical and histopathological indices of toxicity. Kasutri *et al.* (1997) reported that oral administration of 20, 40, 60 mg of dry *Azadirachta indica* leaf powder for 24 days resulted in decrease in the weight of seminal vesicle, nuclear diameter and the secretory material in the lumen. Biochemically they observed decrease in total

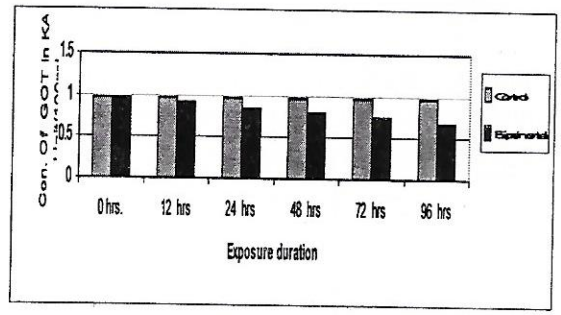

Fig. 5: GOT changes in kidney of *C. batrachus* exposed to 30 ppm Agroneem

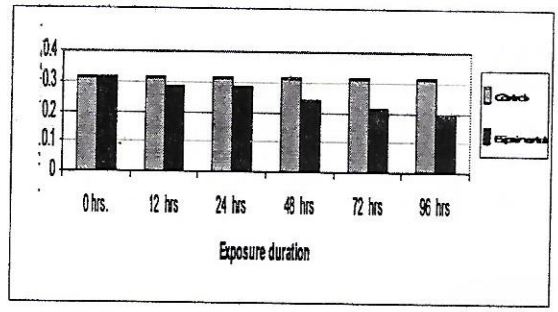

Fig. 6: GPTchanges in kidney of *C. batrachus* exposed to 30 ppm Agroneem

protein, acid phosphatase activities. Kanungo (1996) also observed neem extract as hepeto-toxic and nephrotoxic to poultry birds. Rahman *et al.* (2001) also reported that a neem based pesticide (Vepacide) altered the biochemical profile of serum, kidney and lungs of Albino Wistar rats. In the present investigation Agroneem (30 ppm) exposure

for 96 hrs to *Clarias batrachus* was found toxic as it altered rather decreased all the studied biochemical parameters (RNA, TP, ALP, ACP, GOT and GPT) of kidney and thus support the observation of previous authors. Therefore it is recommended to the user of this bio-pesticide that they should be careful about the dose they are using.

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