

## QUANTIFICATION OF NUCLEIC ACID FROM FRESH WATER FISH *Punctius arenatus* (Day) EXPOSED TO PESTICIDES

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### ABSTRACT

Pesticides have an innate capacity to cause damage to biological system. The range of pesticide toxicity is large in aquatic organisms, among different fish species. In the present investigation, effect of sublethal concentration of two different kinds of pesticides Fenvalerate (synthetic pyrethroid) and Monocrotophos (organophosphate) for 24, 48, 72 and 96 hrs were observed on DNA and RNA contents in selected tissues like gills, liver, kidney and muscle of freshwater fish *Punctius arenatus* (Day). Nucleic acids like DNA and RNA react differently with the various concentration and period of pesticidal exposure. An overall decrease in nucleic acid was noted in these contents which is statistically significant.

**Keywords:** DNA, RNA, pesticides, *Punctius arenatus* (Day)

### INTRODUCTION

Pesticides are one of the most potentially harmful chemicals introduced into the environment. The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance [1]. However, several of the banned pesticides are still used on a large scale in developing countries and continue to pose severe health and environmental problems [2]. Though

they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant. Sublethal adverse effects may result from exposure of aquatic organisms to insecticides at environmentally relevant concentrations [3]. The use of biological change (biochemical, physiological, histological) as an indication of exposure and/or effects of xenobiotic chemicals [4-5]. The great advantage of biochemical

markers is providing evidence of the state of pollution in a comprehensive way based on the synergistic and antagonistic effects of all contaminants involved. The range of pesticide toxicity is large in aquatic organisms, among different fish species it varies, and the variation depends on the age, sex, size, physiological and biological conditions of individual fish and also the environmental factors [6]. Most of the toxic effects in fishes are due to poisoning of metabolism. Biochemical values indicate the intensity of the toxic action of the pesticides.

The cumulative toxicologic impacts of pesticide mixtures are of particular concern for disorders the metabolic activity, alters physiological state and histological changes thereby changing the biochemical constituents of fish [7-8]. Although toxicant impairs the metabolic, physiological and histological analysis in vital tissues. Furthermore, pollutants may get into water in combination with each other, causing additive harmful effects on the fish [5, 9, 10]. The present work deals with the fate of DNA and RNA content under sublethal concentrations two different kinds of pesticides Fenvalerate

(synthetic pyrethroid) and Monocrotophos (organophosphate) in the fish *Punctius arenatus* (Day).

## MATERIAL AND METHODS

The freshwater fish *Punctius arenatus* was collected from Gomai River near Shahada, Nandurbar District and acclimatized for a week in laboratory condition. The test fish with a length ranging from 5 to 8cm and weight ranging from  $10 \pm 5$ gms were chosen for experiments. A concurrent control having no death record was maintained in laboratory conditions.

The collected sample fishes were divided into two groups, which exposed to various concentrations of the pesticides so as to find out the  $LC_{50}$  for 24, 48, 72 and 96 hrs [11], taken as sublethal concentrations. Each group of fishes was further divided into four batches and each of which were treated with different pesticides.

The first group of fishes was exposed sublethal concentrations of Fenvalerate (0.015 ppm, 0.0082 ppm, 0.0024 ppm and 0.0021 ppm) for 24, 48, 72 and 96 hrs. The second group of fishes was exposed to of sublethal concentrations of Monocrotophos (0.075 ppm, 0.038 ppm, 0.0056 ppm and 0.0053 ppm) for 24, 48, 72 and 96 hrs.

At the end of each exposure period of pesticides treatment from each experimental group and control, fishes were dissected carefully. And tissues were blotted dry and weighed to the nearest mg. The estimation of DNA and RNA [12] was carried out in the tissues gills, liver, kidney and muscle. Each observation was repeating at least for five times and results were statistically analysed by student 't' test [13].

## RESULTS AND DISCUSSION

The results were summarized in Table 1 and 2. The percentage reduction of nucleic acid level was statistically significant in selected tissues of freshwater fish *Punctius arenatus* (Day) (Fig. 1 and 2). The relative decrease varies from tissue to tissue. The decrease of DNA and RNA content was found to be significant statistically at  $P < 0.01$  or  $P < 0.001$ .

The level of DNA and RNA was found to be decreased in the gills, liver, kidney and muscle of *Punctius arenatus* (Day) due to period of exposure and different concentration of two different kinds of pesticides. Pesticide toxicity indicates alteration in nucleic acid synthesis. Despite the importance, information on

impact of pesticides on DNA and RNA are scanty. The rapid histolysis of pesticide treated animals is the only possible reason for this. Impairment of nucleic acid metabolism the degradation of cells, resulting in the reduction in the DNA content. Furthermore, inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. The regulatory roles of nucleic acid metabolism as observed in the different animals when treated with the different pesticides were reported earlier [14, 3, 15]. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway [16].

RNA plays significant role in protein synthesis hence depletion in RNA contents also results in depletion in protein level [17]. Hence, there is decrease in RNA level thus reducing protein synthesis. Maruthanayagam and Sharmila, [18] reported similar results. This is because the animal requires more energy to overcome the stress upon exposure to the pesticides. As a result, the animal prefers

glucogenesis to protein synthesis and diverts all the metabolites for carbohydrate biosynthesis. Tripathi [19] reported that significant declines in RNA level were observed in treated fish might also be any obstruction in RNA synthesis. The decrease in RNA may be suggested that the daily addition of pesticides results in the swelling and chromatolysis of Nissle bodies which are rich in RNA.

Tripathi [19] also reported that fish exposed to Dimethoate (organophosphate) exhibited a decrease in nucleic acid (DNA and RNA) content. The reason for decreased nucleic acids levels in liver under the influence of carbosulfan treatment in mice might caused genotoxic action by decreased mitotic index and disturbed cell division [20] or due to inhibitory action of pesticides on DNA and RNA synthesis [21] or by cell death due to focal necrosis [22]. The effects of sublethal concentration of Fenvalerate on DNA, RNA, RNA/DNA ratio and protein contents were estimated in gill and kidney tissues of an air breathing fish, *Clarias batrachus*. Fenvalerate reduced the DNA content in gills, whereas it dose not produce any significant effect on

DNA in kidney. This tissue-specific change in DNA content may be due to differential effects of Fenvalerate or its metabolite(s) on synthesis and/degradation of DNA in gill and kidney cells of the fish [17]. Toxicants that cause genetic effects may be present at very low, sub-lethal concentrations [23]. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway [16].

Thus, from the present investigation, it can be concluded that the marked decrease in the DNA and RNA content upon exposure to pesticides may be due to decrease in protein synthesis, impairment of nucleic acid metabolism, the degradation of cells, resulting in the reduction in the DNA and RNA content. In case of DNA, most decrease was found in the liver and least in the kidney. Thus, it is possible that these pesticides will have inhibited the enzyme necessary for DNA synthesis. On compilation of the results, it appears that the disruption of DNA synthesis might have affected RNA synthesis.

## CONCLUSION

In the present investigation, it can be concluded that under exposure to pesticides the DNA and RNA content decreased in the tissues of the fish, *Punctius arenatus* (Day) leading to a decrease in protein synthesis and cellular degradation. The present data happens to constitute the first report on pesticide

causing depletion in the nucleic acid level amongst the freshwater fishes.

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**Table-1. Effect of pesticides on DNA content of gills, liver, kidney and muscle of *Punctius arenatus* (Day) mg/g wet wt.**

Tissues	Control	Fenvalerate				Monocrotophos			
		24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<b>Gills</b> %	1.85 ±0.065	1.35 ±0.12**	1.18 ±0.042**	0.99 ±0.72*	0.83 ±0.14**	1.41 ±0.021**	1.21 ±0.039**	0.69 ±0.12**	0.62 ±0.15**
		-27.03	-36.22	-45	-55.13	-23.78	-35.59	-62.7	-66.48
<b>Liver</b> %	3.75 ±0.773	2.59 ±0.456*	2.17 ±0.966*	1.66 ±0.986**	1.05 ±0.542**	2.22 ±0.724*	2.02 ±0.924*	1.62 ±0.932*	1.03 ±1.603*
		-30.93	-42.13	-55.73	-72	-40.8	-46.13	-56.8	-72.53
<b>Kidney</b> %	2.71 ±0.062	2.48 ±0.21*	2.19 ±0.059**	1.71 ±0.11**	1.66 ±0.11**	2.51 ±0.18*	2.34 ±0.022**	1.81 ±0.001**	1.78 ±0.12**
		-8.48	-19.18	-36.9	-38.74	-7.38	13.65	-33.21	-34.31
<b>Muscle</b> %	2.63 ±0.89	2.11 ±0.21*	1.91 ±0.24*	1.45 ±0.27**	1.13 ±0.29**	2.26 ±0.22*	1.86 ±0.23*	1.51 ±0.32*	1.16 ±0.034*
		-19.77	-27.37	-44.86	-57.03	-14.07	-29.27	-42.58	-55.89

Values expressed in mg/g wet wt.

Each value is mean of five observations ± SD

Values are significant at P< 0.01\*; P< 0.001\*\* & NS- Not significant

Sign '+' or '-' indicates % increase or decrease over normal respectively.

**Table-2. Effect of pesticides on RNA content of gills, liver, kidney and muscle of *Punctius arenatus* (Day) mg/g wet wt.**

Tissues	Control	Fenvalerate				Monocrotophos			
		24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<b>Gills</b> %	1.85 ±0.065	1.35 ±0.12**	1.18 ±0.042**	0.99 ±0.72*	0.83 ±0.14**	1.41 ±0.021**	1.21 ±0.039**	0.69 ±0.12**	0.62 ±0.15**
		-27.03	-36.22	-45	-55.13	-23.78	-35.59	-62.7	-66.48
<b>Liver</b> %	3.75 ±0.773	2.59 ±0.456*	2.17 ±0.966*	1.66 ±0.986**	1.05 ±0.542**	2.22 ±0.724*	2.02 ±0.924*	1.62 ±0.932*	1.03 ±1.603*
		-30.93	-42.13	-55.73	-72	-40.8	-46.13	-56.8	-72.53
<b>Kidney</b> %	2.71 ±0.062	2.48 ±0.21*	2.19 ±0.059**	1.71 ±0.11**	1.66 ±0.11**	2.51 ±0.18*	2.34 ±0.022**	1.81 ±0.001**	1.78 ±0.12**
		-8.48	-19.18	-36.9	-38.74	-7.38	13.65	-33.21	-34.31
<b>Muscle</b> %	2.63 ±0.89	2.11 ±0.21*	1.91 ±0.24*	1.45 ±0.27**	1.13 ±0.29**	2.26 ±0.22*	1.86 ±0.23*	1.51 ±0.32*	1.16 ±0.034*
		-19.77	-27.37	-44.86	-57.03	-14.07	-29.27	-42.58	-55.89

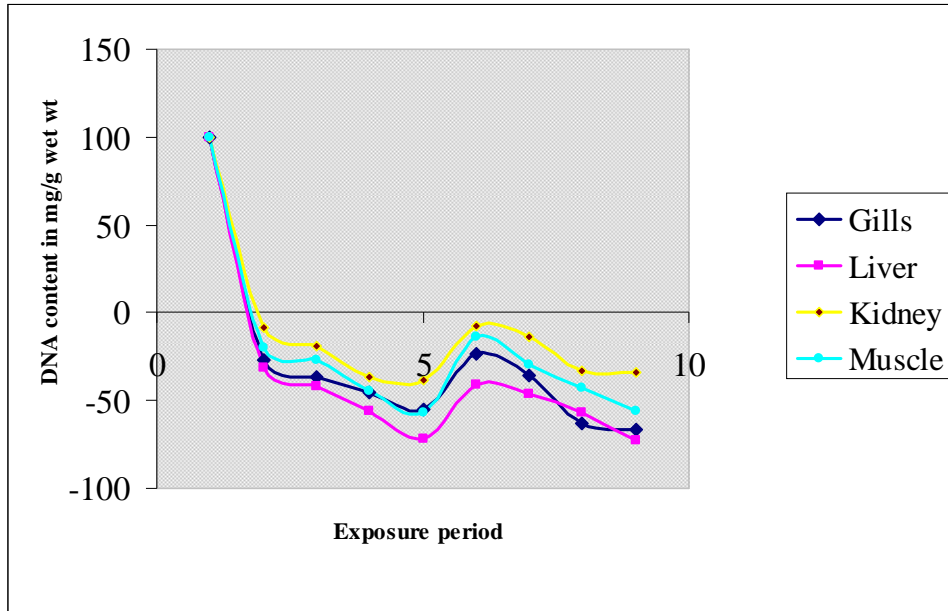
Values expressed in mg/g wet wt.

Each value is mean of five observations ± SD

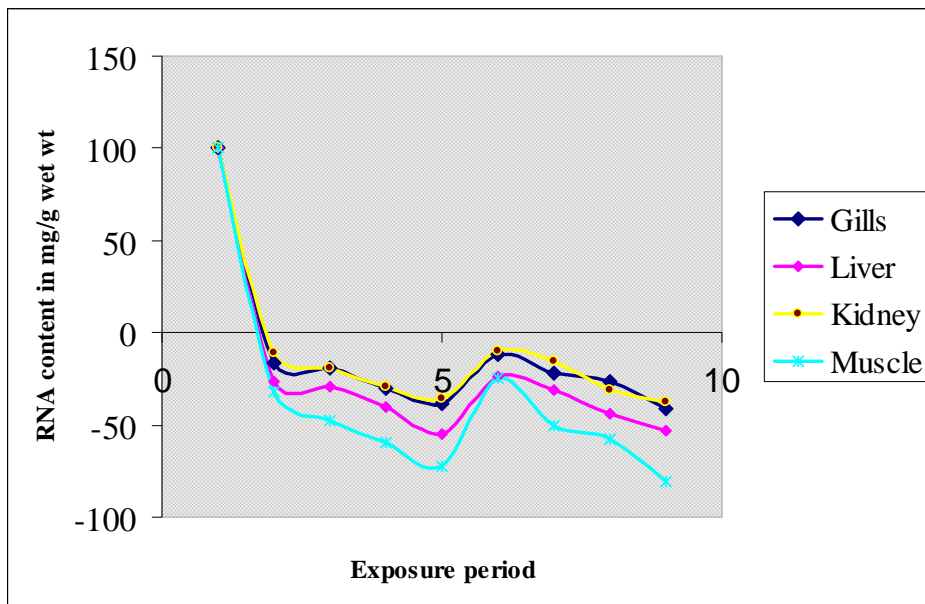
Values are significant at P< 0.01\*; P< 0.001\*\* & NS- Not significant

Sign '+' or '-' indicates % increase or decrease over normal respectively.

**Fig-1. Effect of pesticides on DNA content of gills, liver, kidney and muscle of *Punctius arenatus* (Day) mg/g wet wt.**



**Fig-2. Effect of pesticides on RNA content of gills, liver, kidney and muscle of *Punctius arenatus* (Day) mg/g wet wt.**



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