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Analysis of toxicity effect of DAP on *Anabas testudineus* (Ham.)

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ABSTRACT

DAP is a standard fertilizer widely used in agriculture. The present work was designed to study the effect of sub-lethal concentration of DAP on some haematological parameters of *Anabas testudineus* (Ham.). A comparison of control and treated fish showed a change in blood parameters due to the toxic effects of this fertilizer. The initial increase in both studied total count (TC) of RBCs and haemoglobin concentration and then gradual decrease of these two parameters with the increase of doses indicated slow recovery from adverse effects of DAP in this fish. Differential leukocyte count indicated a significant response due to the toxic effect of this fertilizer, though the count of lymphocytes, heterophils and eosinophils decreased. In addition, an increase in basophils, neutrophils and thrombocytes was taken place with an increasing dose of DAP. Elevation in the number of these cells revealed recovery and exhibited resistance of this fish.

Key Words - DAP, Hematological Parameters, *Anabas testudineus*

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INTRODUCTION

DAP (Diammonium phosphate) is one of the widely used fertilizers for crops globally. The use of chemical fertilizers has increased many folds after the blue revolution in India. Due to indiscriminate use in agriculture, this fertilizer is ultimately washed out and continuously added to the water bodies, affecting the aquatic flora and fauna life. When mixed with a water body, DAP was found to diminish fish production and cause mortality (Jhingran, 1992). DAP also has a negative correlation between fish production and the level of organic nitrogen. In some cases, DAP was reported to cause pernicious physiological changes in fish. *Channa punctatus,* when exposed to sub-lethal concentration of malathion, revealed a declining trend of RBCs, Haemoglobin and increasing trend of WBCs (Singh *et al.* 2006). The experiment was also done with the effect of plant seed extracts on air-breathing fish (Nanda *et al.* 2010). Whereas the growth performance of significant carp was better in DAP treated pond than in untreated fish, as observed by Nanda *et al.* (2010) have reported the prevalence of hypoxic stress and tissue damage in *Heteropneustes fossilis* due to the sub-lethal toxicity of rotenone. In most cases release of the tissue-specific enzyme into the circulation and changes in the haematological profile was observed when fishes are exposed to toxic manifestation of a disease. After entreating the environment, pesticides can be circulated into different ecosystems through the air, water, soil, different food chain and other agents. Bluml *et al.* (2008) reported that nutrient fertilizers and chemical fertilizers were the leading cause of pollution of water bodies. A study was also conducted on freshwater fish to observe the response of plant nutrient, which was considered a chemical pollutant and broad histopathological changes in the liver,

ovary, and testis were observed. The fishes were suffering from several histopathological problems due to changes in the water quality of the river Nile. When fishes are exposed to chemical pollutants, there are either increases or decreases in hematological levels, it depends on species, age and other characteristics of the fish. The present work deals with DAP's effect, a widely used fertilizer in Bihar.

MATERIALS AND METHODS

Live and healthy fish were collected from the local market, having a mean weight of 50.35±0.29 g and length of 20.04±0.56cm. In a sub-lethal toxicity study, the fish have reared in a glass aquaria (60 x 48 x 48) cm^3 containing dechlorinated tap water for seven days. Fish were fed protein-rich fish foods. Each tank in 30L of dechlorinated tap water. The physico-chemical parameters of the water were maintained. In each tank, there was 25 no. of fish of different concentration of DAP. The control tank was treated similarly but without DAP treatment. Fishes were exposed for 7 days and 14 days to different dosages of DAP. Blood samples were collected from the sampled fish by tail ablation, and the blood samples were used to analyze different parameters, viz. Red Blood Cells (RBCs) total count, Differential leukocyte count and Haemoglobin percentage.

Erythrocyte Count: Total count of RBCs was done with the help of the improved Neubauer Haemocytometer slide and studied under a light microscope.

Calculation: The number of RBCs/mm^3 of blood = the total number of cells counted X dilution X 4000 / the number of small squares in which counting has been done.

Differential Count of Leukocyte: Blood film was prepared with Leishman's stain following the standard method used in human blood film preparation.

Haemoglobin Percentage: The Haemoglobin percentage fed was estimated by Sahil's hemoglobinometer.

RESULTS AND DISCUSSION

The study revealed that the RBC count in control was 2.26±0.23 when the fish was treated with 0.078 mg/l DAP the count of RBC's comprised 2.13±0.18 and 2.04±0.17 of blood after 7 days and 14 days of treatment respectively. the count. The result is depicted in Table 1. the result shows a decreasing trend of 0.078mg/l and an increasing trend compared to the average RBC's count. The haemoglobin percentage in control fish was 9.4±0.61 g/100ml of blood. The experiment showed that haemoglobin percentage was 9.04±0.50 and 9.2±0.42 g/100 ml of blood after 7 and 14 days, respectively, in 0.078 g /l of sub-lethal concentration of DAP. When fishes were treated with (0.0156 g /l, 0.03125 g/l, 0.0625 g/l and 0.125 g/l1) higher concentrations of toxicant, the haemoglobin percentage constituted 10.62±0.12 and 10±0.25, 11.06±0.43 and 11.1±0.38, 9.9±0.25 and7.9±0.19, 10.4±0.43 and 9.6±0.36 g/100ml of blood after 7 days and 14 days of exposure respectively.

The percentage of lymphocytes in the control fish was 32%. When the fish was treated with 0.0078 g/l of DAP, lymphocyte percentage lowered to 30% and 31%, whereas treatment with higher concentrations of DAP induced a decrease in lymphocyte percentage. The result clearly shows that the percentage of lymphocytes decreased with an elevation of DAP concentration.

The heterophils percentage of control fish was 18%, whereas in the fish treated with 0.0078 g/l of DAP, the heterophils percentage was 15% and 17% during 7 and 14 days, respectively. So, the study also showed that heterophils percentage was decreased with increased doses.

The differential count also showed that eosinophils were14% in control fish, and the observation showed an increasing trend at 0.0078 g /l. The number decreased with the increasing doses at 7 days duration. However, the percentage came down gradually after 14 days duration. The basophils percentage in control fish was 10%; when the fishes were treated with 0.0078 g/l of DAP, the basophils

Table 1: Total count of RBCs and hemoglobin percentage in control and DAP treated fish after 7 and 14 days of exposure to various concentrations

		Duration	Doses of DAP				
Parameter	**Control**		0.0078 g/l	0.0156 g/l	0.0312 g/	0.0625 g/l	0.125g /l
Hb (g/100ml)	9.40±0.61	7days	9.04±0.50	10.62±0.12	11.06±0.43	9.90±0.25	10.40±0.43
		14days	9.20±0.42	10±0.25	11.10±0.38	7.90±0.19	9.60±0.36
TC of RBCs (10^6mm^3)	2.26±0.23	7days	2.13±0.18	3.02±0.20	3.05±0.14	2.70±0.62	2.72±0.21
		14days	2.04±0.17	2.51±0.13	4.02±0.17	1.92±0.24	2.41±0.12

Table 2: Differential count in control and treated fish after 7 and 14 days of exposure to various concentrations of DAP

Parameters	Control	Duration	Doses of DAP				
Leucocytes			0.0078 g/l	0.0156 g/l	0.0312 g/l	0.0625 g/l	0.125g/l
Lymphocyte	32	7 days	30	28	25	20	18
		14 days	31	30	27	24	21
Heterophil	18	7 days	15	13	11	8	7
		14 days	17	14	12	11	9
Eosinophil	14	7 days	18	16	15	13	11
		14 days	16	12	10	9	7
Basophil	10	7 days	9	12	14	18	21
		14 days	7	6	10	15	20
Monocytes	12	7 days	16	15	14	12	11
		14 days	18	18	17	15	17
Neutrophil	8	7 days	8	10	13	19	21
		14 days	6	10	12	14	15
Thrombocyte	6	7 days	4	6	8	10	11
		14 days	5	10	12	12	11

were 9% and 7%, but this cell showed an increasing trend with the high doses for both 7 and 14 days of duration in comparison to control fish.

The number of monocytes in the control fish was 12%, increasing gradually and then maintaining an almost average level when fishes were treated with 0.0625 g/l and 0.125 g/l for 7 days, respectively. At the same time, the number of monocytes showed an increasing trend with increased doses after 14 days. The percentage of neutrophils and thrombocytes in control was 8% and 6%, respectively. The results clearly show that the percentage of these two leukocytes increased with the elevation of DAP concentration.

The sub-lethal concentration of DAP resulted in the initial increase of RBCs count and Hb at both 7 and 14 days. This conforms with Sasikala *et al.* (2011), who observed significant changes in haematological parameters in Channa striata. The initial increase in both studied parameters and then gradual decrease with the increase of doses indicate slow recovery from adverse conditions in the fishes. Roy and Nath (2011) reported an almost similar observation in the case of Thiamethoxam treated *Oreochromis niloticus*. Then a gradual decrease in the total count of RBCs and haemoglobin percentage indicates anaemia that could be due to the breaking down and destruction of RBCs triggered by an influx of DAP into erythrocytes as in the case of phenol-dosed fishes. Patnaik and Patra (2006) also indicated symptoms of anaemia due to a reduction of the number of erythrocytes and amount of haemoglobin in carbonyl induced *Clarias batrachus*. Similar

phenomena were also reported in tobacco leaves dust treated African catfish.

Haematological parameters have been considered as an indicator of stress-induced by pesticides and variation in RBCs count and haemoglobin concentration due to the deleterious effect of the pollutant on the erythropoietic tissue of *Mystus vittatus*. Goger and Sawant (1989) suggested that leukocytes' differential count (DC) is a reliable haematological index to study the change in environmental conditions.

In differential leukocytes count (DLC) number of basophils, monocytes, neutrophils and throm bocytes were found to increase with increasing doses during 7 days and 14 days period. Such an increase may be due to stress because these cells play an essential role in the body's defence mechanism. Moreover, the gradual increase in count indicated the recovery behaviour from this fertilizer stress.

Such high counts of these leukocytes indicated damage due to infection of body tissues, severe physical stress, and leukaemia. A similar finding documented significantly higher leukocytes in fish exposed to copper. It was reviewed that though DAP is a perturbing solute that inhibits enzyme activity and stability, it acts as a significant osmotic in some species, and some cellular salt like KCl and NaCl plays a crucial role in counteracting the effect of DAP. Eosinophil in 0.0078 g/l does increase in both 7 and 14 days of exposure, then gradually decreases with increasing the doses. On the other hand, lymphocytes and heterophils decrease gradually with the increase of doses on the same day of exposure. In a similar observation, Vasait and Patil (2005) found decreasing lymphocyte count in *Nemacheilus botia* fish treated with organophos phorus insecticide.

HET is a highly phagocytic cell which can prevent antimicrobial activity. In the present study, the number of heterophils decreased with increasing doses, indicating DAP's negative effect on these leukocytes. However, it was reported that HET increased with stress conditions.

DAP is a typical organic fertilizer used throughout India in agriculture. The study indicated that DAP has a deleterious effect on haematological parameters of fish, i.e., the fertilizer has acute and long-term side effects in aquatic animals like fish and may ultimately reach the upper level of the food chain, including humans.

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