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Study of haematological alterations induced by exposure to diazinon in *Channa punctatus* (Bloch)

Somali Sood[1*], QJ Shammi[2]
[1] Department of Zoology, Govt. N.M.V. Hoshangabad, Madhya Pradesh, India
[2] Department of Zoology, Govt. Arts, Commerce and Science College, Harda, Madhya Pradesh, India

Abstract
Pesticides are a necessity these days owing to increasing pathogenic attacks on the plants and fresh water. Hence, to prevent such contamination use of pesticides has been promoted. As a result there subtherapeutic levels have very serious impact on the survival rates of the concerned animal. In the present study, the impact of diazinon, a pollutant of land water habitat, was investigated on fresh water fish *Channa punctatus (Bloch)*. The fish (weight =160± 10g, mean length= 25-40cm) were subjected to 0.1 ppm diazinon and observed for mortality. The alive fishes were then exposed to 5 and 50ppm of diazinon concentration. At the end of study, blood samples were collected from the caudal tail vessels with 21 or 23 gauze needles and 1 or 3 cc syringe before ventilator response was noticeably depressed. The studies on different doses of diazinon were performed and alteration in hematological parameters were observed. RBC, hemoglobin concentration, decreased after diazinon exposure while WBC, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) showed an increase in the values after treatment with diazinon. Our findings showed that hematological parameters can be used as diagnostic indices to evaluate the health status of *Channa punctatus* after exposure to diazinon.

Keywords: blood, *Channa punctatus*, diazinon, pollution

Introduction
The never ending need of increasing population is continuous supply of good and healthy food. This demand can be fulfilled with advancement of technology and procuring the food material for longer duration. In order to have equilibrium between rising supply and demand, the modern day farming has opted to the use of varied natural and synthetic sources of chemicals to produce and preserve large quantity of high quality of food & products. Pests, weeds and various other insects causing disease in plants and animals are the major drawback in achieving this demand. To have a large production, fertilizers, pesticides, cleaners and crop preservatives are profusely used. Pesticides are major player to protect the crops from pests and weeds therefore they are indispensable to modern agriculture.

The importance of pesticides in India can be understood from the fact that agriculture is a major component of the Indian economy: It contributes 22% of the nation's GDP and is the livelihood of nearly 70% the country's workforce. Globally, due to consolidation in the agrochemical industry, the top five multinational companies control almost 60% of the market. In India, the industry is very fragmented, with about 30 to 40 large manufacturers and about 400 formulators. The use pattern is skewed towards insecticides, which accounted for 67% of the total pesticide consumption in 2006. The potential adverse impact on human health from exposure to pesticides is likely to be higher in countries like India due to easy availability of highly hazardous products, and low risk awareness, especially among children and women.

Diazinon: (O,O - diethyl-O-(2- isopropyl-6- methyl- 4-pyrimidinyl) phosphorothioate) (Garfitt *et al*. 2002) [1] is a contact organophosphate insecticide with a faint ester-like odor. Diazinon was developed in the early 1950 and has a wide range of insecticidal activity (U.S. EPA. 2000. Environmental risk assessment for diazinon. (Preliminary.) Washington, DC.

Diazinon is an organo-phosphorus compound with the empirical formula of $C_{12}H_{21}N_2O_3PS$, a molecular weight of 304.35 and has an octanol/water partition coefficient (log P_{ow}) of 3.40 (Hunter *et al*., 1985; WHO 1998) [2]. It is a colorless oil in its purest form with a density greater than water (1.116-1.118 g/mL at 20°C) and is soluble in water at 20°C to 0.006 percent (40 mg/L, WHO 1998) [2]. The technical product is a pale to dark brown liquid of at least 90 percent purity and has a faint ester-like odor. It decomposes above 120°C (Verschueren, 1983; WHO, 1998) [3, 2], is susceptible to oxidation above 100°C, is stable in neutral media, but slowly hydrolyses in alkaline media and more rapidly in acidic media (WHO 1998) [2]. If stored properly, diazinon has a shelf life of at least three years.

Diazinon acts as a contact stomach and respiratory poison. It is used throughout the world to control a wide range of sucking and chewing insects and mites on a range of crops including deciduous fruit trees; citrus fruit, bananas, vegetables, potatoes, beet, sugar cane, coffee, cocoa, tea, tobacco, cotton and rice. It is used to control agricultural soil - dwelling insects and is applied as a sheep dip to control ectoparasites such as sheep scab.

The fate of diazinon in the aquatic environment is thought to be regulated by two main processes - chemical hydrolysis and microbial degradation. Both processes are influenced by the conditions of pH, temperature and the organic content of the

water. Diazinon is stable at pH 7.0 and can persist in the environment for as long as six months. Diazinon is an exception to other organophosphorus insecticides in that it hydrolyzes at both acidic and alkaline pH's (Gomaa *et al*. 1969) [5]. In the laboratory at 20°C, the half-life was determined to be 12, 4436 and 146 hr at the respective pH's of 3.1, 7.4 and 10.4 (Faust and Gomaa, 1972) [4]. Ku *in* 1998 found that hydrolytic decomposition occurred only for the diazinon-H+ species present in acidic solutions, and that breakage of the P-O bond was the major decomposition step for the hydrolysis of diazinon.

Diazinon is characterized by its high durability in the water environment. In water of neutral pH and of temperature of 20°C the pesticide's half-life equals around 6 months. Altering of pH causes shortening this time so, that at pH 5.0 diazinon's half-life equals 31 days and at pH 9.0 - 136 days. Its faster metabolism in an acidic environment makes diazinon an exception among other organophosphate pesticides.

Diazinon is in the large part bonded in soil, but it can migrate to ground waters, particularly if torrent rain occurs after its application. During a simulation it was found that if heavy rainfall occurs within 2 hours after application of diazinon, the pesticide's concentration in runoff water reaching the concentration around 0.6 mg/dm^3. Following diazinon application at a strawberry farm, 0.15 mg of diazinon was found in 1 dm^3 of rainwater.

Material and Methods

Hematologic parameters can be used as valuable tools in evaluating physiological changes in fish since the blood parameters have been widely used for detection of changes in different conditions (Singh 1995) [7] and are good indicators of toxicity (Abhijith *et al.* 2012) [6]. This study was done to evaluate the impacts of diazinon on *Channa punctatus*. Thirty *Channa punctatus* (weight = 160±10, total length 32.5 ± 7.5 cm) were obtained from Narmada river at Hoshangabad. They were kept for two weeks in glass aquarium for acclimatization due to varying laboratory environment.

They were kept fed by commercial pellets (35% protein and 2% biomass). The fishes (take six fishes from each group of glass aquarium n=30) were kept in each aquarium in triplicates for each treatment. The stock solution of 0.1ppm of the solution was introduced separately in each tank. The fishes were observed for 1-3 hours for any mortality during the exposure time. The healthy stock of fishes (n=30, but take six fishes from each group of aquarium) was kept in aquaria containing 50 litre of freshwater as control. During the exposure in different concentrations of pesticide, the behavioural changes of fish were recorded. Those fishes which did not show any tactile response were considered dead. The dead fishes were removed from the aquaria immediately after death to avoid depletion of oxygen. 5 and 50 ppm concentration of diazinon was maintained in the tanks.At the end of the experiment, the fish were weighed and sacrificed and Blood samples were collected from the caudal tail vessels with 21 or 23 gauze needles and 1 or 3 cc syringe before ventilator response was noticeably depressed. In case of diazinon (5 ppm), the blood samples were collected after 5, 10, and 15 hours. In case of diazinon (50 ppm), the blood samples were collected after 8, 16, and 24 hours the samples were collected from caudal peduncle of fishes. RBCs were counted by using Hayem's solution. WBCs were counted using two different solutions having different chemical composition into the Neubauer chamber. Hemoglobin count was done using Haldane's hemoglobin meter.

Differential WBC counts were conducted by Unna-Zeihl staining method as per standard method prescribed by Romies, 1968. Mean corpuscular volume (MCV), Mean Corpuscular hemoglobin concentration (MCHC) were derived from the RBC, PCV and Hb described by (Housten 1990). MCV was calculated into liters. (PCV/ RBC x 10). MCH was calculated in picograms (Hb / RBC x 10) and MCHC in percent (Hb/HCT x 100). Data were expressed as mean ± standard deviation (S.D). Means were analyzed by one way analysis of variance (ANOVA) test using spss.16 and where significant difference were indicated, means were tested using least significant difference (LSD) test to compare the mean of test groups against that of the corresponding control with a confidence range of $P<0.05$.

Results and Discussion

Table 1: Hematological Parameters (Mean ±SD) at 5ppm diazinon exposure for *Channa punctatus* in still water condition at various time interval.

Parameters	Healthy Control	5 hrs. 30 min	10 Hrs. 30 Min	15 Hrs. 30 Min.
RBC (x 10^6/µL)	2.88±0.670	2.60±0.300	2.20±0.332	1.59±0.179
WBC (x 10^3/µL)	6.03±0.356	7.29±0.496	9.01±0.557	12.29±0.349
Hb (g/dL)	10.30±0.158	9.88±0.148	7.28±0.054	5.24±0.297
MCV (fL)	110.20±1.924	128.20±1.304	136.40±1.140	142.20±2.588
PCV (%)	31.27±1.449	28.32±1.816	23.83±0.923	20.54±1.342
MCH (pg)	35.76±2.358	36.50±3.248	34.30±3.178	40.24±3.655
MCHC (g/dL)	32.94±2.874	33.30±3.154	37.40±4.258	35.10±3.265
LYMPHOCYTES (x 10^3/µL)	32.22±1.518	38.25±1.464	41.30±1.493	48.86±1.068
MONOCYTES (x 10^3/µL)	6.60±0.265	6.10±0.150	5.33±0.333	4.22±0.122
BASOPHILS (x 10^3/µL)	10.02±0.338	9.24±0.442	7.81±0.276	6.44±0.341
EOSINOPHILS (x 10^3/µL)	11.70±0.845	10.22±0.654	9.26±0.279	7.34±0.355
NEUTROPHILS (x 10^3/µL)	18.51±0.515	12.37±0.563	9.46±0.204	7.23±0.086
THROMBOCYTES (x 10^3/µL)	28.79±2.860	27.29±1.946	25.91±1.583	21.21±2.002

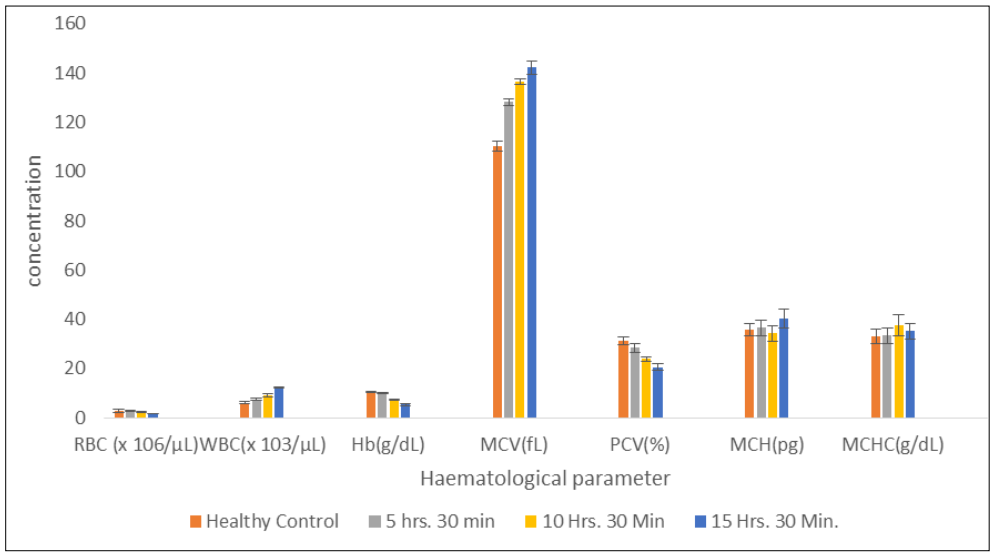

Fig 1: Graphical representation of some hematological parameters of *Channa punctatus*

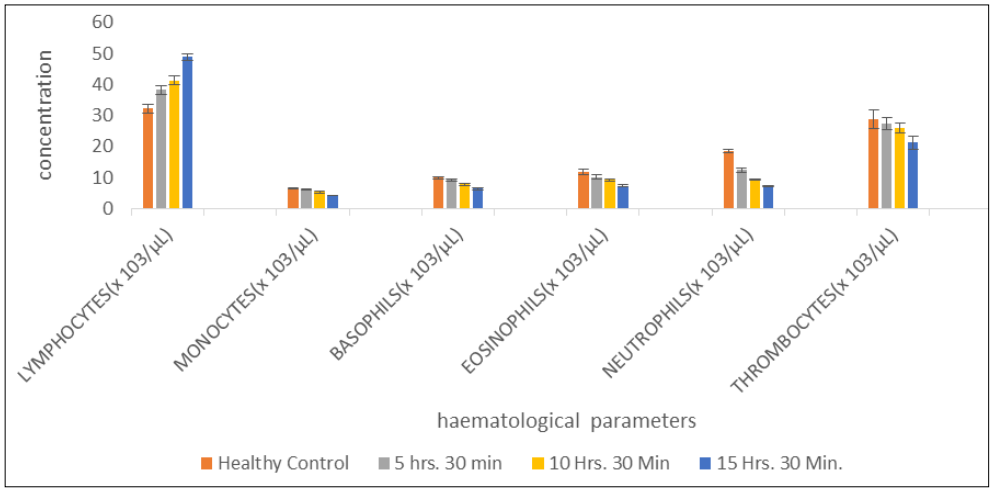

Fig 2: Graphical representation of some hematological parameters of *Channa punctatus*

Table 2: Hematological parameters (Mean ±SD) at 50ppm diazinon exposure for *Channa punctatus* in still water condition at various time interval.

Parameters	Healthy Control	8 hrs.	16 Hrs.	24 Hrs.
RBC (x 10^6/µL)	2.88±0.670	2.75±0.190	2.68±0.189	1.04±0.058
WBC (x 10^3/µL)	6.03±0.356	5.89±0.334	4.49±0.372	3.90±0.418
Hb (g/dL)	10.30±0.158	10.04±0.404	9.20±0.485	7.30±0.381
MCV (fL)	110.20±1.924	99.40±3.647	24.80±2.588	20.87±1.632
PCV (%)	31.27±1.449	30.15±1.531	24.54±1.127	20.83±1.104
MCH (pg)	35.76±2.358	38.00±3.567	33.00±3.120	32.00±3.526
MCHC (g/dL)	32.94±2.874	34.88±3.158	30.54±3.487	25.51±2.145
LYMPHOCYTES (x 10^3/µL)	32.22±1.518	40.14±2.179	47.77±1.948	52.30±1.374
MONOCYTES (x 10^3/µL)	6.60±0.265	6.07±0.515	5.72±0.295	4.91±0.449
BASOPHILS (x 10^3/µL)	10.02±0.338	9.10±0.675	7.88±0.461	6.83±0.495
EOSINOPHILS (x 10^3/µL)	11.70±0.845	10.88±0.739	8.75±0.575	7.05±0.510
NEUTROPHILS (x 10^3/µL)	18.51±0.515	13.11±0.761	10.82±1.171	8.59±0.410
THROMBOCYTES (x 10^3/µL)	28.79±2.860	27.28±2.023	25.25±2.237	22.55±1.892

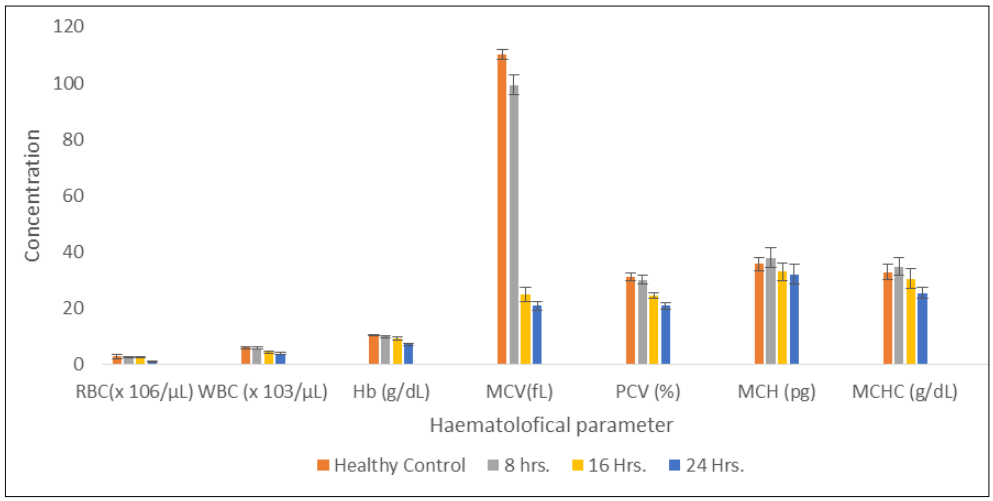

Fig 3: graphical representation of some hematological parameters of *Channa punctatus*

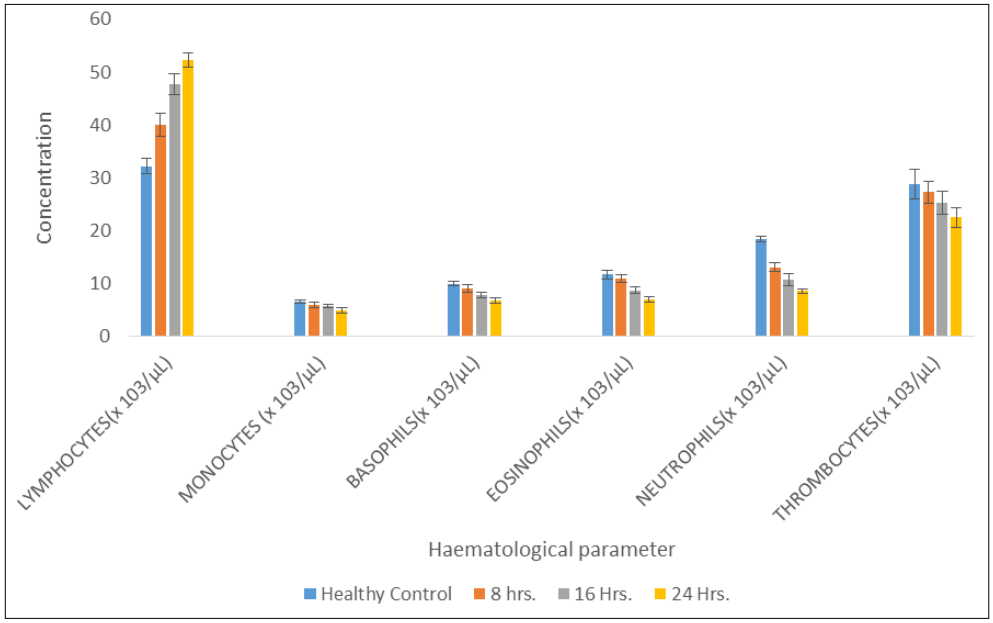

Fig 4: Graphical representation of some hematological parameters of *Channa punctatus*

The effect of diazinon exposure on hematological parameters of exposed fish is show in table one 1. RBCcounts, Hemoglobin concentration, MCHC, MCH and MCV value decreased after diazinon exposure (5ppm) while WBC count showed an increase. The number of lymphocytes increased but monocytes, basophils, eosinophils, neutrophils. Thrombocytes decreased significantly in experimental groups. The number of eosinophil's was not affected by phenol treatment. Dose dependency was only recorded in RBC, WBC, Hemoglobin, MCV, PCV, lymphocytes, monocytes, basophils, eosinophils, neutrophils, thrombocytes counts.

In case of diazinon exposure at 50 ppm, RBC, WBC, Hb, MCV, PCV, MCH, MCHC, decreased significantly after exposure. (Table 2) The differential WBC count was also affected upon exposure. The counts of lymphocytes increased while monocytes, basophils, eosinophils, neutrophils. Thrombocytes also decreased.

Hematological parameters can be used as accurate reliable laboratory diagnostic in diseases by fish breeders, administrators and scientists as to know the state of health of fish population and to make warning about deleterious effects of compounds (Forrest *et al.* 1981) [8]. It has been revealed that may changes in environmental conditions causes a change in the blood parameter (Shreshth *et al.* 2013) [9].

In study a decrease in RBC count and Hb were recorded that is in accordance with the effects of diazinon on hematological properties of Common carp *(Cyprinus carpio)* (Chen 2002; Svodoba *et al.*, 2001). After exposure to 5 and 50 ppm diazinon for 24 hours, a decrease in the number of RBC and Hemoglobin content was observed. Decreased numbers of RBC may be caused by destruction of cells (Javed *et al.* 2013) [10]. The decrease in RBC and Hb concentration is considered as anemia and could be due to decreased haematopoiesis. The anemia could be caused by destruction of RBC (Javed *et al.* 2013) [10]. The observed decrease in hematological parameters could be caused by decreased rate of RBC production (Larsson 1975). Another reason for RBC suppression could also be damage to the hematopoietic tissue.

There was a significant increase in WBC count as well as in MCV, MCHC, MCH, lymphocytes count in the experiment groups after exposure to diazinon (5 ppm). These changes are evidence of increased nonspecific immunity in fish after acute exposure to diazinon. Stressors such as pollutants could evoke nonspecific responses which enable the fish to cope with them for maintenance of the internal Homeostatic state. (Diaz-Resendiz *et al*. 2015) [12].

Ours findings showed that diazinon has deleterious consequences on fish hematologic parameters, so they could be used as indicator of exposure to diazinon and other organ phosphorus compounds.

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