

Effect of Pesticides 2, 4-D on Hematological parameters of *channa punctatus*

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Abstract

Pesticides are chemicals used for pest control in the agricultural fields. Modern agricultural pesticides result in indiscriminate use of various pesticides. Which usually enter into the aquatic environment. They finally reach the surrounding water bodies through surface runoff affecting the aquatic fauna. 2, 4-D is frequently used pesticides due to its effectiveness and rapid breakdown into environmentally safe products. A 96hr static sub-lethal concentration toxicity test was carried out to determine the LC₅₀ value of 2, 4-D on the snake head fish *channa punctatus*. The fish were exposed to 5 different concentrations of 2, 4-D (40, 45, 50, 55 & 60ppm) for toxicity. Control (0.0ppm) was also carried out. The data were subjected to Finney's Probit analysis and processed with Trimmed Spearman-Kärber statistical software. The LC₅₀ values for 2, 4-D for 24, 48, 72 and 96hr were 4.87, 3.12, 2.69 and 1.92 mg l⁻¹ respectively. At higher concentration of 2, 4-D (3.25 mg l⁻¹ and above) the fish showed uncoordinated behavior such as erratic and jerky swimming, attempt to jump out of water, frequent surfacing and effected the all over of body.

Key words: 2, 4-D (Dichlorophenoxy Acetic acid), LC₅₀ values, *channa punctatus*.

1. INTRODUCTION

The pesticides are frequently used on crops and animals in the integrated farming practices, for the eradication of a wide range of insect pests, mites and weeds. The use of pesticides has been recognized as part of agricultural practices throughout the world. The indiscriminate use of the pesticides worldwide for agricultural and other activities poses great threat to the environment. According to 1996-97 market estimates (US EPA, 2001), about 5700 million pounds of pesticides were applies throughout world. Nearly three million cases of pesticides poisoning are reported annually (WHO, 2001). Pollution of water ultimately affects human health. Water is as indispensable natural resource on this earth on which all life depends. The earth's surface is covered by water and most of the animals and plant have 60-65% water in their body. With increasing human population and rapid development like urbanization and industrialization water pollution is also increasing.

Organophosphates (op) are one of the most preferred pesticides due to their high effectiveness and low persistence in the environment. OP pesticides directly inhibit acetyl cholinesterase enzyme activity in fishes and invertebrates (Fulton and Key, 2001; Rao et al., 2005; Agrahari et al., 2006). According to Das and Mukherjee (2000) and Ghosh (1989) reported an increase in blood glucose of *Labeo rohita* and *Clarias batrachus* when exposed to sub lethal concentration of quinalphos and organophosphates, 2,4-D, (Cicik & Engin,2005) reported significant decrease in the levels of glycogen reserves in the proteins, lipids and muscle tissue of *Clarius batrachus* when sub lethal concentration of 2,4-D and cadmium. The principal use is for the control of broad leaf weeds in cereal crops including wheat, maize, rice and sorghum and grassland and turf areas.

2, 4-D has soil persistence. The half-life in soil is less than 7 days (Wauchope et al., 1992) Soil microbes are primarily responsible for its disappearance. Despite its short half-life in soil and in aquatic environment, the compounds have been detected in groundwater supplies in at least five states in Canada. Modern agricultural practices result in indiscriminate use of various pesticides, which usually enter into the aquatic environment. The use of pesticides in the field has the potential to change the aquatic medium affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystems. These pesticides adversely affect the non-target organisms,

especially fish. The present study discusses among other issues, the toxic effects of pesticides on aquatic life with emphasis on fish and the public health implication.

The studies have been made largely with reference to the growth and physiology of the snake head fish species *Channa punctatus*. The extent of the toxicity of pesticides in fishes has been studied at varying concentration of pesticides 2, 4-D (Dichlorophenoxy Acetic acid). The purposes of this study are to evaluate toxic effect of 2, 4-D on snake head fish determining some hematological parameters changes in behaviors and mortality of *Channa punctatus*.

REVIEW OF LITERATURE

The need of the industrial development has grown many folds in the present day's civilized society. Pesticides are categorized according to their target use. The three major group of pesticides and herbicides (weed control). They are widely applied to agriculture crops, forests, lands, gardens and lawns. The principal use is for the control of broad leaf weeds in several crops including wheat, maize, rice and sorghum and grasslands and turf areas. It is also widely used in mixture with other herbicides to provide weed control in forestry, orchards and non-crops areas and for the control of aquatic weeds. 2, 4-D weed control on US wheat relies on little else and global use is predicted to grow over the next decade (1994). In the US where it was the third most used pesticides in the early to mid 1990s, over 31000 tonnes of 2, 4-D were used annually (4 sep. 1993). In aquatic environments, microorganisms readily degrade 2, 4-D. Rates of breakdown increase with increased nutrients, sediment load and dissolved organic carbon. Under oxygenated conditions the half-life is one week to several weeks. 2, 4-D interferes with normal plant growth process. Uptake of the compound is through leaves, stems and roots. Breakdown in plants is by variety of biological and chemical pathways (EPA, 1987). 2, 4-D is toxic to most broad leaf crops, especially cotton, tomatoes, beets and fruit trees (EPA, 1995). There is growing scientific consensus that numerous industrial and agricultural chemicals have the ability to interfere with various activities of all animals including fishes.

MATERIAL AND METHOD

In the present investigation toxicity of 2, 4-D (Dichlorophenoxy Acetic acid) of fresh water fish *Channa punctatus* has been studied. The effect of pesticides in the form of (Dichlorophenoxy Acetic acid) on *Channa punctatus* were determined by APHA (1985) and EIFAC (1983). Fish *Channa punctatus* (wt-28.56±1.78g; length-13.9±1.18cm) were collected from Betwa river of Jhansi. The collected fish were maintained in glass aquaria containing 100L of chlorinated tap water for acclimatization to laboratory conditions for one week. This water in aquaria was aerated continuously then dead animals were removed to avoid any contamination.

They were fed on alternate days with wheat flour mixed together with soya bean flour and mustard cake in ratio of 3:1:1. Test water qualities were as follows:

- Temp : 26.9⁰ C±1.5⁰ C
- PH : 7.5±0.04
- Dissolved oxygen : 6.6±0.2 mg/l
- Alkalinity : 2.4±2.8 mg/l

Determination of LC₅₀ value of 2, 4-D

The 24 hr., 48 hr., 72 hr., 96 hr. LC₅₀ value of 2, 4-D (Dichlorophenoxy Acetic acid) was found to be 3.25 mg/l in *Channa punctatus* using the Probit analysis method described by Finney (1971).

Hematological study:

For hematological study the fish was exposed to various sub lethal concentration (40, 45, 50, 55 & 60ppm) of 2, 4-D for a period of 7 days. At the end of 7 days exposure period blood samples were collected from the fish species from each concentration and preserved with anticoagulant with blood soluble 2ml sample was prepared using 10 to 15 min. A centrifugation for serum protein estimation and studied under a microscope. Then blood sample were prepared using a Barker Hauskley counting slide, Haymen's diluting fluid (NaCl 1% Na₂SO₄) and 2, 4-D(2.5%), hematocytometer, WBC's, RBC'S pipette of help from studied under a microscope and for study out the hemoglobin percentage of fish blood samples with help of N/10 HCl and hemoglobin meter.

It's after single and daily administration of 2, 4-D for intervals of 7 days. It's given to fishes 2, 4-D. The blood samples were collected from each fish by puncturing help of syringe the lateral line of fish with down vertebral column help of capillaries. The collected blood samples help of sterilized needle were analyzed for hematological parameters.

(1) Hematological components:

(A). RBC count (Neubaur's Chamber)

(B). Hematological percentage (shale's apparatus)

(2)Statically analysis: The obtained were exposed as mean +S.E. significance of different compared to the control group of different compared to the control group was determined using student t-test.

(3)Total RBC count: In the present study effect of the *Channa punctatus* at a sub lethal concentration level of 1.5mg/l was studied for 24hr, 48hr, 72hr and 96hrs. During acute administration RBC level is showing decrease at in early duration, which gradually increased at longer duration and reaches at normal value. But during administration RBC count decreased significantly. The EC dependent reduction in number of RBC may have resulted from hemolytic activity of the extract. It could also be a result of suppressive action of the extract on erythropoiesis. Those saponins are known to cause hemolysis of RBC.

The experimental animals were divided in six groups.

Group-I(Control)	Normal
Group-II(Exposure)	2,4-D 40 ppm once daily for 35 days
Group-III(Exposure)	2,4-D 45 ppm once daily for 35 days
Group-IV(Exposure)	2,4-D 50 ppm once daily for 35 days
Group-V(Exposure)	2,4-D 55 ppm once daily for 35 days
Group-VI(Exposure)	2,4-D 60 ppm once daily for 35 days

After 35 days the blood from the control and mercuric chloride treated fishes was obtained and collected in Eppendorf tubes containing EDTA anticoagulants (Mg. Benka *et al.*, 2003). These treated and control blood samples used to estimate the hematological parameters.

MORTILITY:

The fish which showed not activity and did not respond to mechanical stimuli were counted for morality. Death of 3 fishes occurred during acclimatization period.

The number of dead fish was recorded. Fishes were removed immediately from the test aquarium. No mortalities were observed during the experiments.

BEHAVIOURAL CHANGE:

During the period of experiment we daily observe the behavior of all groups of fishes.

BODY WEIGHT:

During the period of experiment we daily observe the body weight of all groups of fishes. At the staining of experiment the average weight of fishes is 18-25gm.

HAEMATOLOGICAL STUDY:

Total Count of RBC

Total red blood cells (tRBCs) were counted using an improved Neuban haemocytometer (Shah and Altindag 2004a). Blood was diluted 1:200 with Hayens fluid (Mishra *et al.*, 1577). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 106mm^{-3} (Wintrobe, 1967).

Total Count of WBC

Total white blood cells (tWBCs) were counted using an improved Neuban haemocytometer (Shah and Altindag 2005; Mg. Banka *et al.*, 2003). Blood was diluted fluid and placed in haematocytometer for large (15q mm) corner square of the haematocytometer were counted under the microscope (Olympus) at 640x. The total number of WBC was calculated in $\text{mm}^3 \times 10^3$ (Wintrobe, 1967).

Estimation of Hemoglobin:

Hemoglobin (Hb) was determined with a hemoglobin test Kit (DIAGNOVA, Ranbaxy, India) using the Cyanmethmoglobin method. Values of treated groups were compared statistically with control by student t-test. Significant was established at P- 0.05 using the Microsoft excel 2000 programme. Significant of data was further checked with percent change (+ increase and – decrease). In blood parameters of *Channa punctatus*.

RESULTS

Total RBC COUNT:

The erythrocyte count of healthy controls showed a mean value of $1.77, 10^6 \text{mm}^{-3}$. He fishes exposed to sub-lethal concentration of mercuric chloride showed mean values of RBCs as 1.63, 1.54, 1.49, 1.36 and $1.24 \times 10^6 \text{mm}^{-3}$ for 40,45,50,55, and 60 ppm treatment, respectively. The treatment with 2, 4-D was found to inflict a drastic reduction was dosage dependent; as concentration of mercuric chloride increased the RBC levels declines (table). The values mentioned above showed a significant decrease when compared to the control (P-0.05).

Total WBC COUNT:

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 6.40, $\text{mm}^3 \times 10^3$. The fishes exposed to sub-lethal concentration showed the mean values of WBC as 6.40, 9.80, 10.90, 11.80, 12.75 and 13.89 $\text{mm}^3 \times 10^3$ for 40, 45, 50, 55 and 60 ppm of 2, 4-D treatment respectively (table). The value mentioned above showed a significant increase when compared to the control (P-0.05).

ESTIMATION OF HEMOGLOBIN:

The control fishes showed mean value of 75.00 g/dL for hemoglobin. The fishes were exposed to sub-lethal concentration of 2, 4-D showed the hemoglobin mean values of 59.85, 50.27, 41.7 and 33.46 and 28.40 g/dL hemoglobin at 40, 45, 50, 55, and 60 ppm treatment respectively (Table 1). The values for treatments showed a significant decrease when compared to the control (P-0.05).

Table: Total count of RBCs, WBCs and Hemoglobin in the control and 2, 4- D treated *Channa punctatus*.

Concentration of 2, 4- D	No. of RBCs (10^6mm^{-3})	No. of WBCs ($\text{mm}^3 \times 10^3$)	Hemoglobin (g/dL)
Control	1.77	6.40	75.00
40 ppm	1.63	9.80	59.85
45 ppm	1.54	10.90	50.27
50 ppm	1.49	11.80	41.70
55 ppm	1.36	12.75	33.46
60 ppm	1.24	13.89	28.40

DISCUSSION

The LC50 value for 96 hours was 1.92mg l^{-1} for a *Channa punctatus* respectively each test was repeated three times. The data obtained were statically evaluated by Finney's Probit analysis method a 96 hours were found to be several abnormal behaviors such as restlessness, loss of equilibrium increased opercula activities surface to bottom movement, sudden quick movement, resting at the bottom etc. were similar to the observation RadhaKrishaniah K and et al;(1992).however swelling in the abnormal region and gas filled stomach were not observed, which is contrary to the finding of Srivastav ,et al(1985).It is an indication that the effect of pesticides is specific. In recent years hematological variables have been used more to determine the sub lethal concentrations of pollutants (Wedemeyer and Yasutake, 1977). The use of immune system parameters to assess alterations in fishes experiencing 2,4-D exposure and interest in defense mechanisms stem from the need to develop healthy management tools o support a rapidly growing aquaculture industry (Jones).

The results of the present investigation show that the 2, 4-D treatment inflicted a drastic reduction in the total count of RBC's. Reductions in the reduction were dosage dependent. In the Plasma protein levels of fish treated with 2, 4-D may lead to the availability of Free State of the pesticide in the large quantity in the plasma which may interfere with the biochemical mechanisms of fish making it more susceptible to the toxicant. The present study shows the high toxic nature of 2, 4-D on fish, the fishes are very sensitive to the presence of even minute quantities of 2, 4-D and are under server metabolic stress. This study also shows the significance of hematological parameters in assessing the pesticide hazards to fish.

BEHAVIOURAL CHANGE:

In the control condition Group-I fishes showing regular swimming pattern. Later change in swimming pattern was observed. Due to the affect of pesticides the movement of fishes observed that hypersensitivity & fast

swimming of fish changed into sluggishness after ingestion of 2,4-D. This change in behavior of the fish may be due to hormonal imbalance caused by the toxic effect of the pesticides as suggested by Sastry (1981). In the present study it was observed that after a brief period of hypersensitivity and fast swimming the fish became sluggish. Howard (1975) and Mc leay (1977) observed the similar phenomenon of impaired swimming stamina in salmon affected by paper mill effluent.

BODY WEIGHT

During the period of experiment the body weight of all groups of fishes was observed daily. After the fishes were treated with sub-lethal doses of 2, 4-D a slight decline in their body weight is observed.

Pesticides contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, *et al.*, 2007; Vosyliene and Jankaite, 2006; Ashraj; 2005). Fish are widely used to evaluate the health of aquatic ecosystem because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et.al.* 2002; Yousuf and El-Shahawi 1999). The decrease in body weight may be due to anaorexia. Similar views have been given by Adams (1977) and Abdel Rehman *et al.*, (1985). In rats after treatment with various oregano phosphorus pesticide.

HEMATOLOGICAL CHANGE

In recent year hematological variables have been used more to determine the sub-lethal concentration of pollutants (Wedemeyer and Yasutake, 1977). The uses of immune system parameters to asses alterations in fishes experiencing heavy metal exposure and interest in defense mechanism stem from the need to develop healthy management tools to support a rapidly growing aquaculture industry (Jones, 2001).

The results of the present investigation show that 2, 4-D treatment inflicted a drastic reduction in the total count of RBCs. The reduction was dosage dependent.

In the fish *Channa punctatus* exposed to 2, 4-D hemoglobin percentage decreased significantly. This may be due to a decreased rate of production of Red blood cells or an increased loss of these cells. Gill and Epple (1993) have attribute anemia to: (I) impaired erythropoiesis due to a direct effect of metal on hematopoietic centers (Kidney/Spleen), (ii) accelerated erythroclasia due to altered membrane permeability and/pr increased due to altered membrane permeability and/pr increased mechanical fragility, and (iii) defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions.

Hematological studies done by Mc Kim *et. al.* (1976) reported that mercury accumulates in the fish blood. Decline in Hemoglobin and heamatocrit was observed in ophicephalus (*Channa*) *punctatus* exposed to Diazinon (Sastry and K.Sharma. (1981). Thus the accumulated could produce certain hematological and biochemical changes in blood. Heamatocrit decreased significantly in the pesticides treated fish when compared with the control fish. The distributed hemoglobin synthesis due to an effect of 2, 4-D on ALA-D may result in Anemia (Santos and Hall, 1990).

White blood cells play a major role in the defense mechanism of the fish and consist of granules. Monocytes, lymphocyte and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Ellis *et al.*, 1978; Wedember and McLeay, 1981).

In the present investigation, leucocytes concentration, eosinocyte concentration showed greater and quite different pattern of change with the effect of mercury when compared with the erythrocyte levels of the control group. Blood of all experimental groups contained higher concentration of leucocytes than those of controls. An increase in lymphocyte number may be the compensatory response of lymphoid tissues to the destruction of

circulating lymphocytes (Shah and Altindag, 2005). Allen, (1994) observed increased WBC (leucocytes) counts in *Oreochromis aureus* after 2, 4-D exposure.

The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by 2,4-D. Gill and Pant, (1985) have reported that the stimulation of the immune system causes an increase in lymphocyte by an injury or tissue damage. Exposure to mercuric chloride induced variations in differential leucocytes counts and caused lymphocytosis neutrophilia, monocytosis, eosinophilia and thrombocytopenia in *Anabas testudineus* (kumar *et al.*, 2004). Total WBC count and leucocytic increased in *Tinca tinca* exposed to lethal and sub-lethal treatments with 2, 4-D (shah and altindag, 2005).

CONCLUSION

In the present investigation, it has been observed that the pesticides 2, 4-D have affected the fresh water fish *Channa punctatus*. The concentration of the pesticides affected the fish. The behavior of the fish including swimming movements was also found to be affected by the pesticides. Body weight was observed to be decreased. Hematological parameters changes of fish been studied and found that these hematological parameters are affected by these pesticides producing hematological parameters are affected by these pesticides pollutants producing hematological parameters lesions. The present investigation show the 2,4-D pesticides caused immunological impairments in *Channa punctatus*, which suggest that the pesticides may be weaken the immune system and may result in severe physiological problems, ultimately leading to the death of fish.

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