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Research article

Haematological changes induced by the deltamethrin a synthetic pyrethroid technical grade and 11% EC (Decis) in the fish *ctenopharyngodon idella* (valenciennes)

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Keywords: *Ctenopharyngodon idella,* Deltamethrin, Technical grade, 11% EC (Decis) blood parameters, RBC, WBC, Hb, Ht, MCV, MCH and MCHC.

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Abstract

Haematological changes induced in the fish, *Ctenopharyngodon idella* after exposure to the two toxicants deltamethrin technical grade and 11% EC (Decis) are studied. They are erythrocytes (RBC) White Blood Corpscules (WBC), Haemoglobin (Hb), Haematocrit (Ht), Mean Corpscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpscular Haemoglobin Concentration (MCHC) when the fish are exposed to lethal and sublethal concentrations of both the toxicants. Among the blood parameters studied changes/alterations are observed as decrement in RBC, HB and Ht whereas WBC is increased. Accordingly, the calculated values also changed. A culturable fish, one, like in the present study, needs to be carefully monitored in cultarable water quality which when contaminated either directly or indirectly.

Introduction

Any impairment of water by adding contaminants either directly or indirectly which is mainly anthropogenic render it unfit for drinking and support the biotic communities, such as fish. Pesticides have a lion's share as agricultural chemicals in its usage and become potentially harmful to these life forms instead of sustaining them [1]. A marketing strategy is that, these are formulated and used. In their usage either by indiscrimination or ignorance which is caused by more than 50% of water pollution and is by such agricultural chemicals only. Being present in water due to such activity, the concentrations are such that, either acute causing immediate death or chronic (delayed) effects which are termed as lethal and sub lethal that will affect the fish which occupy an important group among vertebrates. If such effects are haematological, the poikilothermic / cold blooded vertebrates the sustenance is questionable and there is every loss of population continuum.

Among the studies of alterations, the blood will be an indices serving as a biomarker of the toxicant study. Hence in the present study an attempt is made to evaluate the changes in the blood, after exposure to the toxicants, deltamethrin technical grade as well as 11% EC (Decis) which belong to synthetic pyrethroid group, type II with cyano group induced in the fish, *Ctenopharyngodon idella* (Valenciennes).

Experimental

Material and Methods

Collection and maintenance of test organism

The freshwater fish *Ctenopharyngodon idella* (Ctenopharyngodon and Idella are both Greek words, meaning "comb-like throat-teeth and "distinct" respectively. The grass carp is one of the largest members of the minnow family. The body is oblong with moderately large scales, while the head has no scales. There are three simple and seven branched rays on the dorsal fin. Grass carp are silvery to olive in color, lacking the golden hue of common carp, and they have no barbells) 3 to 5 cm in length 4 to 5 gms in weight irrespective of their sex, have been chosen as the test organisms for the present investigation. Healthy and active fish were obtained from local fish farms, Nandivelugu, Guntur district, Andhra Pradesh, India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°C. Water was renewed every day with 12-12h dark and light cycle and the one used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographical conditions as physical and chemical properties of water were: Turbidity-8 silica units. Electrical conductivity at 28°C – 816 Micro ohms/cm, pH at 28°C-8.1, Alkalinity: Phenolphthalein-Nil mg/l, Alkalinity; Methylorange-172mg/l, Total Hardness (as $CaCO_3$-232mg/l, Carbonate Hardness (as $CaCO_3$)-232mg/l, Non Carbonate Hardness

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(as $CaCO_3$)-Nil mg/l, Calcium Hardness (as $CaCO_3$)-52mg/l, Magnesium Hardness-40mg/l, Nitrite Nitrogen (as N)-Nil mg/l, Sulphate (as $SO4_2$)-Trace mg/l, Chloride (as Cl^-)-40mg/l, Fluoride (as F^-)-1.8 mg/l Iron (as Fe)-Nil mg/l, Dissolved Oxygen-8-10 ppm, Temperature-28+2°C. During the period of acclimatization, the fish were fed *(ad libitum)* with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organism [2-4] were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded. The technical grade which was 95-98% pure which was supplied by the Tagroos Chemical India Ltd., Chennai 600 008. The pesticide 11% EC (Decis) is locally purchased, manufactured by GIDC Industrial Estate Limited 629/630 Gujarat marketed by Sikko Industries Ltd., Ahmedabad. The toxicant pesticides were introduced into water from where the pesticide entered into the fish through gills. A total of 50 fish were taken each in sublethal and lethal concentrations for both technical grade and 11% EC (Decis). The 96 hrs LC_{50} values are determined by Finneys probit analysis (1971) as mentioned in [2-4]. Haematological changes were determined at the end period of exposure to 96 hrs LC_{50} value (0.331) for Technical grade and (0.0331) for 11% EC(Decis) and also $1/10^{th}$ of LC_{50} of 96 hrs exposed for 10 days for both technical grade and 11% EC (Decis) and the concentrations are (0.172) and (0.0172) respectively. 50% of the organisms were dead in lethal concentrations and 1% in sub lethal, while the remaining live organisms were sacrificed during the experimentation in both lethal and sub lethal concentrations for assays of blood parameters.

Sampling of blood

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood sample was collected by caudal severance from the disease free test fish during early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

Haematological examination

The haematological variables analyzed were red blood cells count (RBC), haemoglobin (Hb), white blood cells count (WBC), Haematocrit (Ht), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Determination of red blood corpuscles (RBC) count

RBC count was determined with an improved Neubauer crystalline counting chamber [5]. The blood was sucked up to 0.5 mark on the RBC pipette and immediately Hayem's solution as a diluents stain was drawn upto 101 mark and the pipette was rotated between the thumb and the forefinger to facilitate adequate mixing of the solution (dilution: 1:200). The counting chamber and the cover glass were cleaned thoroughly and cover glass was placed in position over the ruled area. The fluid from the stem of the pipette was expelled as it contains only the diluting fluid. The pipette was then held at an angle of 45° with the tip of the pipette at the junction of the edge of cover glass and the counting chamber. A pint of blood was placed from the tip of the pipette on the central platform near the edge of the cover slip, so that it was sucked up between the central platform and cover slip by the capillary force. The cells were allowed to settle for 2 to 3 min. The ruled area of the counting chamber was focused under the microscope and the numbers of RBC were counted in 80 small squares (4 squares of 16 at the four corners and one of 16 at the centre). The cells touching the upper and left hand lines were counted. The cells touching the lower and the right hand lines were omitted.

The numbers of RBC per sq mm were calculated as follows

The area of Small Square: 1/400 sq mm
The depth of the counting chamber: 1/10 mm
Therefore the volume of a small square is: 1/400 x 1/10= 1/4000cumm
The dilution of blood is 1:200
Total number of RBC = n x 4000 x 200/80
n =Number of cells counted in 80 small squares

Determination of white blood corpuscles (WBC) count

WBC count was determined by using method described by Donald and Bonford [6]. The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to the 101mark above the bulb (the dilution fluid consists of 1.5ml of glacial acetic acid and 1 ml of aqueous gentian violet solution and made up to 100 ml with distilled water). The solution was mixed thoroughly by shaking gently and allowed to stand for 3 min. Cleaned Neubauer counting chamber and cover glass were placed over the ruled area. Excess solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 40° and allowed to stand for 2 to 3 min. The WBC was counted in the four corner square millimetres and the number of WBC per cubicmillimetre was calculated.

Estimation of haemoglobin (Hb)

Hb concentration in the blood was estimated by cyanmet haemoglobin method [7]. Hb is converted into cyanmet haemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank.

Determination of packed cell volume (PCV) or Haematocrit value

Packed cell volume was determined by micro haematocrit method [8]. The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000 rpm for 30min. The relative volume of the height of the RBC's packed at the bottom of the haematocrit tube was recorded as packed cell volume in terms of percentage of total blood column taken in the haematocrit tube.

Determination of mean corpuscular volume (MCV)

MCV indicates the average size of the blood cell in a given sample of blood. MCV was calculated by the following formula and expressed as femtoliter (fL).

MCV = Haematocrit (%) x10/RBC count

Determination of mean corpuscular haemoglobin (MCH)

MCH represents the average content of the HB in each red blood cell. MCH is influenced by the HB concentration and the number of RBC. MCH was calculated by the following formula and expressed in picogram (pg).

MCH = haemoglobin *(*g/dL*)* x 10/RBC count

Mean corpuscular haemoglobin concentration (MCHC)

MCHC reflects the average concentration of the haemoglobin in the red blood cells in the blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g%).

MCHC = haemoglobin (g/dL) x 100/haemoglobin (%)

Results and discussion

Results

The blood parameters alterations in both lethal and sub lethal concentrations of technical and 11% EC (Decis) are presented in table 1 and graphical image as figure 1 & 2.

The RBC count decreased in both lethal and sub lethal concentrations and more percent of decrease in 11% EC. The WBC count increased in both lethal and sub lethal more so in 11% EC.

The haemoglobin content decreased in both lethal and su blethal concentration more so in 11% EC. The haematocrit values altered accordingly to RBC values and also the calculated values MCV, MCH and MCHC showed changes accordingly.

Table 1. Haematological parameters of fresh water fish *Ctenopharyngodon idella* exposed to lethal and sublethal (1/10 of LC_{50} of 96h) concentration of both technical grade and 11% EC of Deltamethrin

Parameters	Control		Exposure			
			Lethal		Sublethal	
			TG	11% EC	TG	11% EC
RBC x 10_6/mm^3	5.02±0.3	>	3.96±0.22 [78.88]**	3.42±0.58 [68.2]**	2.84±0.02 [56.57]**	2.64±0.28 [52.58]***
WBC x 10^3/mm^3	8.50±0.65		7.6±0.18 [89.41]*	9.38±0.3 [110.0]*	10.22±0.18 [120.2]*	12.76±0.52 [150.11]**
Hb g%	20.44±0.98	>	18.4±1.3 [90.02]*	16.82±0.69 [82.29]*	14.04±0.96 [66.68]**	10.84±0.58 [53.03]**
HCT %	61.8±41	>	66.6±5.4 [107.80]*	56.6±1.2 [91.60]^{NS}	53.50±0.75 [86.60]*	47.86±2.57 [77.44]**
MCV μm^3	244±6.0	>	326±8 [133.60]*	230±10 [94.30]*	344.8±16.6 [140.98]**	378.8±24 [155.0]**
MCH (Pg)	84.2±7.0	<	168.4±3.3 [200.00]^{NS}	82.6±21 [98.09]^{NS}	100.40±2.6 [119.24]*	86.72±6.64 [103.00]^{NS}
MCHC (g/dl)	68.2±4.6	>	56.8±1.6 [83.30]*	59.2±1.0 [86.80]*	54.08±0.66 [79.30]*	47.06±1.77 [69.00]**

Mean ± SD value differ significantly P<0.05 Witheir the same column

*Significant value *P<0.05 **p<0.01 ***P<0.001 NS=Non-Significant values in the parenthesis are percentage change over control treated as 100 percent.

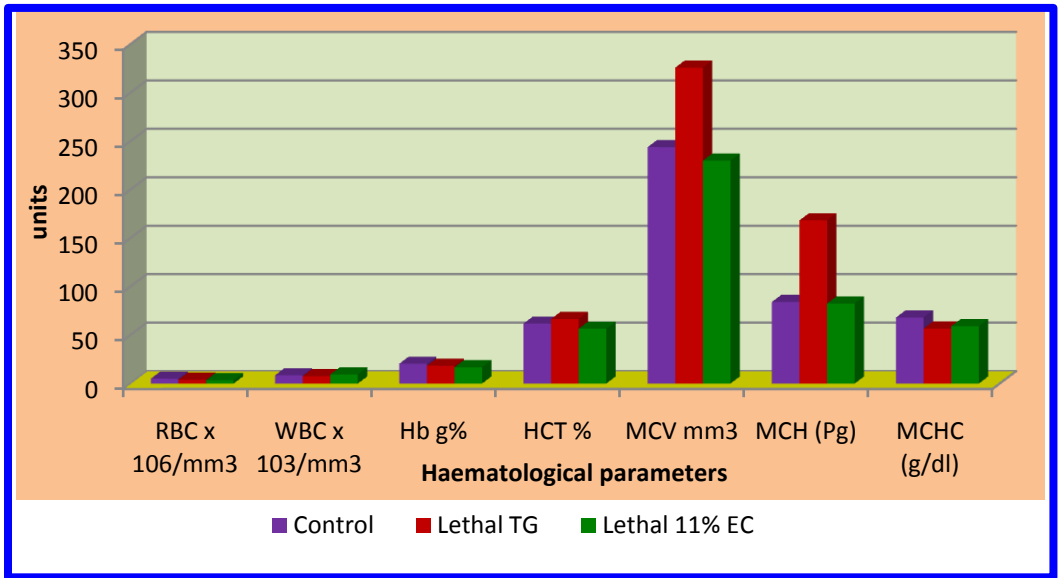


Figure 1. Haematological parameters of fresh water fish *Ctenopharyngodon idella* exposed to lethal and sublethal (1/10 of LC_{50} of 96h) concentration of both technical grade and 11% EC of Deltamethrin.

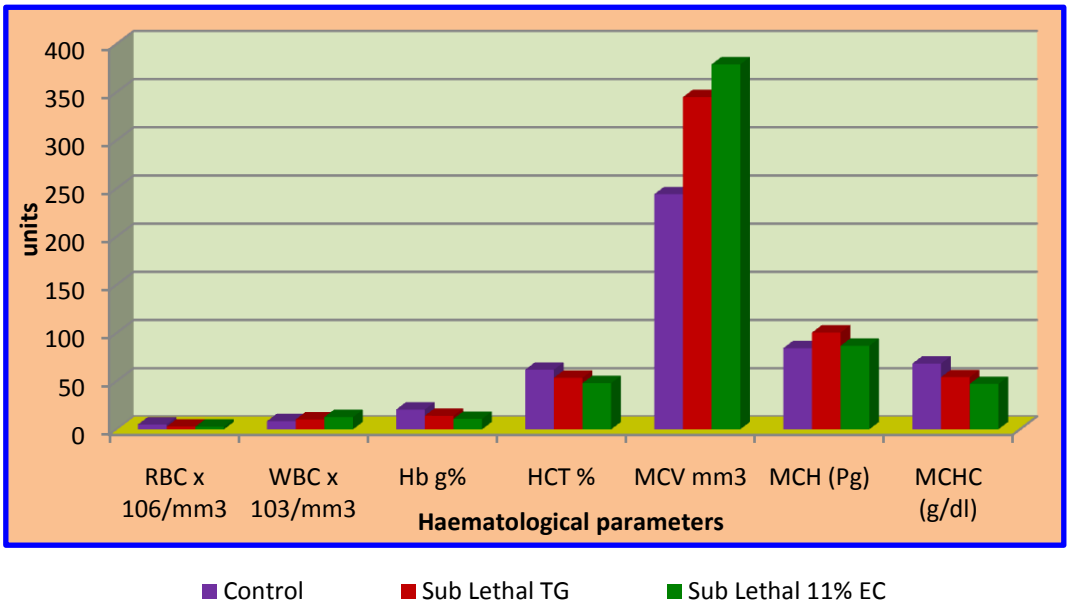


Figure 2. Haematological parameters of fresh water fish *Ctenopharyngodon idella* exposed to sublethal (1/10 of LC_{50} of 96h) concentration of both technical grade and 11% EC of Deltamethrin.

Discussion

The review of the pesticides effects on fish by [9-12] mentioned about the haematological changes which serve as indices of toxicity and are significant biomarkers.

Velisek *et al.* [13] reported the effects of pyrethroids and triazine pesticides on fish physiology. The present study revealed that Deltamethrin exposure to the fish common crap resulted significantly lower values of RBC, HB and PCV, whereas in the rainbow trout significantly higher values of erythrocyte count, haemoglobin content and haematocrit than control group. The results drastically are different in the two fish with the same toxicant with the earliest report of the reference cited.

However, the present study is in agreement with the study of common carp whereas it differs with trout. The study mentioned that the main acute haematological response of rainbow trout and common carp to the effects of pyrethroid was a significant change in RBC, Hb, MCV and MCHC, lymphocyte and segmented neutrophilic granulocyte counts. The report clarified that the reduction in RBC count and PCV value and the higher erythrocyte haemoglobin of fish can be attributed to haemo dilution due to the damage of organs and changes in the haematological parameters PCV, RBC and Hb which can be interpreted as a compensatory response to increase the O_2 carrying capacity of the blood to maintain transfer also indicating a change of the water blood

barrier for gas exchange in gill lamellae. They too opined that the changes of the haematological parameters as results indicated decease in non-specific immunity. The increase of lymphocytes can be attributed to lymphopoesis or altered release of lymphocytes from lymphoid tissue.

According to Ahrar Khan *et al*., [14] a study of Haemato – Biochemical changes induced by pyrethroid insecticide, compared the toxicity and biochemical changes including haematological of Cypermethrin and Deltamethrin. Total erythrocyte count, Haemoglobin, Haemotocrit and Leucocytes at 2 ppm concentration being high along with MCV, MCH and MCHC, but for Deltamethrin at 1.61 µg/L all the values are at low wherein both of them belong to Type II synthetic pyrethroids, with cyano-group but Deltamethrin is more toxic to fish than Cypermethrin. Leucoytosis has been documented after Cypermethrin exposure but for deltamethrin it was leucopenia. But the present study, of *Ctenopharyngodon idella* RBC values decreased more so in 11% EC whereas WBC values are increased more so in 11% EC. Consequently MCH increased whereas Hb, PCV, MCHC, decreased. Such measurement of the toxicity and haematological alterations serve as indices of toxicity. They opined haemotological and biochemical disturbances and damage in the tissues of excretory organ kidney and liver. That is why they recommended doses as permissible limits for this group of insecticides as well to be very cautious related to other insecticides.

Jayaprakash and Shettu [15] reported changes in the haematology of the freshwater fish *Channa punctatus* exposed to Deltamethrin exposed to lower 0.075 mg/l and higher 0.15 mg/l for 15,30 and 40 days. MCV and WBC are increased significantly after the exposure period whereas RBC, Hb, PCV, MCH and MCHC values decreased. The results support the present study. They opined decreased in Hb, TEC and PCV values leading to anemia which is due to impaired absorption of iron. Stress factor leads to changes in the blood parameters.

Tayfun [16] reported the effects of deltamethrin on some haematologicalparameters of brown trout *(Salmo trutta* fario). The fish are exposed for four days, intwo different concentrations of Deltamethrin 0.91 µg/L^{-1} and 188 µg/L^{-1} and that resulted WBC, Hb, PCV, MCV, MCHC decreased however RBC cells increased. The reduction of oxygen carrying capacity in fish may be associated with a decrease in haemoglobin that is affected by Deltamethrin. With this point, the present study is in agreement. The results are quite contrasting of the present study may be due to herbivorous nature of the fish *Ctenopharyngodon idella* in toxic stress behaved differently.

David *et al.,* [17] reported effects of deltamethrin on haematological indices of Indian major carp *Cirrhinus mrigala* (Hamilton). The fish are exposed toboth lethal and sublethal Deltamethrin of 8 mg/L and 0.8 mg/L respectively at 1, 2, 3 and 4 days and 1st, 5th, 10thand 15thday respectively. The results that are reported are RBC, Hb and haematocrit values as decreased, whereas WBC, MCV and MCH were increased MCHC remain unchanged. The results are in agreement of the present study except MCHC. The increase of MCV and MCH values after exposure to deltamethrin indicates that a reduced RBC count which may be due to destruction of erythrocytes (lysis) or erythropenia

Venkata ramudu *et al.,* [18] reported hematological studies in freshwater fish *Channa punctatus* during sub lethal toxicity of deltamethrin in relation to sex. They studied only two parameters RBC and WBC in both males and females of Carnivorous fish *Channa punctatus* (Bloch) exposing the fish to sub lethal concentration for the periods of 24h, 7 day, 15 day, 20 day and 30 day. They reported a decreasing trend in RBC except 24 h period and increasing trend of WBC in all the exposure periods in both sexes. The fish reacted quickly to the stress conditions tried to eliminate the pesticide a detoxification process. They opined presence of pesticide have induced hypoxia which in turn accelerate the *haemopoietic tissue*. They referred Rodriguez *et al., [*19], the decline in RBC count is obviously due to entry of the toxicant into the body of the fish and in turn entitled erythropoesis. They also opined the increase in WBC may be attributed as a work of defensive mechanism against the pesticide that has entered. A homeostatic mechanism change resulted an increase in the WBC. The report is in the agreement of the present study of the fish *Ctenopharyngodon idella* wherein a decrease and increasing trend of RBC and WBC count respectively which ultimately have profound bearing on other parameters Hb, Ht value, MCV, MCH and MCHC.

Haematological alterations of the different toxicants Monocrotophos to the fish *Cyprinus carpio* by Vaiyanan [20], Diazonin by Pourgholam *et al.,* [21] to the fish *Ctenopharyngodon idella.* Renu Bala Singh [22] testing endosulfan as toxicant to *Ctenopharyngodon idella,* Nile tilapia *Oreochromis niloticus* exposed tosublethal concentration of Mercury by Nilton *et al.,* [23], Jaya and Ajay [24]; to the fish *Clarias batracus* by the toxicant Manocozeb, Swarna kumari et al., [25]; Abdul *et al.,* [26] to the fish *Cyprinus carpio* - by the toxicant Dichlorvos; Mallum *et al.* [27] to the fish *Oreochromis niloticus* to the toxicant Dichlorvos. Aliakbar and Niazie [28] to the fish sliver carp by the toxicant Diazion, George *et al., [*29], Jerald felix [30] and Anusirya Devi *et al.,* [31]; Homaira Afreen *et al.,* [32] are noteworthy.

The haematological parameters of the fish altered due to exposure to different types of toxicant synthetic pyrethroids. Type I Permethrin Type II Cypermethrin, Gopala Rao *et al*., [33]; Neelima *et al*., [34]; Tilak and Satyavardhan [35] to Fenvalerate wherein the stress resulted in the blood of the fish, certain parameters swap even in low concentration termed as sublethal. Such

sublethal toxicants slowly is alienation of the time factor to succumb instead of not being in lethal concentration. The ambient waters defilement definitely pose a threat in which, blood changes serve as biomarker as opined by Kaviraj and Gupta [36]. They mentioned higher erythrocyte count haemoglobin haematocrit, MCV, MHC and MCHC fail to harmonize resulting a disturbance of the equilibrium in homeostasis resulting a failure of haemostasis rendering to be unsuitable to lead a normal mode of life. Really, sub-lethal is lethal wherein later there is a happy death but in former is slow suffocative death.

Conclusion

Hence it may be concluded that even in the fish *Ctenopharyngodon idella* grass carp cultured along with the other carps when pesticide contaminate the culture medium alter the constituents of blood and such alterations are more severe in EC due to the ingredients mixed. If RBC is decreased the oxygen carrying capacity is reduced cellular respiration is impaired there by growth is curtailed. Hence stringent measures have to be taken for quality control before giving pesticide representativeness for environmental usage.

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