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Effects of Pesticides on Haematological Parameters of Fish: Recent Updates

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Abstract: **Since the blood takes part directly or indirectly in various physiological processes of the body, haematological parameters are considered important biomarker of alterations in metabolism or physiology. Indiscriminate use of pesticides has polluted different water bodies with adverse effects on the health of aquatic biota including fishes. Toxicological effects of agrochemicals including pesticides can be observed by monitoring haematological parameters. Present review deals with study of effects of pesticides on the important blood parameters such as erythrocyte count, haemoglobin content, packed cell volume, erythrocyte sedimentation rate, absolute values, leucocyte count, coagulation time and thrombocyte count of freshwater fish species. The review also aims to explain possible mechanism of pesticide induced alterations in the parameters and deleterious consequences on fish health. This could help to explore the future prospects of research in the concerned field. Haematological parameters of fish could thus serve as sensitive index to examine health status and to ascertain the toxic effects on ecosystem under pesticide exposure in the era of increased pesticide utilisation.**

Index Terms: **Fish, Pollution, Pesticides, Haematological parameters, Toxic effects**

I. Introduction

Now-a-days, detrimental ecological consequences posed by indiscriminate use of pesticides in agriculture are of great concern in general. Moreover, the increasing trend of Indian population requires self-sufficiency in food production by improved tools and techniques and effective chemicals. Pesticides are one of those agrochemicals that are widely used in agriculture to control different types of pests e.g. insects, unwanted weeds, parasitic nematodes and fungus (Tudi et al., 2021). They are being extensively used not only in the developed countries but also in developing countries to increase food production and its quality. Thus the pesticides have occupied significant and inseparable association with the advance agricultural achievements in our country. In pesticide production and consumption, India is the biggest country in Asia and twelfth in the world while at fourth position for export (Devi et al., 2017).

In past few decades, a considerable number of researches have been carried out in relation to the adverse effects of toxic pesticides on environment and biodiversity. These chemicals are particularly the persistent ones and bring about disturbances in ecological balance in nature and various kinds of health hazards by leaving residues. A very small portion of total pesticides is in fact effective in killing or controlling target pest, while remaining large amount is released in the environment including aquatic ecosystem to have negative impact on non-target species (Tudi et al., 2021; Özkara et al., 2016). There are overwhelming evidences depicting the adverse effects of pesticides on aquatic ecosystem (Barlas 1999; Aktar et al., 2009). They pollute water by discharging surplus formulation after spraying operations into rivers, ponds and lakes, pouring the washing water of spraying equipments into water bodies; extending spread crops to the water's edge, accidental spillage of agrochemical formulations, run-off and erosion from treated areas, fallout from air pollution by agrochemical industrial effluents etc.

Water pollution is the cause of death of several interdependent aquatic forms of life and also a source of bio-magnification of persistent pesticides. This can result in local effect on environment and mortality of fish. Fishes are particularly sensitive to any change in physicochemical as well as biological characteristics of aquatic bodies. The toxic chemicals in aquatic environment are proved to be dangerous for the survival of fish (Caldas et al. 1999; Lamai et al. 1999; Sayeed et al. 2000; Isenring, 2010; Zacharia, 2011). Therefore, fish are regarded as very sensitive biological indicators of any adverse change in the aquatic ecosystem.

Moreover, there are reports of mortality of large number of aquatic animals in different parts of the world by pesticides intoxication. Fish mortality due to pesticides like malathion (organophosphorus pesticide) and aldrin (organochlorine pesticide) groups of chemicals are on the record (Jayaraj et al., 2016; Murthy et al., 2013; Sabra and Mehana, 2015). Types of effects of different pesticides can be variable to a greater extent on fish population. Survival, growth and reproduction of fish are badly affected either by direct death or due to starvation or by destruction of food organisms (Prashanth, 2011; Murthy et al., 2013). Now, it has been established fact that all pesticides are potentially lethal to the fishes even at relatively low concentration (Ullah and Zorriehzahra, 2015).

Further, fish constitute a basic vertebrate fauna with protein rich flesh containing all the 20/22 amino acids. Fishes with good quality protein and essential omega fatty acids occupy an important place in human nutrition as they have high digestibility, biological value and growth promoting value (Nurullah et al. 2003, Ahmed et al., 2020). Therefore, utmost importance should be given to evaluate the adverse effects of toxic pesticides on fish population so as to fulfil the demand of healthy fish. Pesticide resistance is also emerging with the advent of each pesticide in the field. Therefore, this is ever ending race among pest, new pesticide and pesticide resistance (Le Goff and Giraudo, 2019) which further necessitates increased study on the hazard and risk associated with emerging pesticides on non-target species such as fish.

Significant changes in the internal environment e.g. biochemical characteristics, tissue integrity, immune status, blood parameters of fish serve as good biomarkers of stress, toxicant/pesticide exposure, disease onset or any harmful change in water quality. Well established biomarkers studied in various fish species include histopathology, tissue biochemistry, hormones and haematology (Joseph and Raj, 2011). Out of these, study on haematological parameters as biomarker is least invasive as this does not often require killing of fish to obtain blood samples.

The blood may provide accurate information about the effect of pesticides on their exposure in the environment, and in most of the cases, haematological parameters provide significant initial alarm signal of altered physiological condition of fish due to stress, pollution, pesticide exposure or infection. Important haematological parameters of fish include total erythrocyte count (TEC), haemoglobin content, packed cell volume (PCV), erythrocyte sedimentation rate (ESR), absolute values, total leucocyte count (TLC), coagulation time and thrombocyte count (Singh and Srivastava, 2010). Change in the quality of the aquatic environment can be easily assessed by studying the changes in these parameters of fish. In this way, it is a very important tool to study the toxicological effects of pesticides on fish and there are a significant number of reports available in this regard.

In the present review, attempt has been made to compile previously reported scientific information on the changes in haematological parameters of various fish species under the influence of different pesticides. The review would also serve as starting point for future researches to unravel the unexplored and least understood facts of fish haematology under normal or stressed conditions as well as under exposure of newly emerging pesticides and other environmental contaminants.

II. Haematological Parameters of Fish Under Pesticidal Effect

Haematological parameters of fish are becoming indispensable tool to assess the impact of pesticides on fish. Further, it is prerequisite to understand and estimate normal range of haematological parameters of fish under unexposed condition. A review of literature reveals a great variation in the haematological parameters of different fish species (**Table 1**). Pronounced variations in the parameters of the same fish species have also been reported by different authors that could be due to differences in the length and weight of fish sample which they used in their studies. For instance, highly variable data for TEC ($2.16 – 5.96 \times 10^6\ mm^{-3}$) and TLC ($11.64 – 36.22 \times 10^4\ mm^{-3}$) in *Cyprinus carpio* have been reported in different studies (Neelima et al., 2015; Vaiyanan et al., 2015; Rao et al., 2017). Such variations could be attributed to differences in size and weight of experimental fish used by those researchers (**Table 1**). Therefore, these parameters are also subjected to high degree of variation under the influence of different intrinsic factors (viz. age, body size, sex, reproductive cycle, feeding habits, nutritional state) as well as extrinsic factors (viz. season, photoperiod, temperature, dissolved oxygen, water quality, stress, fish density etc.) (Ahmed et al., 2020). Due to such variability, it is very important to maintain all the influencing factors constant while studying the effect of toxic chemicals and pesticides on fish haematology.

A comprehensive review on changes in haematological parameters as bioindicators of insecticide exposure in teleosts has been presented by Singh and Srivastava (2010), a decade before. Therefore, focus of the present review is to encompass recent updates in the field of fish haematology and its relation with pesticidal exposure in the environment. In this connection, a summary of most affected haematological parameters studied in past decade are presented in **figure 1** and in **tables 2, 3 and 4**. Important haematological parameters of fish exposed to different pesticides are discussed under following sections.

A. *Erythrocyte dependent parameters*

1) *Total Erythrocyte Count (TEC)*

It is an estimation of total red blood cells count or total erythrocyte count (TEC) that is a quantitative measure of the population of red blood cells in circulation. Besides erythrocyte count, morphology of RBC is also an important blood parameter that is altered by toxic effect of pesticides. Teleost erythrocytes are nucleated and are of elliptical and biconcave shape. A significant decrease in the total erythrocyte count in different fishes under the influence of various pesticides is reported by Mishra and Srivastava (1984) and Lal et al. (1986) in *Heteropneustes fossilis* due to malathion; and by Kumari and Yadava (1988) in *Clarias batrachus*.

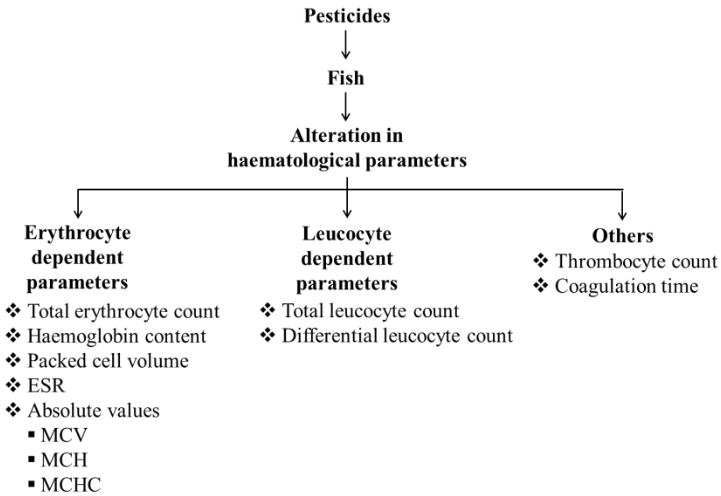

Figure 1. A summary of important haematological parameters of fish studied under the pesticidal exposure. (ESR, erythrocyte sedimentation rate; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration)

Alterations in shape and size of erythrocytes of fishes under pesticidal effects have been reported by Frank (1980) in *Salmo gairdneri* and *Cyprinus carpio*; Kumari and Yadava (1988) in *Clarias batrachus*; and by Sawhney and Johal (2000) in *Channa punctatus*. Pyrethroid insecticide induced decline in erythrocyte count has been observed in *C. carpio* due to cypermethrin (Reddy and Bashamohideen, 1989; Dorucu and Girgin, 2001), *H. fossilis* due to deltamethrin (Ghosh and Banerjee, 1992), *Ctenopharyngodon idella* due to fenvalerate (Shakoori et al., 1996) and in *Sebastes schegeli* due to cypermethrin (Jee et al., 2005).

Comparative analysis of current reports present in the literature reveals that the most investigated agrochemicals for their marked detrimental effects on fish haematology are organophosphorus insecticides followed by pyrethroid insecticides and other pesticides including herbicides and fungicides (**Fig. 2**). In addition, this analysis also suggests that many organophosphates cause significant decline in TEC in most of the fish species investigated. For instance, significant decrease in erythrocyte count was observed in *Channa punctatus* exposed to chlorpyrifos (Ali and Kumar, 2012), *Oncorhynchus mykiss* exposed to Diazinon (Far et al., 2012), *Barbonymus gonionotus* due to quinolphos (Mostakin et al., 2015), *Cyprinus carpio* due to monocrotophos (Vaiyanan et al., 2015), *Ctenopharyngodon idella* due to dichlorvos (Kumari et al., 2018), and in *Oreochromis mossambicus* due to chlorpyrifos (Ghayyur et al., 2019) (Table 2). Exposure to herbicides (atrazine and metolachlor) and fungicide (difenoconazole) also induces anaemic condition due to decreased TEC in *Clarias gariepinus* (George et al., 2017), *Labeo rohita* (Jasmin et al., 2018). Likewise, in recent decade, pyrethroid insecticides cypermethrin, deltamethrin and permethrin have been reported to reduce TEC in different fish species (Jayaprakash and Shettu, 2013; Neelima et al., 2015; David et al., 2015; Rao et al., 2017, 2018).

Significant reduction in erythrocyte count in fishes under pesticidal effect is the reflection of an anaemic condition. Anaemia could be due to destruction of erythrocytes by reactive oxygen species (ROS), which are enormously produced in response to toxic effect of pesticides (Bloom and Brandt, 2008). Erythropoietin is an important factor stimulating erythropoiesis to maintain normal circulating RBC count. Low erythrocyte count in *Sarotherodon mossambicus* has been associated with reduced erythropoietin content due to toxicity of an herbicide diuron (Reddy et al., 1992). Based on these reports, a mechanism of alteration in TEC has been presented in **figure 3**. Prolonged anaemia may be life threatening and often reduce fertility and fecundity of fish. However, significant increase in total RBC count has been reported in *Prochilodus lineatus* exposed to herbicide – clomazone (Pereira et al., 2013) and in *Labeo rohita* exposed to nicotine-mimicking insecticide imidacloprid (Patel and Parikh, 2015). An acute increase in RBC may occur after splenic contraction due to adrenergic stimulus in response to pesticide stress (Heath, 1995).

2) Haemoglobin (Hb) content

Haemoglobin is a respiratory pigment present in blood RBC. In fish, it carries oxygen from gills to the tissues and thereby helps in cellular respiration. Haemoglobin content provides an indirect estimate of the number of RBCs in the blood and is measured in g/dl. Significant decline in haemoglobin (Hb) content of fishes under pesticidal influence have been reported by Frank (1980) in *Salmo gairdneri* and *Cyprinus carpio* due to different pesticides; Mukhopadhyay and Dehadrai (1980) in *Clarias batrachus* due to malathion; Verma et al. (1981) in *Saccobranchus fossilis* and *Mystus vittatus* due to thiotox and malathion; Matthiessen (1982) in *Tilapia* spp due to endosulfan; Dieter (1982) in *Herotilapia multispinosa* and *Tilapia leucosticte* due to organophosphate Lebaycid®; and by Natarajan (1984) in *Channa striatus* due to metasystox.

In recent decade, organophosphate induced anaemia due to significant decrease in Hb content has been reported in *Barbonymus gonionotus* exposed to quinolphos (Mostakim et al., 2015), *Cyprinus carpio* exposed to monocrotophos (Vaiyanan et al., 2015), *Ctenopharyngodon idella* exposed to dichlorvos (Kumari et al. 2018), and in *Oreochromis mossambicus* exposed to chlorpyrifos (Ghayyur et al., 2019) (**Table 2**). Such anaemic conditions have also been observed in fishes exposed to herbicide clomazone (Pereira et al., 2013) and fungicide difenoconazole (Jasmin et al., 2018). Similar trend of reduction in Hb content was reported in several fish species exposed to pyrethroid insecticides cypermethrin, deltamethrin and permethrin (Jayaprakash and Shettu, 2013; Neelima et al., 2015; David et al., 2015; Rao et al., 2017, 2018).

Table 1. Normal range of haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) content, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC) and differential leucocyte counts (neutrophils, basophils, eosinophils, lymphocytes and monocytes) of different fish species as reported by various workers in the field.

Fish species (with length and weight)	TEC ($\times 10^6$ mm^{-3})	Hb content (g/100 ml)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g%)	TLC ($\times 10^4$ mm^{-3})	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)	References
Barbonymus gonionotus (8.11±1.44cm; 5.9 ± 3.61g)	5.19 ±0.54	12.57 ±0.23	46.22 ±2.16	89.05 ±4.12	24.21 ±1.89	27.19 ±1.43	2.84 ±0.13	N.D.	N.D.	N.D.	N.D.	N.D.	Mostakin et al., 2015
Channa punctatus (18.56 ± 2.4g)	3.165 ±0.023	12.537 ±0.309	34.366 ±0.23	108.6 ±0.01	39.62 ±0.10	36.48 ±0.88	18.80 ±0.55	N.D.	N.D.	N.D.	N.D.	N.D.	Jayaprakash and Shettu, 2013
Channa punctatus (N.D.)	2.120 ±0.062	N.D.	N.D.	N.D.	N.D.	N.D.	6.12 ±0.22	62.14 ±0.36	5.25 ±0.42	4.11 ±0.30	22.25 ±0.56	6.25 ±0.36	Parkash, 2016
Channa punctatus (14 ± 3.0cm 30 ± 2.0g)	3.78 ±0.26	12.96 ±0.44	33.80 ±0.53	N.D.	N.D.	N.D.	64.87 ±0.63	N.D.	N.D.	N.D.	N.D.	N.D.	Ali and Kumar, 2012
Cirrhinus mrigala (15-20cm; 20-30g)	1.773 ±0.01	7.386 ±0.01	25.652 ±0.01	71.63 ±0.11	22.961 ±0.12	22.53 ±0.04	10.47 ±0.01	N.D.	N.D.	N.D.	N.D.	N.D.	David et al., 2015
Clarias batrachus (16 ± 2cm; 35 ± 5g)	2.81 ±0.23	9.06 ±0.05	N.D.	91.23 ±0.09	33.03 ±0.13	38.16 ±0.05	0.143 ±0.81	N.D.	N.D.	N.D.	N.D.	N.D.	Shahi and Singh, 2014
Ctenopharyngodon idella (3 to 5cm; 4 to 5g)	3.18	9.53	28.62	90.06	29.78	33.36	14.17	N.D.	N.D.	N.D.	N.D.	N.D.	Kumari et al., 2018*
Ctenopharyngodon idella (3 to 5cm; 4 to 5g)	5.02 ±0.3	20.44 ±0.98	61.8 ±41	244 ±6.0	84.2 ±7.0	68.2 ±4.6	8.50 ±0.65	N.D.	N.D.	N.D.	N.D.	N.D.	Rao et al., 2018
Cyprinus carpio (6 ±2cm; 6 ±2.5g)	2.16 ±0.04	0.46 ±0.33	3.2 ±0.33	N.D.	N.D.	N.D.	36.22 ±0.21	N.D.	N.D.	N.D.	N.D.	N.D.	Vaiyanan et al., 2015
Cyprinus carpio (6 –8cm; 6.5 –7.5g)	02.98 ±0.18	07.06 ±0.64	27.18 ±0.34	62.19 ±0.22	20.54 ±0.14	28.32 ±0.46	11.64 ±0.15	N.D.	N.D.	N.D.	N.D.	N.D.	Neelima et al., 2015
Cyprinus carpio (3 – 5cm; 3 to 6g)	5.96 ±0.36	14.12 ±1.28	54.36 ±0.68	124.38 ±0.40	41.08 ±0.28	56.64 ±0.92	23.28 ±0.30	N.D.	N.D.	N.D.	N.D.	N.D.	Rao et al., 2017
Heteropneustes fossilis (18 ± 2cm; 41 ± 2g)	4.22 ±0.02	4.23 ±0.13	73.75 ±1.75	178.5 5±1.8	21.5 ±1.09	5.73 ±0.002	10.85 ±0.78	31.78 ±1.34	12.48 ±1.10	1.88 ±0.30	25.32 ±1.26	27.78 ±1.62	Tiwari et al., 2017
Labeo rohita (17.31±2.20cm; 111.25±12.13g)	1.01 ±0.10	2.66 ±0.21	14.0 ±1.15	137.2 ±8.65	26.3 ±4.21	18.9 ±1.11	6.64 ±1.12	N.D.	N.D.	N.D.	N.D.	N.D.	Jasmin et al., 2018
Labeo rohita (25 ±3cm; 110 ± 5g)	0.7 ±0.003	3.5 ±0.029	9.8 ±0.03	139.9 ±1.34	35.7 ±0.69	50.00 ±0.05	6.23 ±0.56	79.1 ±0.36	N.D.	N.D.	102.4 ±0.11	N.D.	Patel and Parikh, 2015
Oncorhynchus mykiss (264±40g)	1.02 ±0.16	63.62 ±10.63	0.34 ±0.08	331.14 ±72.54	62.19 ±4.83	199.02 ±54.36	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Li et al., 2010
Oncorhynchus mykiss (2 ± 0.1g)	1.15 ±0.165	7.2 ±0.58	40.7 ±3.9	356.9 ±31	63.6 ±5.1	17.69 ±5.1	1.272 ±0.10	16.1 ±4.8	N.D.	N.D.	82.8 ±4.5	N.D.	Far et al., 2012
Oreochromis mossambicus (12 ±2cm; 25 ±1.9g)	1.95 ±0.04	7.34 ±0.19	23.3 ±0.21	137.2 ±0.42	43.43 ±0.46	29.53 ±0.29	1.047 ±0.09	17.85 ±0.18	N.D.	N.D.	65.49 ±0.18	N.D.	Patel and Parikh, 2015

(Abbreviation: mm, millimetre; g, gram; ml, millilitre; fl, femtolitre; pg, pictogram; N.D., not determined by authors in their study. Values are mean ± standard deviation/error; *, standard deviation/error is not reported

Table 2. Summary of recent updates on alterations in total erythrocyte count (TEC), haemoglobin (Hb) content, packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) in different fish species exposed to various pesticides.

Fish species	Pesticide	Total erythrocyte count	Hb content	PCV	ESR	References
Oncorhynchus mykiss	Carbamazepine	No change	No change	No change	N.D.	Li et al., 2010
Channa punctatus	Chlorpyrifos	↓	↓	↓	N.D.	Ali and Kumar, 2012
Oncorhynchus mykiss	Diazinon	↓	↓	↓	N.D.	Far et al., 2012
Cyprinus carpio	Fenthion	↓	↓	N.D.	↓	Muralidharan, 2012
Channa punctatus	Deltamethrin	↓	↓	↓	↑	Jayaprakash and Shettu, 2013
Prochilodus lineatus	Clomazone-based herbicide – Gamit® 500	↑	↓	↓	N.D.	Pereira et al., 2013
Clarias batrachus	Mancozeb	↓	↓	N.D.	N.D.	Shahi and Singh, 2014
Cirrhinus mrigala	Deltamethrin	↓	↓	↓	N.D.	David et al., 2015
Hypophthalmichthys molitrix	Diazinon	No change	↑	↑	N.D.	Hedayati and Niazie, 2015
Barbonymus gonionotus	Quinolphos	↓	↓	↓	N.D.	Mostakin et al., 2015
Cyprinus carpio	Cypermethrin	↓	↓	↓	N.D.	Neelima et al., 2015
Oreochromis mossambicus	Imidacloprid	↓	↓	↓	N.D.	Patel and Parikh, 2015
Labeo rohita	Imidacloprid	↑	↑	↑	N.D.	Patel and Parikh, 2015
Cyprinus carpio	Monocrotophos	↓	↓	↓	↓	Vaiyanan et al., 2015
Clarias gariepinus	Atrazine and Metolachlor	↓	↓	↓	N.D.	George et al., 2017
Cyprinus carpio	Permethrin (technical grade)	↓	↓	↓	N.D.	Rao et al., 2017
Labeo rohita	Difenoconazole and thiamethoxam	↓	↓	↓	N.D.	Jasmin et al., 2018
Ctenopharyngodon idella	Dichlorvos	↓	↓	↓	N.D.	Kumari et al., 2018
Ctenopharyngodon idella	Deltamethrin (technical grade)	↓	↓	↓	N.D.	Rao et al., 2018
Oreochromis mossambicus	Chlorpyrifos	↓	↓	↓	N.D.	Ghayyur et al., 2019

(Abbreviations and symbols: N.D., not determined by authors in their study; ↑, significant increase; ↓, significant decrease)

Decrease in Hb content in fish could be due to either its destruction or reduced synthesis (Mostakim et al., 2015). Oxidative damage caused by ROS under pesticide stress may lead to oxidation of Hb molecules (Bloom and Brandt, 2008) thereby reducing its oxygen carrying capacity. Another aspect of toxic effect of pesticides is the disturbance in enzyme system responsible for conversion of methaemoglobin into haemoglobin. Accumulation of methaemoglobin accompanied with hypoxia has been observed in *S. mossambicus* exposed to carbamate insecticide sevin (Manthirasalam 1993; Singh and Srivastava, 2010) (**Table 2; Fig. 3**).

3) Packed Cell Volume (PCV)

It is an estimation of percentage/proportion of RBC in circulating blood. It is also called haematocrit value. The packed cell volume (PCV) of fishes after pesticidal treatment have been studied by Mahajan and Juneja (1979) in *Channa punctatus* due to aldrin; Frank (1980) in *Salmo gairdneri* and *Cyprinus carpio* due to different pesticides; Mukhopadhyay and Dehadrai (1980) in *Clarias batrachus* due to malathion; Verma et al. (1982) in *Mystus vittatus* due to different pesticides; Hilney et al. (1983) in *Anguilla vulgaris* and *Mugil* *cephalus* due to organochlorines – endrin and DDT; and by Dabral and Chaturvedi (1984) in *Heteropneustes fossilis* due to organophosphate folidol. Recently, Pereira et al. (2013) in *Prochilodus*, Vaiyanan et al. (2015) in *Cyprinus carpio*, Mostakim et al. (2015) in *Barbonymus gonionotus*, Kumari et al. (2018) in *Ctenopharyngodon idella*, and Jasmin et al. (2018) in *Labeo rohita* reported significant decrease in PCV under the influence of different classes of pesticides including organophosphates (**Fig. 2**).

Further, pyrethroid induced concomitant decrease in TEC, Hb content and PCV has been observed in *C. carpio* due to cypermethrin (Reddy and Bashamohideen, 1989; Dorucu and Girgin, 2001), *H. fossilis* due to deltamethrin (Ghosh and Banerjee, 1992), *Ctenopharyngodon idella* due to fenvalerate (Shakoori et al., 1996), and in *Sebastes schegeli* due to cypermethrin (Jee et al., 2005). Decrease in these parameters in pesticide exposed fish could be either due to shrinkage or hemolysis of RBCs leading to anaemia (Mostakim et al., 2015) or due to disruption of haematopoietic tissues (Vaiyanan et al., 2015) (**Table 2**).

On the other hand, simultaneous increase in Hb content and PCV values have been reported in *Hypophthalmichthys molitrix* exposed to an organophosphate diazinon (Hedayati and Niazie, 2015) and in *Labeo rohita* exposed to nicotine-mimicking insecticide imidacloprid (Patel and Parikh, 2015). Elevated Hb content along with increased PCV value is considered compensatory mechanism to increase oxygen carrying capacity of blood to meet metabolic under stress conditions in fish (Gbore et al., 2006).

4) Erythrocyte Sedimentation Rate (ESR)

It is the rate at which RBCs in uncoagulated whole blood descends in a standardized tube over a period of one hour. In general, ESR is a non-specific sickness indicator and often depicts the state of inflammation as well as infection (Tishkowski and Gupta, 2020). Increased erythrocyte sedimentation rate (ESR) in fishes under the influence of pesticide has been reported by Srivastava and Mishra (1983) in *Heteropneustes fossilis* exposed to fenthion; Kumari and Yadava (1988) in *Clarias batrachus* due to various pesticides; Ahmad and Ahsan (1989) in *Amphipnous cuchia* exposed to carbaryl; Singh et al. (1992) in *H. fossilis* exposed to sublethal concentration of propoxur; Swarnlata (1995) in *C. batrachus* exposed to carbaryl and carbofuran; Nath and Banerjee (1999) in *Anabas testudineus* exposed to Nuvan®; and by Kumar et al. (1999) in *H. fossilis* when subjected to deltamethrin for 30 days.

A review of recent literature in the field also points towards the elevated ESR in different groups of fish exposed to toxic pesticides. For instance, significantly increased ESR has been reported in *Channa punctatus* exposed to sub lethal concentrations of deltamethrin for 15, 30 and 45 days (Jayaprakash and Shettu, 2013). Vaiyanan et al. (2015) in *Cyprinus carpio* reported significant decrease in ESR value due to the toxic effect of monocrotophos (**Table 2**).

However, Mukhopadhyay and Dehadrai (1980) have reported unaltered ESR in *Clarias batrachus* exposed to malathion; and Verma et al. (1982) have observed decreased ESR in *Mystus vittatus* exposed to different pesticides. An insignificant decrease in ESR has also been observed in *C. batrachus* exposed to sublethal concentration of endosulfan (Venkateshwarlu et al. 1990). In recent decade, decreased ESR has been observed in *Cyprinus carpio* exposed to fenthion for 60 days (Muralidharan, 2012).

Altered ESR is often associated with certain physiological distress in fish. There is a negative correlation between ESR and total erythrocyte count (TEC) i.e. lower the TEC and higher will be the ESR (**Fig. 3**). In addition, increased concentration of certain plasma proteins such as fibrinogen and haemolysis may also contribute to increase in ESR (Jagtap and Mali, 2012). Increased ESR is considered as a strong indicator of tissue damage (Britton, 1969).

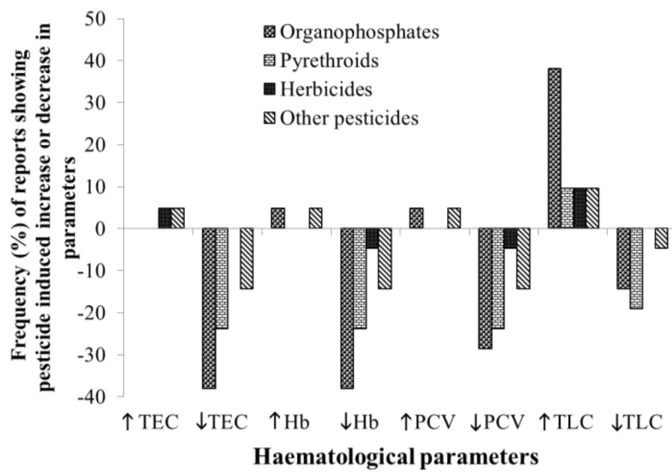

Figure 2. Frequency (%) of reports (based on literature) showing alterations in total erythrocyte count (TEC), haemoglobin (Hb) content, packed cell volume (PCV) and total leucocyte count (TLC) in fish exposed to organophosphate, pyrethroid insecticides, herbicides and other pesticides. Upright bars above baseline show reports of significant increase in parameters whereas inverted bars below the baseline show reports of significant decline in parameters. Most investigated pesticides are organophosphates followed by pyrethroids insecticides, herbicides and others including fungicides. Majority of pesticides have tendency to reduce TEC, Hb and PCV leading to anaemia, and to elevate TLC leading to leucocytosis in fish. (Symbols: ↑, increase; ↓, decrease)

5) Absolute values

Absolute values are studied in terms of three parameters – MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) (Wintrobe, 1967). MCV defines the size of the RBC. It is a measure of the average volume of a red blood corpuscle (RBC). MCH is the average mass or amount of haemoglobin per RBC in a sample of blood. It`s value is decreased in hypochromic anaemia.

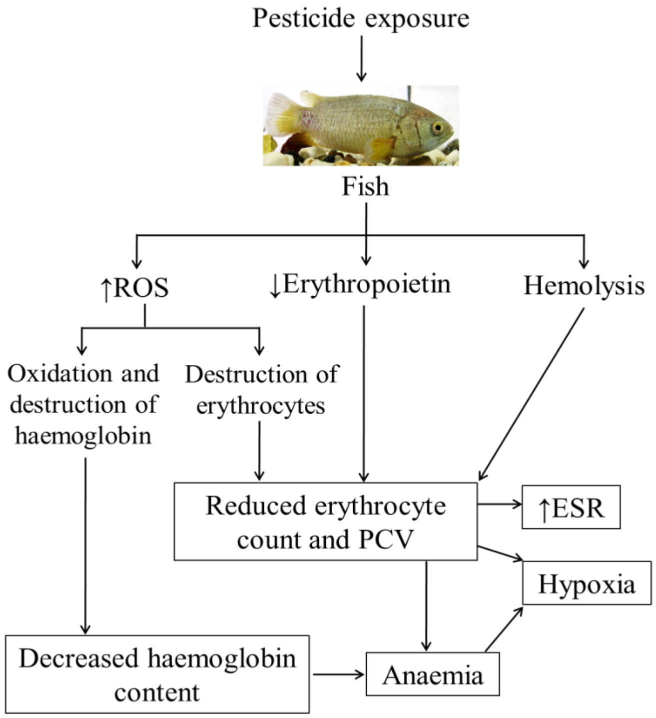

Figure 3. A proposed mechanism of alterations in the total erythrocyte count, haemoglobin content, and packed cell volume (PCV) of fish under pesticidal exposure. Pesticide toxicity may cause increased reactive oxygen species (ROS) production, decreased erythropoietin synthesis and hemolysis, which could result in reduction in erythrocyte count and haemoglobin contents leading to anaemia and subsequent hypoxia in fish. Reduced erythrocyte also results in increased erythrocyte sedimentation rate (ESR). (Symbols: ↑, significant increase; ↓, significant decrease)

MCHC defines average concentration of haemoglobin in a given volume of RBC or it indicates the percentage situation of RBC with haemoglobin.

Studies on MCV, MCH and MCHC values in several teleostean fish spp. treated with sublethal concentrations of various organophosphorus and organochlorine pesticides have shown that there is no fixed pattern of variation in absolute values (**Fig. 4**). Increased absolute values in fishes after pesticidal and other agrochemical treatment have been reported by Lone and Javaid (1976) in *Channa punctatus* due to some organophosphorus insecticides; Mahajan and Juneja (1979) in *Channa punctatus* due to aldrin; Verma et al. (1982) in *Mystus vittatus* due to different pesticides, Natrajan (1984) in *Channa striatus* due to metasystox, and by Dabral and Chaturvedi (1984) in *Heteropneustes fossilis* due to folidol (Table 3).

The absolute values are useful in the determination and classification of anaemia on the basis of size and haemoglobin content of RBCs. Pesticidal intoxication causes alterations in the size and shape of RBCs which are called anisocytosis and poikilocytosis, respectively. Alteration in absolute values may also be characterised by the normocytic RBCs of normal shape and size with normal MCV values, enlarged macrocytic RBCs with higher MVC values or by the small microcytic RBCs with lower MCV values. Likewise, on the basis of haemoglobin (Hb) content, erythrocytes are also categorised into three classes – normochromic with normal Hb content, hypochromic with little amount of Hb and hyperchromic RBCs with higher levels of Hb. Pesticide induced changes in these parameters in fish may occur due to interference with erythropoiesis or haemopoiesis or iron content.

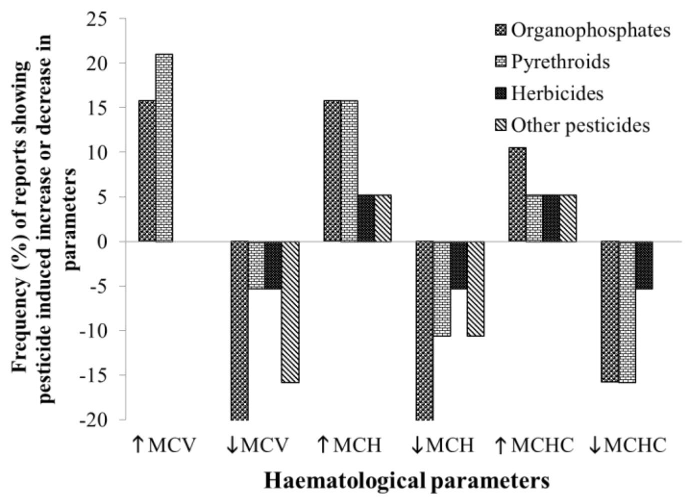

Figure 4. Frequency (%) of reports (based on literature) showing alterations in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) of fish exposed to organophosphate, pyrethroid insecticides, herbicides and other pesticides. Upright bars above baseline show reports of significant increase in parameters whereas inverted bars below the baseline show reports of significant decline in parameters. Different pesticides have both decreasing as well as increasing effect on MCV, MCH and MCHC depending upon fish species. (for details, please see the main text and Table 3) (Symbols: ↑, increase; ↓, decrease)

Increase in the size of RBCs has been reported by Kumari and Yadava (1988) in *C. batrachus* due to certain pesticides. However, decreased MCV values showing microcytic RBCs have been reported in *L. rohita* under the effect of chlordane (Bansal et al., 1979), and in *C. striata* due to metasystox (Natarajan, 1984). In recent years, significant decrease in the values of MCH and MCHC has been reported in *Prochilodus lineatus* due to clomazone-based herbicide – Gamit® 500 (Pereira et al., 2013), in *Channa punctatus* due to deltamethrin (Jayaprakash and Shettu, 2013) and in *H. fossilis* due to chlorpyrifos (Tiwari et al., 2017). On the contrary, significant increase in MCH and MCHC has been reported in *L. rohita* due to exposure to difenoconazole and thiamethoxam (Jasmin et al.,

2018) and in *Barbonymus gonionotus* due to quinalphos (Mostakim et al., 2015). A significant decrease in MCV value has been observed in *L. rohita* due to difenoconazole and thiamethoxam (Jasmin et al., 2018) and in *H. fossilis* due to chlorpyrifos (Tiwari et al., 2017). On the other hand, significant increase in MCV was reported in *Channa punctatus* due to deltamethrin (Jayaprakash and Shettu, 2013) and in *Barbonymus gonionotus* due to quinalphos (Mostakim et al., 2015). Simultaneous decrease in MCV and MCH with increased MCHC was observed in *Ctenopharyngodon idella* due to Nuvan (76% EC DDVP) (Kumari et al., 2018) (Table 3). Decline in MCH and MCHC values are associated with swelling of erythrocytes and subsequent hypoxia (Amaeze et al., 2020).

Table 3. Summary of recent updates on alterations in erythrocyte related absolute values such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) in different fish species exposed to various pesticides.

Fish species	Pesticide	MCV	MCH	MCHC	References
Oncorhynchus mykiss	Diazinon	↑	↑	No change	Far et al., 2012
Channa punctatus	Deltamethrin	↑	↓	↓	Jayaprakash and Shettu, 2013
Prochilodus lineatus	Clomazone-based herbicide – Gamit® 500	No change	↓	↓	Pereira et al., 2013
Clarias batrachus	Mancozeb	No change	↓	↓	Shahi and Singh, 2014
Cirrhinus mrigala	Deltamethrin	↑	↑	No change	David et al., 2015
Hypophthalmichthys molitrix	Diazinon	Initially ↑, later ↓	Initially ↑, later ↓	↑	Hedayati and Niazie, 2015
Barbonymus gonionotus	Quinolphos	↑	↑	↑	Mostakin et al., 2015
Cyprinus carpio	Cypermethrin	↓	↓	↓	Neelima et al., 2015
Oreochromis mossambicus	Imidacloprid	↑	↑	Fluctuation	Patel and Parikh, 2015
Labeo rohita	Imidacloprid	↑	↑	↑	Patel and Parikh, 2015
Clarias gariepinus	Atrazine and Metolachlor	↓	↑	↑	George et al., 2017
Cyprinus carpio	Permethrin (technical grade)	↑	↑	↑	Rao et al., 2017
Heteropneustes fossilis	Chorpyrifos	↓	↓	↓	Tiwari et al., 2017
Labeo rohita	Difenoconazole and thiamethoxam	↓	↑	↑	Jasmin et al., 2018
Ctenopharyngodon idella	Dichlorvos	↓	↓	↓	Kumari et al., 2018
Ctenopharyngodon idella	Deltamethrin (technical grade)	↑	↑	↓	Rao et al., 2018
Oreochromis mossambicus	Chlorpyrifos	↓	No change	No change	Ghayyur et al., 2019

(Symbols: ↑, significant increase; ↓, significant decrease)

B. Leucocyte dependent parameters

1) Total Leucocyte Count (TLC)

It is the measure of total number of white blood cells or leucocytes in the blood. TLC may increase or decrease due to the effect of pesticide. Pathophysiological condition of increased TLC is leucocytosis, and that of decreased TLC is leucopenia.

An increased TLC as leucocytosis under the influence of different pesticides has been observed in *Tilapia zillii* due to pollution (Sadd et al., 1973), *Channa punctatus* due to aldrin and other pesticides (Lone and Javaid, 1976; Mahajan and Juneja, 1979), *Mystus vittatus* due to different pesticides (Verma et al., 1982), *Heteropneustes fossilis* due to different insecticides (Srivastava and Narain, 1982), *H. fossilis* due to malathion (Mishra and Srivastava, 1983), *Anguilla vulgaris* and *Mugil cephalus* due to endrin and DDT (Hilney et al., 1983), *Heteropneustes fossilis* due to DDT (Mustafa and Murad, 1984), *Ophiocephalus punctatus* by endosulfan (Tyagi et al., 1989), *Clarias batrachus* due to organochlorine BHC poisoning (Thakur and Pandey, 1990), in *Heteropneustes fossilis* exposed to sublethal concentration of chlordecone (Srivastava and Srivastava, 1994) and in *Clarias batrachus* exposed to sublethal concentrations of actellic (Mgbenka et al., 2005).

Reports on TLC in recent years also reveal a significant increase in its value in *Channa punctatus* under the influence of sublethal concentrations of deltamethrin (Jayaprakash and Shettu, 2013), in *Cyprinus carpio* exposed to sublethal concentrations of fenthion (Muralidharan, 2012), in *Cyprinus carpio* due to sublethal concentrations of monocrotophos (Vaiyanan et al. 2015), in *Channa punctatus* exposed to sublethal concentrations of endosulphan and dimethoate (Prakash, 2016), in *Heteropneustes fossilis* due to sublethal concentrations of chlorpyrifos (Tiwari et al., 2017), and in *Ctenopharyngodon idella* exposed to lethal and sublethal doses of dichlorvos and its technical grade (Kumari et al. 2018). Increased leucocyte count could be a compensatory and defense response against the toxic effect of pesticides on fish (Mostakin et al., 2015) (**Table 4**).

Leucopenia has been reported in *Channa striatus* exposed to sublethal concentration of metasystox (Natrajan, 1984), in *Heteropneustes fossilis* due to the effect of chlordane, lindane, heptachlor, dimethoate, methyl parathion and cypermethrin (Mishra and Srivastava, 1984; Srivastava and Mishra, 1985, 1987; Ghosh and Banerjee, 1993; Nath and Banerjee, 1996), in *C. carpio* exposed to diazinon (Banaee et al., 2008), and in *Clarias gariepinus* exposed to diazinon (Adedeji et al., 2009). Recently, significant decrease in TLC value has also been reported by Mostakin et al. (2015) in *Barbonymus gonionotus* due to quinolphos and by Jasmin et al. (2018) in *Labeo rohita* exposed to difenoconazole and thiamethoxam (**Table 4**). Decreased TLC in fishes has been associated with stress caused by pesticidal exposure (Jasmin et al., 2018). Thus decline in the leucocyte count is considered a signal of compromised immunity of fish under pesticidal toxicity (Far et al., 2012). Thus pesticides have mixed effect on TLC – most of the pesticides cause leucocytosis while some pesticides are reported to cause leucopenia depending upon fish species investigated (**Fig. 2**).

2) Differential Leucocyte Count (DLC)

DLC is the percentage estimate of each type of leucocyte present in the blood. The differential leucocyte count in fishes under the treatment of certain pesticidal intoxicants has received the attention of several workers. Increase in neutrophils and small lymphocytes along with decrease in large lymphocytes and basophils have been reported in *C. batrachus* and *Cirrhinus mrigala* exposed to aldrin (Mukhopadhyay and Dehadrai, 1980). Significant increase in large and small lymphocytes combined with decreased monocytes was observed in *H. fossilis* due to malathion exposure (Mishra and Srivastava, 1983) and in *Channa punctatus* due to DDT and dieldrin (Lone and Javaid, 1976). Similar findings along with increased neutrophils and basophils have been observed in *H. fossilis* exposed to B.H.C. (Srivastava and Narain, 1982). According to Roitt (1977), pesticides disrupt the immunological responses of animals as reflected by altered lymphocyte count.

Recently, significant alterations in DLC have been reported in *Channa punctatus* due to endosulphan and dimethoate for 96 hr (Parkash, 2016). They observed significant decrease in basophils, eosinophils, lymphocytes and monocytes with simultaneous increase in neutrophils due to endosulfan. In the same study, they also reported significant decrease in monocytes and neutrophils with simultaneous increase in basophils, eosinophils and lymphocytes in the fish exposed to dimethoate. Increased levels of neutrophil, basophil and eosinophil with decreased lymphocytes and monocytes have been observed in *Heteropnuestes fossilis* exposed to chlorpyrifos (Tiwari et al., 2017) (Table 4).

C) Other parameters

1) Thrombocyte Count (ThC)

Thrombocyte count measures the number of thrombocyte or platelets in unit volume of blood. It is usually expressed as platelets per cubic millimetre of whole blood. Increased ThC is referred to thrombocytosis, and significantly lowered ThC count, thrombocytopenia.

Increase in thrombocytes following pesticidal treatments has been reported in *Channa punctatus* due to aldrin (Mahajan and Juneja, 1979), *Clarias batrachus* due to aldrin (Dalela et al., 1980), *H. fossilis* due to nuvacron and dimecron (Srivastava and Narain, 1982), *H. fossilis* due to malathion (Mishra and Srivastava, 1983), *H. fossilis* exposed to formothion (Singh and Srivastava, 1984), *Clarias batrachus* exposed to endosulfan and kelthane (Venkateshwarlu et al. 1990), *H. fossilis* exposed to aldrin (Singh et al., 1991), *H. fossilis* exposed to chlordecone (Srivastava and Srivastava, 1994), and in *Clarias gariepinus* exposed to diazinon (Adedeji et al., 2009). Recent study also reveals increased ThC content in *Cyprinus carpio* exposed to fenthion (Muralidharan, 2012). Thrombocytosis might be a response to overcome the excessive bleeding in eventual tissue damage or haemorrhage under pesticidal effect (Mahajan and Juneja, 1979; Kumari and Yadava, 1988).

Significantly decreased thrombocyte count was observed in *H. fossilis* due to fenthion (Srivastava and Mishra, 1983), *H. fossilis* exposed to chlordane (Mishra and Srivastava, 1984), *H. fossilis* exposed to propoxur (Singh et al., 1992), *Clarias* *batrachus* exposed to certain carbamate pesticides (Swarnlata, 1995).

Table 4. Summary of recent updates on alterations in total leucocyte count (TLC) and differential leucocyte counts (neutrophils, basophils, eosinophils, lymphocytes and monocytes) in different fish species exposed to various pesticides.

Fish species	Pesticide	TLC	Differential leucocyte count					References
			Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	
Oncorhynchus mykiss	Diazinon	↓	Fluctuation	N.D.	N.D.	Fluctuation	N.D.	Far et al., 2012
Cyprinus carpio	Fenthion	↑	N.D.	N.D.	N.D.	↑	↑	Muralidharan, 2012
Channa punctatus	Deltamethrin	↑	N.D.	N.D.	N.D.	N.D.	N.D.	Jayaprakash and Shettu, 2013
Clarias batrachus	Mancozeb	↑	N.D.	N.D.	N.D.	N.D.	N.D.	Shahi and Singh, 2014
Cirrhinus mrigala	Deltamethrin	Initially ↑, later ↓	N.D.	N.D.	N.D.	N.D.	N.D.	David et al., 2015
Hypophthalmichthys molitrix	Diazinon	↑	Initially ↑, later ↓	No change	N.D.	↑	No change	Hedayati and Niazie, 2015
Barbonymus gonionotus	Quinolphos	↓	N.D.	N.D.	N.D.	N.D.	N.D.	Mostakin et al., 2015
Cyprinus carpio	Cypermethrin	↓	N.D.	N.D.	N.D.	N.D.	N.D.	Neelima et al., 2015
Oreochromis mossambicus and *Labeo rohita*	Imidacloprid	↑	↑	N.D.	N.D.	↑ (small lymphocytes); ↓ (large lymphocytes)	N.D.	Patel and Parikh, 2015
Cyprinus carpio	Monocrotophos	↑	N.D.	N.D.	N.D.	N.D.	N.D.	Vaiyanan et al., 2015
Channa punctatus	Endosulphan	↑	↑	↓	↓	↓	↓	Parkash, 2016
Channa punctatus	Dimethoate	↑	↓	↑	↑	↑	↓	Parkash, 2016
Clarias gariepinus	Atrazine and Metolachlor	↑	↑	N.D.	N.D.	↓	↓	George et al., 2017
Cyprinus carpio	Permethrin (technical grade)	↓	N.D.	N.D.	N.D.	N.D.	N.D.	Rao et al., 2017
Heteropneustes fossilis	Chorpyrifos	↑	↑	↑	↑	↓	↓	Tiwari et al., 2017
Labeo rohita	Difenoconazole and thiamethoxam	↓	N.D.	N.D.	N.D.	N.D.	N.D.	Jasmin et al., 2018
Ctenopharyngodon idella	Dichlorvos	↑	N.D.	N.D.	N.D.	N.D.	N.D.	Kumari et al., 2018
tenopharyngodon idella	Deltamethrin (technical grade)	↓	N.D.	N.D.	N.D.	N.D.	N.D.	Rao et al., 2018
Oreochromis mossambicus	Chlorpyrifos	↑	Increased granulocyte count			↑	↑	Ghayyur et al., 2019

(Abbreviations and symbols: N.D., not determined by authors in their study; ↑, significant increase; ↓, significant decrease)

2) Coagulation Time (CT)

It is defined as the time period (minutes) required for a sample of blood to coagulate in vitro under standard conditions. Decreased coagulation time (CT) under pesticidal effects has been reported in *Channa punctatus* due to some organophosphorus insecticides (Lone and Javaid, 1976); in *Heteropneustes fossilis* due to various insecticides (Srivastava and Narain, 1982); in *Heteropneustes fossilis* due to fenthion (Srivastava and Mishra, 1983); in *Clarias batrachus* due to different pollutants (Dhillon and Gupta, 1983); in *Cyprinus carpio* due to fenthion (Muralidharan, 2012). On the other hand, increased clotting time has been observed in *Channa punctatus* due to deltamethrin (Jayaprakash and Shettu, 2013).

CONCLUSION

Aforementioned reports indicate that the pesticides greatly distress the haematology of aquatic fauna with particular reference to fishes. In general, there is no fixed pattern of ill effect of different classes of pesticides on haematological parameters. However, toxicity of most pesticides has resulted into anaemia and leucocytosis in fish. Altered haematological parameters in turn detrimentally change vital physiology of fish e.g. respiration, feeding, reproduction etc. Any change in the physiology of fish could have catastrophic consequences like decrease in productivity of fish. Decrease in the production of fish may affect the food constituents of human population as well as has detrimental consequences on ecosystem and biodiversity. Therefore, it is of utmost importance to check and reduce the indiscriminate usage of pesticides and may look in to other environmental friendly approaches of agrochemicals to increase crop productivity. This will assist to succeed in the nutritional war and will also provide pollution free aquatic body as well as aquatic variety of fishes. However, further studies are needed to unravel precise cellular and molecular mechanism of pesticide induced alterations in haematological parameters of fish.

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